For these reasons, this research study involved production of surfactin by using local isolate of *B. subtilis* MSH1 and investigation the antifungal activities of surfactin against pathogenic fungi. Results obtained in this study can assist in potential new application of surfactin as an alternative for antibiotic and anti-infective treatment in medical and pharmaceutical purposes.

1.2 Objectives

1. To produce surfactin by using local isolate of *B. subtilis* MSH1.

2. To investigate antifungal activities of surfactin against several pathogenic fungi.

CHAPTER I

INTRODUCTION

Bacillus is considered as one of the species abundantly presence of soil and characterized by their ability to produce many antibiotics compounds that have a lot of importance in biochemical fields the most important characteristics of these compounds derivatives peptide are more studied (Stein, 2005). Higher production of strains that have the ability to produce anti-microbial compounds may be associated with environmental role and with a defensive action of strains microbial created (Strahl *et al.,* 2002). *B. subtilis* produces many biological active peptides, which have great significance in the biotechnological and biopharmaceutical applications. These include surfactin, fengycin, iturin, mycosubtilins, and bacillomycins. These lipopeptides biosurfactants are amphiphilic and membrane-active by nature, which has antibacterial activities (Sriram, 2011). It is known that surfactin compound a lot of chemical properties among which anti-activity of positive and negative gram bacteria alike and also has an anti-activity of fungi have been discovered surfactin in 1968 when he was examined broth *B. subtilis*, it was noted the presence of a new compound is biologically active (Arima *et al.,* 1968).

Genus *Bacillus* includes many important species that have a significant role in the industrial areas that have proven safe to used in foods industry. The most important products available commercially that produced by *Bacillus spp*, are enzymes amino acids, antibiotics and antifungal peptides. Surfactin has been applied in many potential applications in the fields of medicine and vitality due to its activity against microbes and discourage fibrin formation and activities antiviral tumors and fungi as well (Singh, 2004). The gene responsible for the production of surfactin has been organized in a large operon in *B. subtilis* and the sfp gene, responsible for production of enzyme 4′-

phosphopantetheinyl transfers was characterized as essential to production of surfactin (Nakano, 1992; Hsieh, 2004).

Surfactin considered as one of the most active surfactants and characterized by their effectiveness and often used as an antibiotic. It is a bacterial cyclic lipopeptide, largely prominent for its exceptional surfactant power (Mor, 2000). The most important characteristic of surfactant is amphiphilic in both hydrophobic and hydrophilic environments. Surfactin is produced from the formation of spores gram positive bacteria *B. subtilis* and it is used as an antibiotic and domain-wide for its ability to antibacterial activity, viruses, fungi, anti-mycoplasma and hemolytic activities (Singh, 2004).

Considered fungi is one of reasons that lead to the contamination of food crops, materials raw and industrial products. The risks and damage caused by the fungus contamination are economic losses, industrial pollution and human and animal diseases (Hawksworth, 2001). It is more traditional problems facing us in the clinical field that treatment of infectious diseases caused by fungal strains (Mehregan, 2010). These problems might have encouraged many researchers to examine the inhibitory new compounds the most effective and safe (Emami, 2008) to resolve these problems and fungal control and reduce the spread. Despite all attempts to fight this scourge and negative effects and the emergence of resistant bacterial and fungal nevertheless the researchers, have many of the studies on natural products from microorganisms to discover new compounds are sophisticated and safe (Mehrgan, 2010).

To resolve the problems caused by fungi should search for new bacterial strains capable of producing inhibitory compounds and this is the first step to discover new compounds to antibiotics that contributes to the medical fields (Imada, 2007). Many of the antibiotics available in the original resulting from bacterial and fungal strains (Mannanov, 2001).

Antibiotics is a chemical substance produced by microorganisms, which have the ability to inhibition the growth of bacteria and even smashing the bacteria and other microorganisms at certain minimal inhibition concentration (MIC) level.

The prevalence of pathogens that have the ability to drug resistance of the most serious threats to the production of successful treatment of microbial diseases. For the treatment of diseases caused by microbes can use the antimicrobial agent as a chemotherapeutic agent (Tepe *et al.,* 2004). Conducted many necessary applications to isolate bacterial strains and proof their ability to produce inhibitory compounds for pathogens growth (Mannanov, 2001; Tamehiro, 2002).

Surfactin and nature of antibiotics, has antibacterial characteristic very large for, it is also able to break through the cell membranes of all kinds of bacteria. There are two main types of bacteria, Gram-negative and they are Gram-positive. Second kind of bacteria varies in their membrane formation. Gram-positive bacteria lack the outer membrane and holds thicker peptidoglycan layer as well as the bi-layer phospholipids (Bergey *et al.,* 1994). While, and Gram-negative bacteria have lipopolysaccharide membrane in the outer layer thin peptidoglycan followed by bilateral phospholipids layer, and this is essential factor contributing to such detergents activity surfactin as it is capable of create transmittance bilateral fat layer environment and cause disruption to the membrane stability.

For surfactin to complement its antibacterial property successfully, the bacterium requires to be treated with a high concentration. In fact, surfactin require being in concentrations between 12–50 µg/ml in order for it to conduct minimal antibacterial effects (Heerklotz H *et al.,* 2001). This is also known as (MIC).

Most important characteristic of this activity is surfactin's ability to dismantle and enveloped viruses. When examining the anti-viral activity surfactin found that surfactin does not analysed lipid enveloped viral and only, but also the capsid of the virus through ion channel formations. This process proved during test on several envelops viruses

such as HIV and HSV (Jung M *et al.,* 2000). Also, the isoforms of the fatty acid chain containing 14 or 15 carbon atoms showed an improvement in disabled of the viral envelope. Unfortunately, surfactin is able only affected cell-free viruses and those that had hacked the cell were unaffected. Concurrently, if surfactin were exposed to a high medium of protein or lipid concentrations, its antiviral activity would be limited. This is also known as the buffer effect and is a significant disadvantage in surfactin's antiviral activity.

For these reasons, this research project will be involve production of surfactin by *B. subtilis* MSH1 and to asses antifungal activities against *Aspergillus niger, Colletotrichum gloeosporioides, Candida albicans , Candida tropicalis , Candida parapsilosis and Candida Krusei.*

1.1 Problem Statement

The number of drug-resistant pathogenic fungi steadily poses a constant threat; some forms of alternative treatment are very much required. Several recent studies have also showed various inhibitory effect of surfactin against various types of bacteria carrying great medical, environmental or agricultural significance. Surfactin is a molecule of heptapeptide, during study the surfactin has been showed activity powerful antibiotic with a spectrum antifungal wide (Maget-Dana, 1994). For this reason is surfactin of more importance of antibiotics because to find out the most important anti-fungal materials and more effective, safer to limit the growth of fungi.

Today, antimicrobial peptides produced by microorganisms to a large extent possible answer to the problem of the increasing resistance of conventional antibiotics, and fungal infections and antibiotic residues that are considered. Nowadays the antimicrobial peptides obtained from microorganisms are considered of possible solution to the growing problem of increasing of resistance to conventional antibiotics, fungal infection and antibiotics residue.