CHAPTER II

LITERATURE REVIEW

2.1 *Bacillus subtilis*

B. subtilis is a gram positive bacterium that has the capability to produce large number of peptide antibiotics with non-proteinogenic constituents and unusual linkages. Surfactin, mycosubtilin, bacillomycin and fengycin are small cyclic lipopeptide that non-ribosomally synthesized by large multiprotein complexes named non-ribosomal peptide synthetises (NRPS). Always these peptide antibiotics are also known as secondary metabolites and which are produced at the end of bacterial exponential growth phase (Stein, 2005). According to (Seydlova & Svobodova, 2008), *B. subtilis* have the capability to encounter the physical nutritional fluctuations due to its characteristics as a soil-dwelling organism. Besides, it also controls fatty acid and polar head group in response to its stationary phase conditions and surfactin production at the same time; which surfactin was produced during stationary phase.

2.2 Surfactant

Surfactants are amphiphilic compounds which have the capability to accumulate between fluid phases such as water in oil or oil in water, and it's also known as a surface-active substance. They also able to the reduction of surface tension as well as interfacial tension and form emulsions (Desai & Banat, 1997). By having both hydrophobic and hydrophilic, surfactants are from within the most distinguished chemical products, used in varieties of applications such as agriculture and household (Deleu & paquot, 2004).

Surfactant able to reduce of the free energy of a system through substituting bulk higher energy molecules at interface. Their capability to assemble contaminations has been the reason why surfactants are so popular in soil washing or flushing. It

hydrophilic group has high affinity towards the bulk medium, while the hydrophobic part has not or less affinity. Because of this amphiphilic property, surfactants have been utilized widely in industries as emulsifiers, de-emulsifiers, adhesives, wetting and forming agents, flocculating (Mulligan & Gibbs, 1993).

In addition, lowering surface tension, increasing solubility, having detergency power, wetting capability and forming capacity have been synonymous with surfactants. Referring to the characteristics surfactants have, no wonder surfactants has become the main option in petroleum industry particularly in promote oil removal application through increasing the solubility of petroleum ingredients (Falatko, 1991). Although that, surfactants are also utilized in pharmaceutical industries and mineral flotation (Mulligan & Gibbs, 1993).

Among the most important characteristics of surfactants are solubility promotion surface tension reduction and low critical micelle concentrations. Between two immiscible phases an interfacial boundary presents and surfactants concentrate at the interface either in liquid-solid conditions, liquid-liquid of steam-liquid. The hydrophobic moieties concentrate at the surface, while the hydrophilic parts are oriented to solution. Capability to decrease thus lower surface tension is a measure towards effectiveness of surfactants through determining the surface energy per unit area required to achieve a molecule from the bulk phase to the surface (Rosen, 1978).

The presences of surfactants need less energy to bring molecules to the Surface and the surface tension is lowered. For example, can reduce the surface tension of water from 72 to 35 mN/m and the interfacial tension; which refers to the tension between polar and non-polar liquids, for water against n-hexadecane from 40 to 1 mN/m. Usually The surface tension linked with the concentration of the surface-active compound until the critical micelle concentration is achieved (Mulligan, 2005).

The lower critical micelle concentration, the more efficient surfactant is and less surfactant is needed to lower the surface tension. The critical micelle concentration is defined as the minimum concentration necessary for micelle formation while in the

practical; the critical micelle concentrations is the maximum concentration of monomers of surfactant in water and are influenced by temperature, ionic strength and pH (Becher, 1965).

In general, the utilizations of surfactants is to provision energy and also provision energy costs, for example the energy required for pumping in pump and for processed techniques. Within the most essential section critical for surfactants are responsible for type, physicochemical behaviour, solubility and adsorption behaviour. As well as organic solvents, chelating agents bases and acids , surfactant have been utilized to promote metals removal, while in the same time new markets are currently being developed for utilize in biological treatment of contaminated areas sites (Oberbremer *et al.*, 1990).

2.3 Biosurfactants

Biosurfactants are amphiphilic compounds produced in living surfaces, and also they able to either stay adherent to microbial cell surfaces or be secreted in the culture broth. The increasing interest in biosurfactant refers to its ability to be a substitute to synthetic surfactants affiliate a number of features such as their diversity, environmentally friendly nature, and the possibility for large-scale production, selectivity, effectiveness at extreme temperatures, pH, and salinity, and which makes these molecules for implement applications in cosmetics, food preservatives, pharmaceuticals, and detergents. In spite of various advantages of biosurfactants, compared to chemical surfactants, it is still not competitive in vary wide of applications cause to rising cost of production together with weak strain productivity and the need for costly substrates (Deleu, 2004).

Biosurfactants are produced by various types of microorganisms; include gram-positive and gram-negative bacteria, and fungi (Ron, 2001). The most commonly reviewed biosurfactants are from bacteria, particularly those produced by members of the Pseudomonas and *Bacillus* genera (Ron & Rosenberg, 2001). Biosurfactants are classified mainly by their chemical structure and microbial origin. Biosurfactant structure consist of hydrophilic moiety which includes an acid, peptide cations or anions, mono-, di- or

polysaccharides and a hydrophobic moiety of unsaturated or saturated hydrocarbon chains or fatty acids. Some main classes of biosurfactant include glycolipids, lipopeptides and lipoproteins, phospholipids and fatty acids, polymeric surfactants and particulate surfactants (Desai *et al.,* 1997).

In the late 1960s, biosurfactants have been attracted attention as hydrocarbon dissolution agents, and their applications have been greatly extended in the past decades as an evolved as replacing to chemical surfactants (carboxylates, sulphonates and sulphate acid esters), especially in oil industry and food, pharmaceutical (Banat *et al.,* 2000; Desai *et al.,* 1997). The reason for their popularity as high value microbial products is mainly due of their certain action, low toxicity, higher biodegradability, effectiveness at extremes of temperature, salinity, pH, and widespread the possibility of the application, and their unique structures offering new characteristics that classical surfactants may lack (Desai *et al.,* 1997; Kosaric, 1992). Biosurfactants possess the characteristic property to reduction the surface and interfacial tensions utilize the same mechanisms as chemical surfactants. Through microbial fermentation processes these molecules can be produced by utilized cheaper agro-based substrates and waste materials, unlike chemical surfactants, which are mostly derived from petroleum, feed stock.

Through used the bulk agro-waste to produce biosurfactants as a medium with the presence of respective bacterial as great producer or synthesizer. Improving environmental compatibility, greater forming characteristic, higher selectivity and ability to biodegradation all the awards of surfactants have been granted with their unique assets compared with the chemically synthesized surfactants (Mukherjee *et al.,* 2006). Biosurfactants able to be produced from several low-cost waste substrates, and therefore resulting to reduction their production cost (Makkar & cameotra, 2002). It's well also known the biosurfactants have many several of groups, each group has natural role in the growth of the organisms in which they are produced. These including increasing the surface area and bioavailability of hydrophobic water-insoluble substrates, pathogenic, heavy metal binding, quorum sensing and biofilm formation (Ron & Rosenberg, 2001).

Biosurfactants can be classified mainly into four categories, which are glycolipid (Kitamoto, 2002), fatty acid, lipopeptide (Peypoux *et al.,* 1999) and polymer (Rosenberg & Ron, 1999). The classification depending on the structure of their hydrophilic part (Muthusamy *et a.l,* 2008). Biosurfactants have huge potential in the fields antimicrobial applications especially in antiviral antibacterial, anti-mycoplasma, and antifungal (Singh & Cameotra 2004).

Since biosurfactants are biologically produced and safer compared to chemically synthesized surfactants, biosurfactants become suitable alternatives against synthetic medicine and antimicrobial agents. Besides, biosurfactants can be used safely and effectively as therapeutic agents or especially while drug resistance among causative organisms for many life-threatening diseases keeps on increasing (Singh & Cameotra, 2004).

2.4 Lipopeptide

Lipopeptide biosurfactants are mostly produced by *Bacillus genus* and usually manifest themselves as mixtures of isoforms with slight variations in the length of fatty acid chains and amino acid substitutions. The lipopeptides that were discovered until now can be divided into surfactin (Peypoux *et al.,* 1999; Mulligan, 2005), iturin (Tsuge, *et al.,* 2005) and fengycin families (Ongena *et al.,* 2008). They are made up of seven α-amino acids (iturin and surfactin) or ten α-amino acids (fengycins). Surfactin is of particular importance in this study and is further discussed.

2.5 Surfactin

Surfactin is a cyclic lipopeptide produced by *B. subtilis* as a secondary metabolite during their growth and helps in mobility and colonizing surface (Maget-Dana & Ptak, 1995).

Surfactin was discovered in 1968 when Arima analysed the *B. subtilis* broth, where he discovered a new compound is biologically active and it was named surfactin because of its exceptional surfactant activity and it's also been clarified structure as that of a macrolide lipopeptide (Kakinuma *et al.,* 1969). In spite of, the discovery several types lipopeptides, but surfactin considered of the most important their and also considered the main representative of this groups, and it also has attracted the attention a large number of researchers to be a substitute for the types of chemical surfactants which have effects harmful to the environment.

Surfactin of the most important characteristics is its ability to differentiate between viral cells from mycoplasma cells, and there have been a number of proposals in to how to use it to ensure the safety of pharmaceutical products (Vollenbroich *et al.,* 1997). Kumar *et. al.,* (2007) reported surfactin mycoplasma was eliminated from an extensively infected irreplaceable hybridoma cell line. There were apparent indications of limited elimination, suggesting the possible usefulness of surfactin in achieving decontamination. Has been examined the effect of surfactant on the proliferation of LoVo cells, a human colon carcinoma cell line, by (Kim *et al.,* 2007). Where surfactin preventing the spread of the cells LoVo and that by stimulating the pro-apoptotic activity and wresting the cell cycle. Results have shown that surfactin has the ability to create anti-cancer proteins as a result its ability to suppress the survival and also its ability to down-regulate the cell cycle. (Whang *et al.,* 2008) Results confirmed the potential application of the two biosurfactants, surfactin (SF) and rhamnolipids (RL), in order to promote the decomposition of diesel contaminated soil and water, high efficiency of biodegradation in soil/diesel systems.

2.6. Biological Properties of Surfactin

Surfactin produced by *B. subtilis* was first identified as a potent inhibitor of fibrin cloning (Arima *et. al,* 1968) and later found to lyse erythrocytes as well as spheroplasts and protoplasts of some bacterial species (Bernheimer & Avigad, 1970). Surfactin is antibacterial, antitumor and hypocholesterolemic agent. It is the most powerful

biosurfactant known lowering the surface tension of water from 72 to 27 mN/m (Cooper *et al.,* 1981). Biosurfactants provide an advantage over synthetic surfactants because most are biodegradable and less toxic. Since the biosurfactants are natural compounds they have industrial importance. They are distinguished by excellent surface and membrane-active properties along with superior emulsifying and foaming properties that can be utilized in food biotechnology and in the agricultural sector (Peypoux *et al.,* 1999).

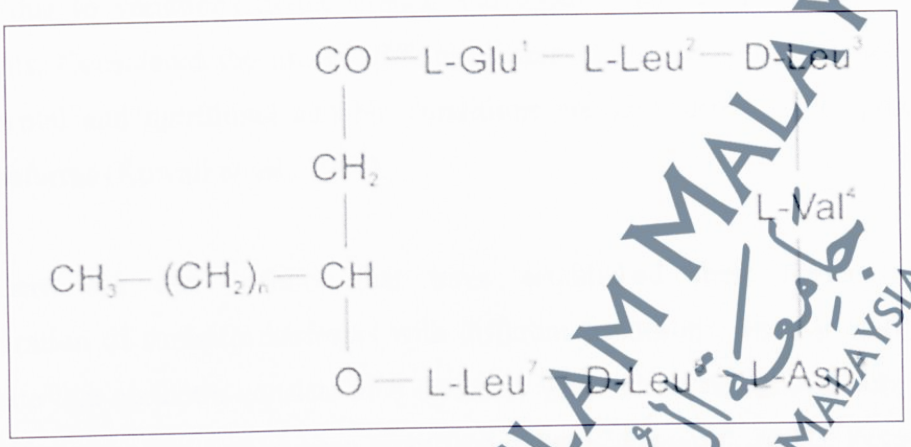
Of the most diverse activities for surfactin including: Inhibition of antiviral, starfish acolyte maturation and anti- mycoplasma activities (Peypoux *et al.,* 1999), emulsification, foaming (Razafindralambo *et al.,* 1993), insecticidal activity against the fruit fly *Drosophila melanogaster* and there are some viruses which are coated by a lipoprotein membrane such as herpes and retroviruses are efficiently inactivated through this biosurfactant (Assie *et al.,* 2002).

Platelet aggregation was inhibited and the density of platelet-rich plasma (PRP) clots was decreased by the preincubation of PRP with surfactin. Among the advantages for surfactin is its ability to inhibit platelet aggregation, which leads to the formation of fibrin clot and also the dismantling of fibrin with facilitated diffusion of fibrinolytic agents (Lim *et al.,* 2005).

2.7. Structure and physicochemical properties of surfactin

Surfactin was discovered by (Arima *et al.,* 1968) from the culture broth of *B. subtilis* and is named as surfactin due to its excellent surfactant activity (Peypoux *et al.,* 1999). The surfactin structure is characterized by a heptapeptidic moiety ($_{L}$-aspartic acid, $_{L}$-leucine, $_{L}$-glutamic acid, $_{L}$-leucine, $_{L}$-valine and two $_{D}$-leucines) linked to a beta hydroxyl-fatty acid of the chain lengths 12 to 16 carbon atoms resulting in cyclic structure. Figure 1 shows the primary structure of surfactin. Based on the figure, hydrophobic amino acids remains are located at positions 2,3,4,6 and 7, while the glutamyl and aspartyl remains at position 1 and 5, respectively introduce two negative charges to the molecule (Seydlová *et al.,* 2008).

FIGURE 1: Primary structure of surfactin.



Although lipopeptide antibiotics have been studied; However, the for the advantage surfactin ability to collect various interests and because of the multi-faceted interactions with biological systems that lead to different physiological and biochemical.

When considering the structure of surfactin has the capability to form micelles self-way these micelles tend to form large totals. Micelles Surfactin are heterogeneous with respect to volume of distribution with different configurations. It was noted that the quantity of assembling micelles-like field is smaller than that traditional surface microbial any 11 (Zhou *et al.*, 2010) or 20 (Shin *et al.*, 2009). The structure of the micelle is of the core shell type, with the hydrocarbon chain and the hydrophobic residues leads to the formation the core of the micelle (Shin *et al.*, 2009). However, it has been detected many different kinds of micelles might be more than 170, and even with the various forms oval, spherical or cylindrical (Heerklotz & Seeling, 2001; Zhou *et al.*, 2010).

2.8. Surfactin isoforms

Surfactin consists of lipopeptides with similar chemical structure called as isoform. Although there are the large number of isoforms for surfactin it can differentiate between them in terms of physical properties due to differences the variations at the peptide ring which the substitutions of amino acid components occurred and also due to variations in the branch and chain length of its hydroxy fatty acid components. Considered the use of different forms of thin strains of *B. subtilis* under environmental and nutritional suitable conditions are key factor in the production of various isoforms (Kowall *et al.,* 1998).

There are few authors that have established their research regarding characterization of surfactin isoforms with different conditions. Several authors able to demonstrate that surfactin consists of 6 isoforms while some others mentioned about at least 9 different isoforms have been identified (Abdel-Mawgoud, 2008). Peypoux *et al.,* (1994) suggests that alteration of peptide sequence affects the surface-active properties of surfactin, hence producing new isoforms. The surfactin isoforms is isolated from *B. subtilis* S 499 is differ from the surfactin standard in position 4 by the replacement of L-Valine residue with L-Alanine residue which related to the culture medium containing L-Alanine as nitrogen source.

A another study carried indicated that the separation of mixtures surfactin from local isolates *B. subtilis* OKB15 through utilized RP-HPLC for separation of surfactin isoforms and continue with NMR as well as MALDI-TOF to characterize new isoforms into its structural form (Kowall *et al.,* 1998)

Abdel-Mawgoud *et al.,* (2008) have identified a number of surfactin isoforms of which was isolated from *B. subtilis* BS5 was discovered a number of six isoforms are similar to standard surfactin (9 isoforms) after comparing the chromatogram results, thus there is relationship between strain variations and differences in relative abundance of surfactin isoforms being produced.

2.9 Biological Application of Surfactin

B. subtilis is one of the most famous bacterium that is well-known and has been using these bacteria in many research and domains due to the ability of these bacteria to produce surfactin substance (Maget-Dana & Ptak, 1995). Surfactin has the largest amount of interactions with cell membranes and lipid bilayer membranes thus surfactin used in a large number of different medical fields (Sheppard *et al.,* 1991), blocks the activity of cyclic adenosine mono-phosphate (Hosono *et al.,* 1983) inhibits platelet and spleen cytosolic phospholipase A2 (PLA2) (Kirn, 1998), and exhibits antiviral (Kracht *et al.,* 1999) and antitumor activities (Kameda *et al.,* 1974).

2.9.1 Antibacterial Activity

In fact, there are a large number of pathogenic bacteria that have the ability to resist the medications and these bacteria pose an ongoing threat to it must resort to treatment substitutionary. Research reported the inhibitory effects of a substance surfactin against different types of pathogenic bacteria and proved that his high medical importance. Thus surfactin is an effective alternative for many medications industrial, and can be utilized as safe and effective therapeutic factors.

Has been studying the impact of surfactin to suppress the influence of inflammatory Lipopolysaccharide (LPS) and by interact with the fact that the nucleus cells showed. Whereas surfactin compounds have ability to disable activity LPS activity can potentially can be a new anti-inflammatory agent. Surfactin proves to be a good indicator for the LPS-induced expression of inflammatory mediators (IL-1β and iNOS) (Hwang *et al.,* 2009), reduces the plasma endotoxin and TNF-α and nitric oxide levels in responding to rodent-related septic shock (Hwang *et al.,* 2007).

2.9.2 Anti-viral Activity

Surfactin antiviral activity against the various types of viruses, including semliki forest virus, herpes simplex virus (HSV-1 and HSV-2), simian immunodeficiency virus, felinecalici virus, vesicular stomatitis virus and the murine encephalomyocarditis virus. Of the most important properties of the antiviral activity surfactin is a physicist interaction between the membrane surfactin and the lipid membrane of the virus (Vollenbroich *et al.,* 1997).

2.9.3 Antitumor Activity

Kim *et al.,* (2007) reported surfactin anticancer effects due to its ability on DNA fragmentation and loss of enhanced polarity and membrane cell death and cell cycle arrest. There is also a recent study has shown the impact of surfactin on the colon cancer through its ability to inhibit the proliferation of cells carcinogens. The inhibition of cancerous cell growth through surfactin was due to the induction of apoptosis and cell cycle arrest via the suppression of cell survival regulating signals such as ERK and PI3K/Akt (Kim *et al.,* 2007).

There are many studies that indicate that surfactin, through a ROS/JNK-mediated mitochondrial/caspase pathway, inhibit proliferation and leads to apoptosis of MCF-7 human breast cancer cells. It characteristics surfactin its ability to generate reactive oxygen species necessary for continuous stimulation of the median survival ERK1/2 and JNK, which are key regulators of stress-induced apoptosis. Considered also stimulate the cells to apoptosis new strategy, which will have a great future for the prevention and treatment of cancer.

2.9.4. Anti-Mycoplasma Effect

Surfactin showed positive results in the mammary cells contaminated as reproduction rates have improved and led to a radical change in the cell morphology, and therefore the low cytotoxicity of surfactin to mammalian cells that allows specific inactivation of mycoplasmas without harmful effects on cell metabolism in the culture (Vollenbroich *et al.,* 1997).

Recently were studied surfactin's ability to killing mycoplasma cells (MIC 25 pM) independently of the target cell concentration, it is considered the hallmark of the working method of antibiotics. Surfactin offered a synergistic effect and resulted in mycoplasma activity, killed at about two orders higher than those of the entire particle size used separately in combination with the enrofloxacin (Fassi *et al.,* 2007).

2.9.5 Antifungal Activity

Surfactin produced by *B. subtilis* containing heptapeptide-and-B-hydroxy potential fatty acids and bioagents this appearance spectrum antifungal, anti-viral, anti-tumor and broad, and the activities hypocholesterolemic (Yao *et al.,* 2003). Heptapeptide molecules, showed surfactin and iturins activity powerful antibiotic with a spectrum antifungal and wide, which makes it possible agent of biocontrol ideal in order to reduce the utilized of chemical preservatives in foods (Maget-Dana & Peypoux 1994; Sandrin *et al.,* 1990). The clinical trials have shown to humans and animals also as the value of the drug to be spectrum to have a large anti-fungal, low toxicity, and low impact sensitivity (Yao *et al.,* 2003). *Bacillus* causing discourages growth of several fungi pathogenic of the plant, including *Fusarium spp*., *Aspergillus spp*., and *Bipolaris sorokiniana.*

2.10 Toxicity

This is due to hemolytic activity, which could be interpreted the capacity of surfactin to destabilize the integrity of the structure of cell membranes and is one of the defects of the most understandable surfactin use in medical applications. Effect has been described depend on concentration of hemolytic surfactin as surfactin concentration to explode 50% of red blood cells (HC50), which is equivalent to 300 micromoles / liter (Dufour *et al.,* 2005). On the other hand, the concentrations of surfactin used in various biomedical studies is much lower than the threshold, ie, 30 micromoles / liter. The minimum concentration of surfactin that hampered growth quite mycoplasmata after 48H (MIC) 25 micromoles / liter (Fihri *et al.,* 2007). The treatment showed 30 micromoles / liter of surfactin anti-proliferative activity of a large in human colon cancer cells (Kim *et al.,* 2007), and was capable to bring about programmed cell death in human breast cancer cells (Cao *et al.,* 2010). Has been reported for any toxicities related surfactin in stay alive, clinical signs, blood measurement and observations pathological appliances haematopoietic yet (Hwang *et al.,* 2009).

2.11 Fungi used in this study

2.11.1 Aspergillus niger or A. niger is a fungus and one of the most common species of the genus *Aspergillus*. This fungus lead to black rot disease on many types of fruits and vegetables, which include peanuts, grapes, apricots, onions and is a common contaminant of food. Reports indicate that the fungus is present in all kinds of soil, especially in indoor environments, because of the similarity between the black colonies of *A. niger* and *Stachybotrys*, it most often happens mixing because *Stachybotrys* has a kinds of mold called black mold. (Samson *et al.,* 2001)

Although the cases rate of this rare fungus, but they may infect the spaces containing air like bronchus or pulmonary cavity and usually harmless (Bennett, 1979). Patients with immune deficiency are the most susceptible to this fungus and thus susceptible to otherwise common and usually harmless microorganisms. The use of

supraphysiological doses of adrenal corticosteroids and chemotherapy, considered of factors that may lead to immunosuppression (Bennett, 1980).

2.11.2 Glomerella cingulata is a type of a plant pathogenic fungus that causes disease on many of the hosts including apple bitter rot and quince and anthracnose on many types of fruit and vegetable such as mango (Sangeetha & Rawal 2008) & (Abd-AllA & Wafaa 2010), or cultured plants like St. John's Wort (Hypericum perforatum) (Penz. & Sacc 2008). It also causes leaf spot on Hevea brasiliensis (Adekunle & Enobakhare 2007). Danielson is a natural phenol and a phytoalexin produced by the papaya carica papaya to fight *Colletotrichum gloeosporioides Glomerella cingulata* is the sexual stage (teleomorph) while the asexual stage (anamorph) is called *Colletotrichum gloeosporioides* (Echeverri *et al.,* 1997).

2.11.3 Candida albicans is a type of fungus that grows exponentially and it considered the main reason for genital infections, especially in women vaginal and oral infections and candidal onychomycosis. Fungal infections may lead to death, especially patients with immunodeficiency (e.g., AIDS, cancer chemotherapy, organ or bone marrow transplantation). *C. albicans* has the ability to grow and the formation of membranes vitality on the surface of medical devices that are used in the examination of the human body from the inside, such as the telescope thus became a hospital-acquired infections of the most important the main reasons that threats to health.

Infection of this fungus is limited only in the mucous membranes such as the mouth and vagina which is known as thrush and can be cured easily, especially in people who are not immunocompromised. When injury by this fungus initially appears as a single yeast cell usual then automatically turn into filiform and multicellular This phenomenon is called the dimorphism (Ryan & Ray 2004).

2.11.4 Candida tropicalis this fungus is widely spread in marine environments in tropical and subtropical areas were obtained it from the mud and seawater and marine sediments and also the small intestine for fish, and from several types of fruits, faeces and soil. *C. tropicalis* is one of the most common *Candida* causing human diseases in tropical countries; *C. tropicalis* represents 4% of yeasts. Considered the classification for *C. albicans* closely with *C. tropicalis* and they share many pathogenic qualities. Causes bloodstream infection and less common tissue invasive *candidiasis* and also seldom cause biofilm infections and oral or vaginal thrush often more pathogenic than *C. albicans* (Berkhout *et al.,* 1923).

For the treatment of *Candida* in the blood are advised to utilization amphotericin B or an echinocandin with extended-spectrum triazoles alternatives solution acceptable and considered fluconazole resistance main reason of uncommon, but it may be an incentive for exposure. Physicians in the areas where *C. orbital* is a common need to take into account the this pathogen less description (chai *et al.,* 2010).

2.11.5 Candida krusei is a budding yeast, considered this fungus is a fungi emerging pathogens And more people are prone to him immunodeficiency patients and those suffering from oncology and especially leukemia and this fungus has natural immunity against to fluconazole. The mortality rate of *C. krusei* higher than the more common *C. albicans*. The positive thing for this fungus is involved in the production of chocolate (Pfaller *et al.,* 2008).

2.11.6 Candida parapsilosis is a type of fungal species of the yeast family which became an important reason to sepsis and of wound and tissue infections in patients with HIV. Considered the immune system is a major player in *C. parapsilosis* infections. Reverse *C. albicans* and *C. tropicalis*, *C. parapsilosis* is not an obligate human pathogen, was isolated from non-human resources such as pets, insects or soil.

C. parapsilosis is also a normal human commensal and it is one of the fungi most of the time isolated from the human hands. But there are several risk factors which can help *C. parapsilosis* to colonize human host. Considered Immuno-compromised individuals and surgical patients, particularly those having surgery of the gastrointestinal tract are more vulnerable to the risk of infection with *C. parapsilosis*. Currently there are no cure agreed upon for *C. parapsilosis* diseases by researchers in spite of all attempts to extract any foreign bodies and the administration of a systemic antifungal. Considered amphotericin B the most commonly used antifungal. Fluconazole is an often administered substitute to amphotericin B (Segal *et al.,* 1996).

2. 11. Local Isolates of *B. subtilis*

This research focusing on reducing the cost of surfactin production by using Malaysian isolates of *B. subtilis* strain. The main three reasons behind focusing on these local isolates are their abundance, possible production of new isoforms and producing high yield of surfactin. These three main reasons can contribute to reducing the cost of surfactin production, which is one of the main hindrances in its commercialization due to utilization of expensive substrates strategies for downstream processing and low surfactin yield. We can take advantage of local isolates of *B. subtilis* tension due to the differences pedestals, which allows the possibility of producing a new kind of isoform of surfactin. New composition new surfactin isoform can have different degree of hydrophobicities which can result to new biological properties and applications.