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Health Care Applications of Different Biosurfactants: Review

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Abstract: ***Biosurfactants are amphiphillic compounds produced by certain microorganisms. Bio surfactant molecules arrange themselves in water-oil interface and reduce surface tension. Biosurfactants have some advantages over chemical surfactants in market with respect to biodegradability, low toxicity, better surface and interfacial activity. There are different application of biosurfactants in health care purposes both oral and dermal. Among this some of the important field of application are medicine and cosmetics.***

Keywords: Biosurfactants, microorganisms, applications in pharmacology, cosmetics and personal care.

1. Introduction

Surfactants are generally active ingredients which have the ability to form concentrate at the air- water and oil-water interface. It can separate oily substances from a media by reducing their interfacial tension at water- oil interfaces and form bubbles by reducing air-water interfacial tension. This mainly happens due to increase in aqueous solubility of the Non Aqueous Phase Liquids (NAPLS) (H Yin *et al.* 2009). Surfactants are amphihillic moieties, both lipophillic and hydrophilic parts. It is widely used in different fields like industrial, agricultural, food, cosmetic, pharmaceutical etc. These compounds are chemically synthesized and create environmental and toxically problems (Schramm *et al*. 2003, Makkar and Rockne, 2003). In the past few decades' surface active molecules having microbial origin has gain interest in this fields. These surface active metabolites are called biosurfactants (Desai and Banat 1997). These are amphipillic compounds produced on microbial cell surfaces or extracellular both hydrophobic and hydrophilic. These can accumulate between fluid phases and reduce surface and interfacial tension respectively (CD Cunha *et al.* 2004). Biosurfactants constitute a diverse group of surface active molecules and variety of chemical structures like glycolipids, lipopeptides, fatty acids, phospholipids etc. (Desai and Banat, 1997). Biosurfactants has potential application in industrial process as it act as surface active agents in emulsion (Amaral *et al*.,2009, Huang *et al*., 2010 ,Sil *et al*., 2013). But biosurfactants also found in several health care applications. It possesses several properties in biomedical and therapeutic fields. It has antibacterial, antifungal and antiviral properties (Joshi *et al*., 2008). Due to its biocompatibility and digestibility biosurfactants also have application in cosmetics and functional food additives (Kosaric 2001). Surface active molecules are mainly two types one that reduce surface tension at the air-water interface known as biosurfactants. Secondly, that reduce the interfacial tension between immiscible liquids or at the solid -liquid interface known as bioemulsifers(Konishi *et al*. 2007, Langer *et al*. 2006, Freitas *et al*., 2009). Biosurfactants can reduce both surface tension and exhibits emulsifying capacity (Langer *et al*. 2006, Freitas *et al*., 2009). As per studies of Desai and Banat (1997) and Gautam and Taigi (2006) depending on the hydrophilic part, biosurfactants are classified on their composition five types

1) Glycolipids: carbohydrates with long chain aliphatic acids. Best known are rhamnolipids, sophorolipids, trehalolipids, mannosylerythritol and cellobiose lipids, liamocins and exophilins, alkyl and acyl glycosides, polymeric exopolysaccharides (Morita *et al*., 2006, Sullivan, 1998; Kitomoto *et al*., 1993).
2) Lipopeptides and lipoproteins: includes decapeptides (gramicidins) a and lipopeptide antibiotics (polymyxins) are important.
3) Fatty acids, phospholipids and neutral lipids: Several bacteria and yeasts produce growth on n-alkanes.
4) Polymeric biosurfactants: Emulsan (also under glycolipid class), liposan, mannoprotein and other polysaccharide-protein complexes (also under lipoprotein class).
5) Particulate biosurfactants: form micro emulsion and plays important role in in alkane uptake by microbial cells (Monteiro *et al*., 2007; Mukherjee *et al*., 2006; Ortiz *et al*.,2006).

Biosurfactants have huge structural diversity. Majority includes low molecular weight glycolipids (GLs), lipopeptides (LPS), flavolipids (FLs) and phospholipids. While high molecular weight polymers like lipoproteins, lipopolysaccharide protein complexes, polysaccharides protein fatty acid complexes etc. (Rodrigues *et al*., 2006b; Ruiz-Garc *et al*., 2005; Singh *et al*., 2007).

2. Application of biosurfactants in health care purposes

There worldwide production of surfactants and expected increase growth rates of 3-4% per year globally (Rahman *et al*. 2008). But chemically synthesized surfactants are mainly petroleum based and non biodegradable and toxic to living beings. So to protect the ecosystem scientists are interest in surfactants formed from microbial origin i.e. biosurfactants as a alternatives of the chemical ones (Benincasa 2007). These biosurfactants have several applications in agriculture, medicine, petroleum and industries. Here we will discuss on the heath care benefits of biosurfactants and their application for different purposes.

Biosurfactants have different bioactivities. Some are like it inhibit bacterial growth (Flagas and Makris, 2009), toxic effects,, tumor growth inhibition , antibiotics, cell lysis (Dehghan-Noudeh et al., 2005), food digestion (Nitschke and Costa 2007), fungicidal properties(Joshi et al., 2008) or enzyme stimulation etc. These bio molecules have different uses in health benefits: pharmaceuticals, testing quality of condoms, cosmetics, beer and beverages (Mukherjee *et al*., 2006) and food processing (Arauz *et al*., 2009).

3. Antimicrobial activity

Biosurfactants have manifold activities ranging from antibacterial, antiviral, antimycobacterial to antimycoplasmal activities (Vollenbroich *et al*., 1997a; Rodrigues *et al*., 2006b; Rahman *et al*. 2003). Some antimicrobial lipopeptide like iturin, surfactin, lichenysin etc. are active against some human pathogens *Candida* sp. Marine *B. circulans* produces lipopeptide biosurfactant had a potent antimicrobial activity against Gram positive and Gram negative pathogens (Gharaei-Fathabad 2011, Das *et a*l. 2008a). Surfactin a best known lipopeptides produce by *Bacillus subtilis* has antimicrobial activity. Antimicrobial lipopeptides produced by some *Bacillus* species inhibit the growth of pathogenic microorganisms in gastrointestinal tract (Hong *et al*., 2005) .*Bacillus subtilis* fmbj produces lipopeptides mainly composed of surfactin and fenygin antimicrobial substances. It has the ability to inactivate the endospores of *B. cereus* (Huang *et al*., 2007). Rhamnolipid biosurfactant produced from soyabean oil waste has antimicrobial activity against several bacteria and fungi (Nitschke *et al*. 2009b). Antimicrobial activities of glycolipids are effective against *Staphylococcus* sp. mainly on methicillin resistant *Staphylococcus aureus* (MRSA) (Das *et al*., 2008a). *Candida antarctica* produces mannosylerythritol lipids (MEL-A and MEL-B) which has antimicrobial action against Gram-positive bacteria (Kitomoto *et al*., 1993).

4. Anti-Adhesive Activity

According to Rodrigues *et al*. (2006) biosurfactants inhibits the adhesion of pathogenic organisms to solid surfaces or to the infection site. Again absorption of biosurfactants to the substratum alters the hydrophobicity of the surface and causes microbial adhesion and desorption processes (Desai and Banat, 1997; Bai *et al*., 1997). Biosurfactants reduces the microbial population on prostheses and decreases the airflow resistance that occurs on voice prostheses after biofilm formation (Rodrigues *et al*., 2004). Biosurfactant can alter the physical and chemical condition of the environment where biofilm is forming and has direct action against pathogens (Mireles *et al*., 2001, Merck *et al*., 2005). It has been found that two lipopeptide biosurfactants produced by *B. subtilis* V9T14 and *B. licheniformis* VI9T2I1 has interesting anti-adhesive activity that inhibit the biofilm formation by two pathogenic strains mainly *S. aureus* ATCC 29213 and *Escherichia coli* CFT073 (Rivardo *et al*., 2009). Serotypes of group II capsular polysaccharides produced by uropathogenic *E. coli* (UPFC strain CFT073) behave like surface active polymers and has good anti-adhesive properties. It inhibits mature bio film development of broad range Gram positive bacteria and Gram negative bacteria (Valle *et al*., 2006) . It has been found that biosurfactant of *Streptococcus mitis* inhibits addition of *Streptococcus sorinus* HG 1025 and *S. mutans* ATCC 25175 to bare enamel (Van Hoogmoed *et al*., 2004). Also, *S. mitis* inhibits the adhesion of *S. sornius* HG 1025 to salivary pellicles.

5. Immunological Adjuvant

Certain bacterial lipopeptides are potent non-toxic, non pyrogenic immunological adjuvant when mixed with conventional antigens. Low molecular weight antigen iturin AL, herbicolin A and Microcystin coupled with poy-L-lysine enhances humoral immune response in rabbits and chickens (Rodrigues et al., 2006b). Emulsan has been approved for use as adjuvant because it has immunomodulatory potential in human body.

6. Antiviral Activity

Surfactin and its analogues show antiviral activity (Naurse *et al*., 1990). Vollenbroich *et al*. (1997) suggested that antiviral activity of surfactin on enveloped viruses are more prominent than on non-enveloped viruses mainly due to physico-chemical interactions between virus envelope and the surfactant. Biosurfactants have been known to inhibit growth of human immunodeficiency virus in leucocytes (Desai and Banat 1997; Krishnaswamy *et al*., 2008). According to Krishnaswamy *et al*. (2008) sophorolipids from *C. bombicola* and its structural analogues such as the sophorolipid diacetate ethyl ester is potent anti-HIV, cytotoxic activities and virucidal agent.

Cell free virus of porcine parvovirus, pseudo rabies virus, Newcastle disease virus and bursal disease virus are inactivated by antimicrobial lipopeptides produced by *B. subtilis* fmbj. This lipopeptide also has the ability to inhibit the replication and infectivity of the Newcastle disease virus and bursal disease virus but not effective on pseudo rabies virus and porcine parvovirus (Huang *et al*., 2006). Rhamnolipids and their complexes with alginate produced by *Pseudomonas* sp. have antiviral activity against Herpes simplex viruses type 1 and 2 (Remichkova *et al*., 2008).

7. Anti-Fungal Activity

Biosurfactants have anti-fungal activities against human pathogens. Yeast like fungus *P. flocculosa* produces a glycolipid known as flocculosin shows *in vitro* anti-fungal activity against pathogenic yeasts and also effective against human mycoses (Mimee *et al*., 2005). This product can inhibit pathogenic strains in acidic condition and has synergistic activity against amphotericin B. There are different glycolipids like cellobiose lipids (Teichmann *et al*., 2007; Kulakovskaya *et al*., 2009, Kulakovskaya *et al*. 2010) shows antifungal activity against phytopathgenic fungi. Other biosurfactants are Rhamnolipids, cyclic lipopeptides, surfactin, iturin and fengycin also has anti-fungal activities (Debode *et al*., 2007; Varnier *et al*.,2009, Tran *et al*., 2007, 2008, Velmurugan *et al*., 2009; Snook *et al*., 2009; Mohammadipour *et al*.,2009; Grover *et a*l.,2010).

Anti-cancer activity, Anti-tumor and immunomodulatory action

Some biosurfactants are involved in cancer cell proliferation and apoptosis (Gudina *et al*., 2013). Some glycolipids have been found to induce cell diferentiation in the human promyelocytic leukemia cell line and act as novel reagents for cancer cells treatment (Krishnaswamy *et al*., 2008). These glycolipids arrest the cell growth and induce apoptosis of mouse malignant melanoma B16 cells. It was found that with increase in concentration of the MELs, B16 cells showed tendency of accumulation in the sub - G0/ G1 phase indicating cell death (Zhao *et al*., 2000) as shown in figure 2. Apoptosis induced by MELs activate protein kinase C (PKC) which leads to a multiplication of cellular responses. MELs also induce the differentiation of human promyelocytic leukemia HL60 cells towards granuloytes. Hence MEL biosurfactants trigger both apoptotic and differentiation mechanisms (Gudina *et al*., 2013; Zhao *et al*., 2000; Isoda *et al*., 1997). Sophorolipids also found to trigger cell differentiation and to inhibit PKC activity in the HL60 human leukemia cell line (Isoda *et al*., 1995). Sophorolipid produced by *Wickerhamiella domercqiae* induces apoptosis in H7402 human liver cancer cells by blocking the cell cycle at G1 phase shown in figure 2, activating caspase-3 and increasing Ca^{2+} concentration in the cytoplasm (Chen *et al*., 2006). According to Kim *et al*. (2007) surfactin blocks cell proliferation by inducing proapoptotic activity and arresting the growth in G2/M phase of the cell cycle as in figure 1. It has been found that surfactin is a best biosurfactant that induces apoptosis in human breast cancer MCF-7 cells through JNK-mediated caspase pathway (Cao *et al*., 2010). Surfactin also induces apoptosis in HepG2 cells through ROS-endoplasmic reticulum stress (ERS)-Ca^{2+}- extracellular signal-regulated protein kinase pathways (Wang *et al*., 2013). There are other bacteria sp. Like *Bacillus*, *Pseudomonas*, *Serratia* produces diferent lipopeptides which exihibit antitumoral activities against various human cancer cells (El-Sersy *et al*., 2012).

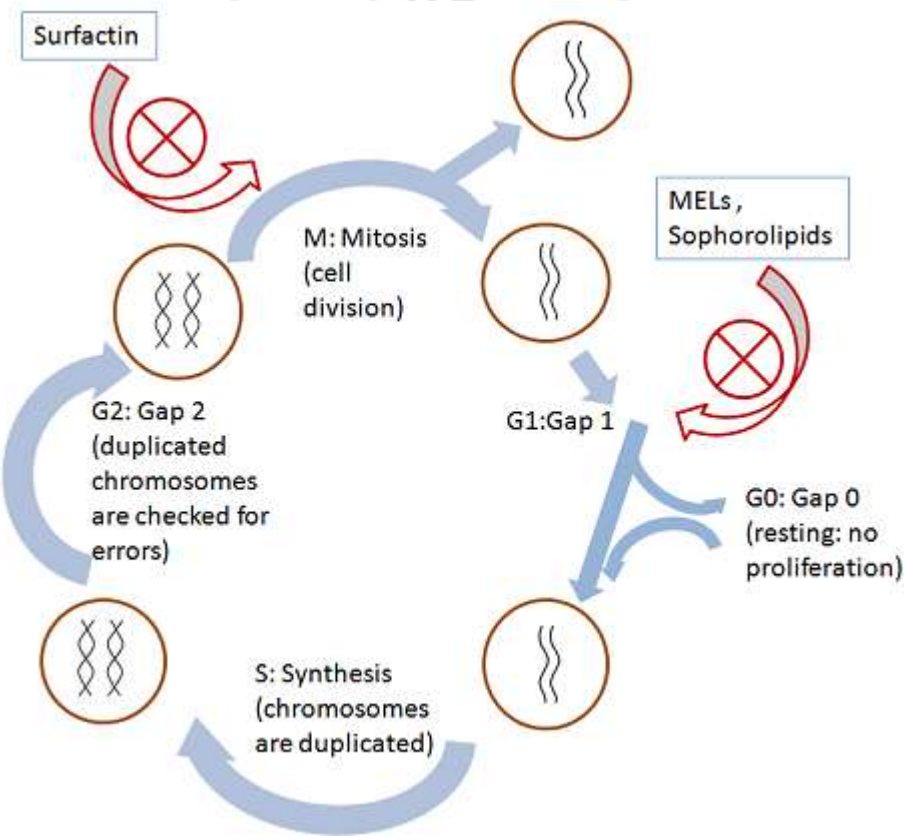

Figure 1

Cord factor or Trehalose-6, 60-dimycolate (TDM) exhibits anti-tumor and immunomodulatory activities. But Mycobacterial TDM has limited use due to high toxicity of the molecule and potential pathogencity of the producer strains. TDM is also produced by *Rhodococcus* sp. 4306 but low toxicity (Francetti *et al*., 2010). Surfactin, a biosurfactant has powerful anti-inflammatory, antibiotics and anti-tumor function (Seydlová and Svobodová 2008). Surfactin inhibits the immunostimulatory function of macrophages by blocking NK-κB, MAPK, AKt pathway (Park and Kim 2009). Surfactin also plays immunopharmacological role in autoimmune disease and transplantation. Surfactin also has potent immunosuppressive capability which shows therapeutic application in autoimmune disease and transplantation. It has been found that *Bacillus subtilis* PB6 which produce surfactin. This surfactin inhibits enzymes phospholipase A2 that release fatty acids from second carbon group of glycerol . This involes in pathophysiology of inflammatory bowel disease (Selvam *et al*., 2009). Kinetic study in interaction of carbohydrate ligand system composed of self-assembled monolayers mannosylerythritol lipid-A(MEL-A) produced by *P. antarctica* easy to handle . It serve as low cost ligand system for IgM and IgG and also lectins(Konishi *et al*., 2007a). A fungus *Exophiala dermatitidis* SK80 produces a biosurfactant monoolein which can inhibit the

proliferation of cervical cancer (HeLa) and leukemia (U937) cell lines depending on doses(Chiewpattanakul et al., 2010) .

There are differences in the cell membrane lipid profile of normal and cancerous tissues. It is known that lipids are surface active in nature, thus with the change in lipid profiles surface activity profiles also changes in the cells. Thus when biosurfactants interact with lipid profiles which is also surface active in nature it lead to cell membrane modifications and ultimately to cell death. Lipid composition mainly determines the structure, function, integrity of biological membranes, phosphatidylcholine (PC) and sphingomyelin (SM) which plays important role in stabilizing the bilayer structure of the cells. It has been reported that changes in PC and SM levels might affect the permeability of the cancer cell membranes. Thus research has designed a new therapeutic strategies that use of biosurfactants might alter lipid content (specially PC and SM) which in turn fluidize rigid cancerous tissues and also modulate interfacial properties (Preetha *et al*., 2005).

8. Gene Delivery

Introducing exogenous nucleotides into mammalian cells for basic sciences and clinical applications such as gene therapy. Various methods for gene transfection, lipofection (Zhang *et al*., 2010; Fuijita *et al*., 2009; Liu *et al*., 2010) using cationic liposomes to deliver foreign gene to the target cells without side-effects. It has been found that liposomes based on biosurfactants show increasing efficiency of gene transfection than commercially available cationic liposomes (Kitamato *et al*., 2002). New techniques and methodologies are developed for the liposome-based gene transfection by introducing biosurfactants (Ueno *et al*., 2007). Cellular association and efficiency of gene transfection mediated by cationic liposomes increased by MEL-A (Igarashi *et al*., 2006). It was observed by Ueno *et al*., 2007, that among MEL-A, MEL-B and MEL-C- containing liposomes only MEL-A has high activity in DNA capsulation and membrane fusion with anionic liposomes. These are important properties of for gene transfection. Maitani *et al*., 2006 was successful in producing a liposome vector containing beta-sitosterol beta- D- glucoside biosurfactant complexed DNA used for herpes simplex virus thymidine kinase gene therapy. 2009 Nakanishi *et al*., was able to produce nano-vectors containing a biosurfactant that increase the efficacy for gene transfection *in vivo* and *in vitro*. Surfactin - mediated gold nanoparticles has opened a new and fascinating application in the field of drug , gene delivery, and targeted therapy etc.(Reddy *et al*., 2009).

9. Recovery of intracellular products

According to Singh *et al*., 2007 biosurfactants used for lysis of cells after fermentation as part of the protocol for recovery of intracellular products. Overall releases of intracellular proteins from microbial cells are done by aggressive mechanical cell disintegration methods. For selective permeabilization reagents are used which render the cell envelope more porous which is beneficial for selective release of target proteins and to obtain an extracted product with a high specific activity or for further protein purification. As demonstrated by Desai and Banat, 1997, biosurfactants can be used as reagents for membrane permeabilization. It is important to note that biosurfactant has no negative impact on the stability or activity of the product as it binds to the proteins and other bioactive molecules (Desai and Banat, 1997; Singh *et al*., 2007) .

Other uses of biosurfactants in medicine are used for stimulating stem fibroblast metabolism etc (M. Krishnaswamy *et al*., 2008).

10. Application and benefits of biosurfactants in cosmetics and personal care:

A ideal surfactant to be used in cosmetics must have properties like detergency, wetting, emulsifying, solubilising, dispersing and foaming effects (Rieger *et al*., 1997). Biosurfactants which are produced by microorganisms, have drawn wide attention regarding their biodegradability, low toxicity, ecological acceptability and availibility from renewable sources (Makkar and Cameotra, 1999). Most widely used biosurfactants are glycolipids in nature due to their physiochemical properties, biological activites, biocompatibility and biodegradability (Desai and Banat, 1997). Potential glycolipid biosurfactants used as multifunctional ingredients in the formulation of cosmetics are sophorolipids, rhamnolipids and mannosylerythritol lipids.

Sophorolipids- It is originally obtained from the yeast *Candida* species. Main strains were *C. bomicola* ATCC 22214 (Spencer *et al*., 1970; Ashby *et al*., 2006) and *C. apicola* IMET 42747 (Hommel *et al*., 1994) produces sophorolipids as a mixture of 2-O-β-D-gluco-pyranose derivatives acetylated at the 6' and/or 6" positions. Beside *Candida* sp. *Wickerhamiella domericquae* (Chen *et al*., 2006; Chen *et al*., 2006) as well as *Cryptococcus curvatus* (Daniel et al., 1998) also synthesize sophorolipids. It was found that mono- and di-acetylated lactones showed strongest inhibitory effects and also could find numerous applications than the acidic forms of sophorolipid (Lang *et al*., 1989).

Application- It has shown several cosmetic applications like good skin compatibility and have excellent moisturizing properties (Yamane 1987; Brown 1991). Due to its emulsifying functions act as potent bactericidal agents and used in the treatment of acne, dandruff and body odours (Mager *et al*., 1987). Sophrolipids act as desquamating and depigmenting agents (Hillion *et al*., *1998*; Borzeix *et al*., 2003; Maingault *et al*.,1999). So several patients are related to sophorolipids in cosmetics (Kawano et al., 1981). Commercially sophorolipds are produced by the Kao Co. Ltd. as humectants and present make-up as "Sofina"and " Soliance" which are sed for skin application. Beside these products , sophorolipids are also used in pencil-shaped lip rouge, lip cream, eye shadow and compressed powdered cosmetics and aqueous solutions (US Patent 4 305 931, US Patent 4 305 929).

Rhamnolipids- Rhamnolipids generally produced by *Pseudomonas* sp., and *P. aeruginosa* produces leading commercial biosurfactant which is suitable form in case of

combined application / cost perspective and is produced on an industrial scale(Lang et al., 1999; Matsufuji et al.,2005). Rhamnolipids are not only good biosurfactant but also have antimicrobial activities against fungi (Abalos et al., 2001). These lipids contain one or two rhamnose units linked glycosidically to a hydroxy acid hence ester bond further with hydroxy acid.The structure shown in figure 2a.

Application- Surface and biological properties of this biosurfactant used in many health care applications and cosmetics e.g insect repellents, antacids, acne pads, anti-dandruff products, contact lens solutions, deodorants, nail care products, toothpastes etc. (Klecker et al., 1993; Maier *et al*., 2000). It is also used for anti- wrinkle and anti-ageing products(Piljac *et al*., 1999) and launched in different forms for commercial skin care cosmetics (Desanto 2008).

Mamnosylerythritol lipids - It is also derived from bacteria and yeast. They consists of fatty acids linked to 4-O-$^{\beta}$-D mannopyranosylerythritol or 1-O-$^{\beta}$-D mannopyranosylerythritol as hydrohillic head group as shown in figure1c. Mannose residues are acetyleted by one or two hydroxyls and there are one to three esterified fatty acids which are arranged in odd or even number C_6 to C_{10} in chain length (figure 2c). It has excellent surface activity (Kitomoto *et al*., 2002) and good emulsifers, dispersants and detergents (Fukuoka *et al*., 2007). Kanebo cosmetics has develop new technology by using MEL-B(Mamnosylerythritol lipid B) for production of cosmetics .

Application- It has also found good use in cosmetic industry. It is used for anti-wrinkle purposes and prevents skin roughness (Masaru *et al*., 2007). As MEL-B has high moisturizing capability. So it is being added in cometic skin care products like skin lotions and creams (). In 2009 Morita *et al*., used a three dimensional cultured human skin model. It was found that the viability of the SDS- damaged cells improved by addition of MEL-A which was depend on a dose used. It was also observed that MEL-A had ceramide-like moisturising activity and helpful for cosmetic industry.

Sodium surfactin(SFNa) Figure 2b - It is produced by *Bacillus subtilis* which has high surface -activating function.

Application - This biosurfactant have future application which has been authorized use in quasi-drug products. Kanebo Cosmetics recently has succeeded in elucidating properties of this biosurfactants. It has been found that this SFN-a is extremely hydrophillic and forms a transparent gel in wider range. So this application of SFNa can be used in skin care cosmetic products.Recently it has been found *Alcaligenes piechaudii* CC-ESB2, diesel-oil degrading bacterium produce a novel biosurfactant that has wide application in beauty care products. This bacteria was incubated in BH media containing soyabean oil from which the novel biosurfactants was obtained. There was found no adverse effect in skin prick and allergic test. Due to its good surface activity and high potential used for commercial applications(Lin *et al*.2011).

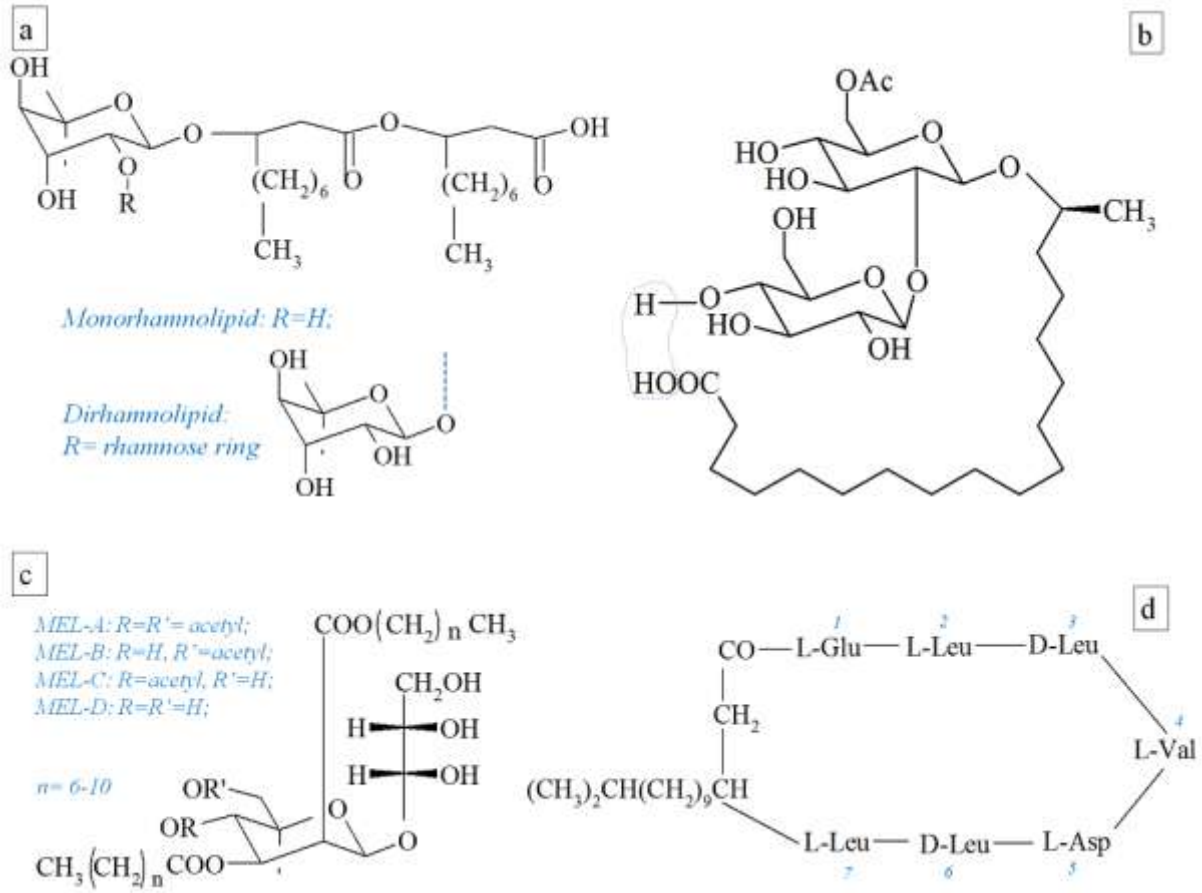

Figure 2

Besides these potential surfactants it is found that highly biosurfactants derived from yeasts act as moisturizing activity which is equivalent to that of natural ceramides at one tenth of the cost of current products. Dai Kitamoto, group Leader of the Biochemical Materials Group of Research Institute for innovation in Sustainable Chemistry, of the National institute of AIST (Advanced Industrial Science and Technology) collaborated with Toyobo Co. Ltd and has succeeded in developing a highly functional biosurfactants from yeast cells and vegetable oils. These developed biosufactants present super skin moisterizing characteristics same as natural ceramides. These biosurfactants are only sugar and fatty acids and eco-friendly and easily form a variety of crystals in aqueous solutions (AIST press release september 7, 2006).In figure 3 we have tried to compare the mechanisms of normal skin, dry skin and skin applied with biosurfactants. It has been found that when the ceramide level remains normal in the skin the water level in the skin also remain normal shown in figure 3A. Skin is found normally moisturised and smooth. But when the ceramide level decreases the normal water content also decreases slowly in the skin. This makes the skin dry and rough in nature and also aging & wrinkling problems comes with it shown in figure 3B. In case of using artificial ceramide if we use biosurfactant containing natural ceramide it will moisturise the skin and retain water level as in figure 3C.

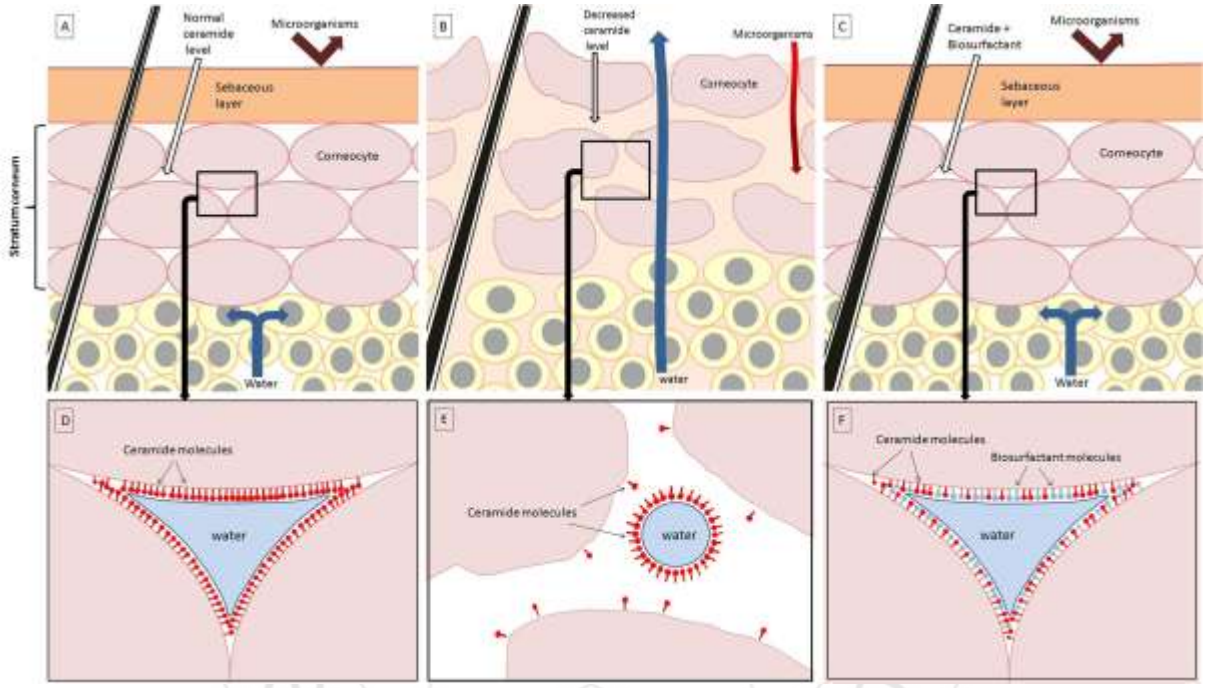

Figure 3

11. Conclusion

Chemically synthesized surface active compounds are though easily available in the market and widely used in different industry. But biosurfactants has advantages of biodegradability and production on renewable resource substrates which replaces the chemical surfactants. There are different health care applications and benefits of biosurfactants that replaces chemical surfactants. But there is also need to give for attention on the improvement of the physiology, genetics and biochemistry of biosurfactant producing strains (Youssef *et al*., 2004, 2007; Zang and Miller, 1992; Zouboulis *et al*., 2003). So that we can get more yield and available in market in reduce production costs.

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