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# A Review of Biosurfactants (Glycolipids): The Characteristics, Composition and Application

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***Abstract---*** *Biosurfactants are natural products with surface-active propriety, it could produce by different various types of microorganisms. The Biosurfactants as biological compounds had various aspects of applications in different field of life including environmental applications, oil recovery and bioremediation, as well as in pharmaceutical and agricultural industries. Biosurfactants could be classified interdepend to the molecular weight M.W, physic-chemical exclusivity and mode of their action. Glycolipids one of most important biosurfactants, includes rhamnolipids, trehalolipids and sophorolipids enhanced widely advertence due to their low toxicity, friendly to environment and readily of biodegradation.*

***Keywords---*** *Biosurfactant, Biodegradation, Rhamnolipids, Trehalolipids, Sophorolipids, Glycolipids.*

## I. Introduction of Biosurfactants

The surface-active particles derivative from different genera of microorganisms are called Biosurfactants. These particles are biological amphipathic mountings composed of hydrophilic and hydrophobic moieties. The hydrophobic moiety could be long chain of fatty acid, hydroxy fatty acid, or alkyl-b-hydroxy fatty acid while the hydrophilic moiety could be, carbohydrate, amino acid, cyclic peptide, phosphate, a carboxylic acid or alcohol. (Saharan *et al*, 2011). Biological Surface Active Compounds BSACs could be grouped into three groups:

I biosurfactants, II amphiphilic polymers and III polyphilic polymers, these compounds distributed in the nature in a wide variety of chemical structures and possess the capability to debasing the surface tension between two fluid phases. (Franzetti *et al*, 2010).

The interesting of biosurfactants due to its roles in biodegradability, low toxicity, ecological acceptability and ability to be generative from natural resource and tackiest substrates (Vatsa *et al*, 2010). These Biological compound are different from synthetic surfactants in being non-toxic, more effective and environment-friendly, the existence of surfactants might increase microbial degradation of pollutants of pesticides in soil and water environment, these proses becoming very interesting recently. They are widely utilized as detergents, solubilizers or emulsifying agents in many industrial fields such as petroleum, food, pharmaceutical and agricultural industries (Paulino *et al*, 2016 and Gudina *et al*, 2016).

The identification and description of such compound outputted by different species of microorganisms have been broadly described by researchers (Chong and Li 2017). Composition and quantity of the yield of biosurfactants producer depend on the type and condition of the fermenter, pH, nutrients, substrates and temperatures employed the production (El-Sheshtawy *et al*,2014),other factors like nitrogen and carbon source, aeration and trace elements could be critical factors affecting on the production (Onwosi and Odibo 2012). The aim of this study was to

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focusing on most interesting group of biosurfactants the Glycolipids, its characteristics, composition and application on different aspect of life.

***Classification of Biosurefactant***

Biosurfactants are classified into groups according to their microbial origin and chemical composition; most biosurfactants are either anionic or neutral, whereas those that contain amine groups are cationic. The hydrophobic moiety has long-chain fatty acids and the hydrophilic moiety can be composed of different chemical group. The molar mass of biosurfactants generally ranges from 500 to 1500 Da (Bognolo, 1999). The majeure groups of Biosurfactants are low molecular weight surface active compound known as biosurfactant and the second group was the high molecular weight substance called bio-emulsifier that is exclusive used as emulsification of hydrocarbons. Further classification of these groups is divided in to six others groups known as glycolipids, lipopolysaccharides, lipoproteins-lipopeptides, phospholipids, hydroxylated and cross linked fatty acids.((Franzetti *et al*,2010).

**Glycolipids***:* Generally most known bioemulisifers are glycolipid in nature. Glycolipids exist of one or more carbohydrates in combination with one or more fatty acids, hydroxy fatty acids or fatty alcohols (figure 1). Because of their high production yield and the possibility to use renewable resources, they are the most promising for commercial production and utilization. The best known of glycolipids are rhamnolipids, trehalolipids and sophorolipids (Shoeb, 2013). (Abdel-Mawgoud, 2010)

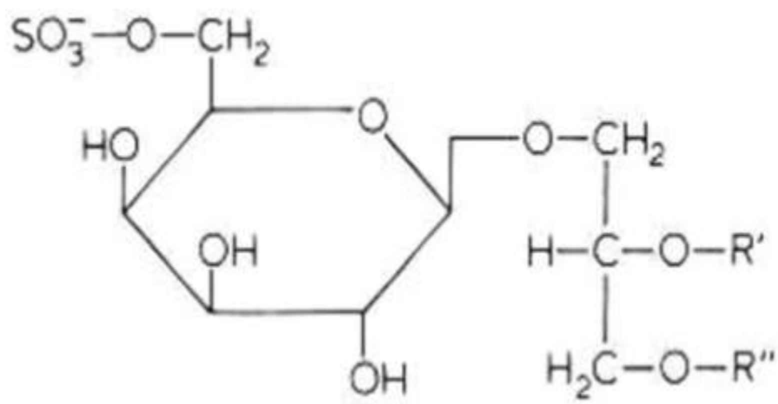


Figure 1: Chemical Structure of Glycolipids

**1- Rhamnolipids***:* the Biosurfactants Rhamnolipids consists of rhamnose molecules which are linked with one or two molecules of β-hydroxy decanoic acid, The -OH group of one of the acids is involved in glycosidic linkage with the reducing end of the rhamnose disaccharide, the -OH group of the second acid is occupied in ester formation (Figure 2). *Pseudomonas aeruginosa* Considered one of the most interesting genera of bacteria having the ability to produced rhamnolipids. (Amani, Mehrnia, 2010 and Hörmann *et al* 2010),

Rhamnolipid having the property of surface agent and its emulsifying properties which increase the capability to debasing the water surface tension, the oil – water surface tension, (Hassan *et al*,2016).increase the cornered oil transferred. Rhamnolipids could reduce the surface tension of water from 72.80 $N{\cdot}m^{-1}$to 25 to 30 $N{\cdot}m^{-1}$,. It is the most effective biosurfactant (Câmara *et al* 2019).

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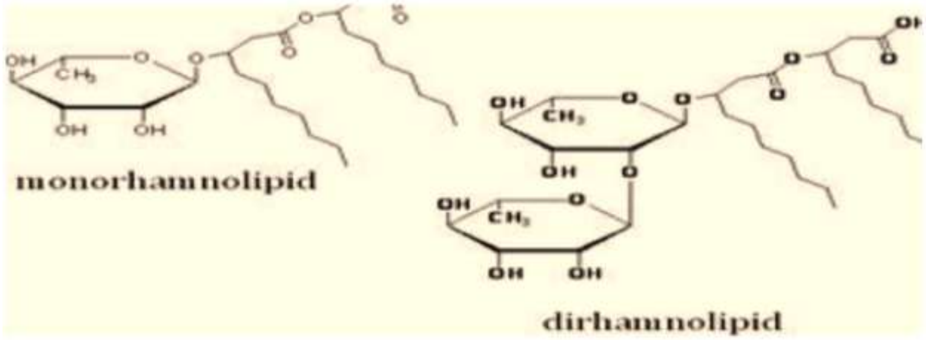


Fig. 2: Chemical Structure of Mono and Di Rhamnolipid (Hassan *et al*, 2016)

The synthesis and regulation of Rhamnolipid has been broadly measured in pseudomonas spp and in *P. aeruginosa* specificity to understand the genes responsible for biosynthesis of rhamnolipid. the sugar (dTDP-l-rhamnose) are precursors for rhamnolipid synthesize while the hydrophobic moieties could be 3-(3-hydroxyalkanoyloxy) alkanoic acid (HAA). The sugar half can be composed from d-glucose, while the hydrophobic half can be mounting thru the fatty acid synthesize pathway, brewing with two-carbon units [16]. Most bacteria contain the demandable enzymes for synthesizing the precursors in rhamnolipid biosynthesis but the enzymes associated in the synthesizeof HAA, mono- and di-rhamnolipids are present virtually aggregative in Pseudomonas sp. and Burkholderia sp. (Fig. 3). Synthesizing of rhamnolipids and their pre substrate generate on induction of the relevant gene products to express the key enzymes for the rhamnolipid (Abdel-Mawgoud, 2014)

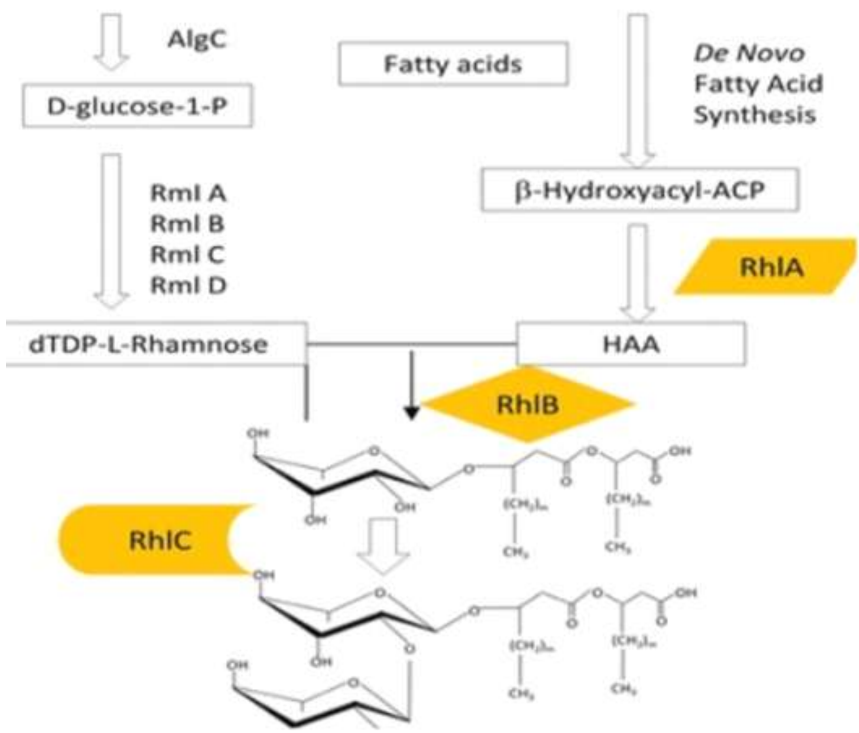


Figure 3: The Pathway of Synthesis the Rhamnolipid Production in Pseudomonas Aeruginosa, and Appeared three enzymes RhlA: RhlB and RhlC requisite for rhamnolipid synthesize.adopted by (Chong and Li, 2017)

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### *The Applications of Rhamnolipid*

Rhamnolipids can be broadly applied in many manufacture fields, in Bioremediation of petroleum rhamnolipids can improved the solubility of hydrocarbons to facilitate degradation due to the biocompatibility and microbial degradation, as well as they can be used in tertiary petroleum recovery (Parra, *et al*, 1989, Husain, 2008, Bertrand *et al*, 1994, Roy *et al*,2015) and removed the contaminated of heavy metal and pesticides using foaming-surfactant technology (Sachdev, Cameotra, 2013)

Rhamnolipids could play impotrtent roles in biocontrol agent against several phytopathogenic fungi; like. *Fusarium. oxysporum,, Botrytis Cinerea Mucor spp*. and many others by stimulated plant immunity system; induced genes involved in plant's defense system in tobacco, wheat and *Arabidopsis thaliana* (Vatsa *et al*,2010) in food preservation Rhamnolipids could prevented food spoilage through antimicrobial agent activity; rhamnolipids inhibit the growth of bacteria causing foodborne agent like Listeria monocytogenes, *Bacillus subtilis*; it could also prevent formation of biofilms due to their anti-adhesive propriety. (Magalhaes and Nitschke, 2013), rhamnolipids prevent biofilm formation by synergistic effect with caprylic acid to inhibit biofilms of most resistant bacteria, e.g. *Pseudomons aeruginosa and Staphlycoccus aureus* (Schooling *et al*,2004)

The most important strain of microorganisms rhaminolipid production are pseudomonas spp such as Pseudomonas chlororaphis NRRL B-30761, Pseudomonas fluorescens HW-6,Pseudomonas fluorescens Migula 1895, Pseudomonas indica MTCC 3714, Pseudomonas putida BD2, Pseudomonas stutzeri, Burkholderia glumae. Burkholderia kururiensis KP23T,Acinetobacter sp. YC-X 2, Acinetobacter calcoaceticus NRRL B-59190, Acinetobacte calcoaceticus NRRL B-59191, Pseudoxanthomonas sp. PNK-04,Renibacterium salmoninarum 27BN, Serriatia rubidaea SNAU02, Streptomyces sp. ISP2-49E, Tetragenococcus koreensis sp. nov., Thermus sp. CCM 4212, Meiothermus ruber CCM 2842 these strains were reviewed in (**Chong and Li, 2017)**

### *Trehalolipids*

Trehalolipids are important emulsifying compounds with application in microbial-enhanced oil recovery and oil empties treatment (Anderson,and Newman 1983; Zaragoza *et al*,2009). the chemical structural of trehalolipid biosurfactants is composed of Disaccharide trehalose linked at C-6 and C-6 to mycolic acid (figure 4), this structure is related with most species of *Mycobacterium*, *Corynebacterium, Nocardia, Rhodococcus erythropolis* and *Arthrobacter sp*. Trehalolipids from diverse organisms vary in the size and structure of mycolic acid, the number of carbon atoms present and the extent of unsaturation. (Takayama *et al*, 2005; Bouchez-Naïtali, and., Vandecasteele 2008, Liu and Liu, 2011).

Trehalolipids obtained from Rhodococcus spp generally produce trehalolipid biosurfactants in the presence of hydrophobic substrates with both cell-bound and extracellular production demonstrated (Rosenbergand Ron, 1997). Various researches found the *Rhodococcus* genus, had numerous kind of trehalose (Lang and Philp 1998), it differs in number and length of Carbone chain, its varied from (C20 to C90) of the ester in fatty acids, the chemical diversity of trehalolipids produced by Rhodococci is vast and includes trehalose monomycolates, dimycolates and trimycolates (Niescher t al, 2006). The capacity of varied microorganisms to interior hydrocarbons relied on the hydrophobic of cell surface. In mycolates, the layer of mycolic acid which consisted of long chain of α-branched and

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β-hydroxy fatty acids promptitude high hydrophobicity to the cell surface, causing the main hydrocarbon acceding style permitting microorganisms to closely login the oil dribbles and annealed hydrocarbons (White *et al*,2013).

The researchers mentioned the regulation during growth stages of microbes producing of trehalolipids can changing the substrate access mode in hydrocarbons (Franzetti *et al*.2008). They noticed that the cells of the genus *Gordonia* were hydrophobic throw early logarithmic stage of germinating cells on n-hexadecane and access to large oil drops throw direct contacting. During this phase, the alters occur and the cell surface becomes hydrophilic. as well as expurgates extracellular bioemulsifier permitting cells to bind to hydrophilic outer stratum of the emulsified oil drops (Jitendra and Banat 1997; Satpute, *et al*,2010).

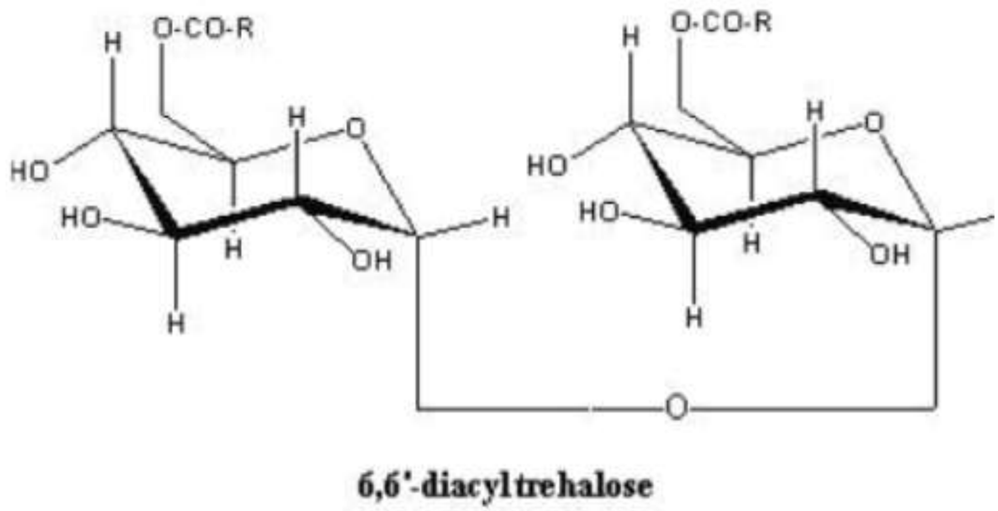


Figure 4: Chemical Structure of Trehalose Lipid

## II. BIOSYNTHESIS OF TREHALOLIPIDS

the constituting of mycolic acid residues it thought to be a Claisen-condensation of the eventual resulting sugar residue, the trehalose-6-phosphate concedered the key reaction for synthesis trehaloliped is controlled by the enzyme trehalose- 6-phosphate synthetase which bonds 2 molecules of D-glycopyranosyl at C1, UDP-glucose and glucose-6-phosphate play important role as the promptly gestures (Suleman and Mussa,2018). InAlkanotrophic *Rhodococcus ruber*, the enzyme trehalose- 6-phosphate syntheses induction with n-alkanes (Ortiz *et al*, 2008). In the additionally reactions associated with synthesis of trehalolipids have been conspicuously articulated of trehalose dimycolates (TDM). The production of trehalolipids in *Mycobacterium tubercolosis* could happened in the eventual step of the biosynthesis of cell wall (Mutalik *et al*, 2008). The synthesized mycolic acids transposed and seated in to the complex consist of peptidoglycan and arabinogalactan of the cell wall,then formation of trehalose dimycolates eventuates with four various reactions (Figure 5). The synthesis proceeds through the transfer of the mycolyl group to D-mannopyranosyl-1-phosphoheptaprenol. The activation of mycolyltransferase II, transferred mycolyl group to formed Trehalose Mono Mycolate (TMM)-phosphate and by dephosphorilation process, causing in formation the TMM. A rapid and efficient transfer of TMM from the inside to the outside of the cell is very important in process of the synthesis of cell wall arabinogalactan-mycolate and TDM. (Kuyukina *et al*, 2001)

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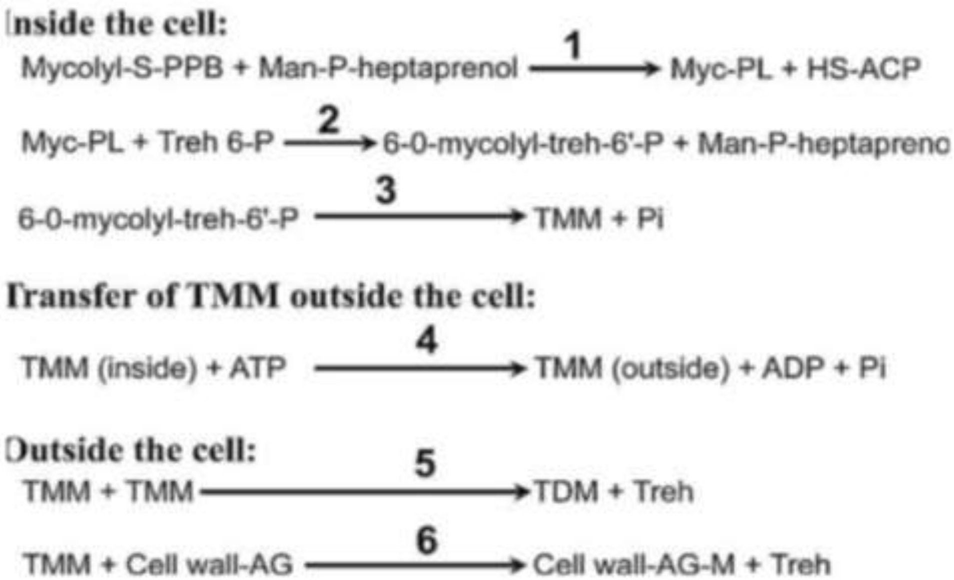


Figure 5: Processing of Newly Synthesized Mycolic Acids in M. tuberculosis in Reactions 1, apperead mycolyltransferase I; and in reaction 2, mycolyltransferase II; in 3, TMM-P phosphatase; 4, TMM transporter; 5 and 6, Ag85 as the mycolyltransferase (FbpA, FbpB, and FbpC). man-P-heptaprenol, mannosyl-phosphoryl-heptaprenol; treh, trehalose; AG, arabinogalactan; AG-M, arabinogalactan-mycolate.adepted by ((Ortiz et al,2008)

## III. Application of Trehalolipids

Trehalolipids have acquired battened attention applications in a number of scope in agriculture, food, bioremediation and antimicrobial activity, and these is due to the activity of trehalolipids in lowering interfacial tension and increase pseudosolubility of hydrophobic installer. The most consequent applicability in bioremediation, by optimizing degradation of hydrocarbons (White *et al*, 2013). In comparable to other microbial glycolipids, trehalolipids have commonly appeared contrasting consequences and accomplishment with the cases of preventing and activating of biodegradation averages. (Liu and Liu 2011) from a medical viewed, Trehalolipids plays a determining management during infectiousness. TDM(trehalose dimycolates) appeared considerably effectiveness as biological activities, such as activity against tumor cells (antitumor effectiveness) (Natsuhara,*et al*,1990; Orbach-Arbouys *et al*,1983) enhanced the effectiveness on immune cell in infection caucused by microorganisms (Parant *et al*,1977);increased the functionality of immunomodulating as formation of granuloma (Bekierkunst *et al*,1969); inducting the murine macrophages to outputted nitric oxide($NO^-$) (Chami *et al*,2002); exhortation the cytokines productions of and consolidation the antigenic activity (Sakaguchi *et al*,2000) the toxicity of *Mycobacterial* TDM is limited the applicability in pharmaceutical aspects, *and* (van Dyke *et al*,1993) mentioned that the *Rhodococcus erythropolis* biosurfactants could conduced assailable in desorption tests of hydrophobic composite from soil.

***Sophorolipids***

Sophorolipids are considered one of the most promising and attractive biosurfactant, Sophorolipids are secondary metabolites classified as extracellular glycolipids, synthesis essentially by varied kind of yeast includes *Torulopsis bombicola, T. petrophilum* and *T. apicola* and Starmerella bombicola (former Candida bombicola), from

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carbohydrates and lipids, being excreted as a mixture of related chemical structures (Freitas *et al*,2018, Morya *et al*,2013).

Sophorolipids structure are amphiphilic molecules composed of hydrophilic moiety, consists of a dimeric carbohydrate sophorose (O β-D-glucopyranosyl-2-1-β-D-glucopyranose) linked by a glycosidic linkage bound between the carbon 1' and the terminal (ω) or sub-terminal (ω-1) carbon of a fatty acid chain, sophorolipids are found as a mixture of free acid and macrolactones form (Chen *et al*, 2011; Desai and Banat 1997 ; Hu, & Ju2001). These biosurfactants are a combination of six to nine varied hydrophobic sophorolipids. Figure-6.

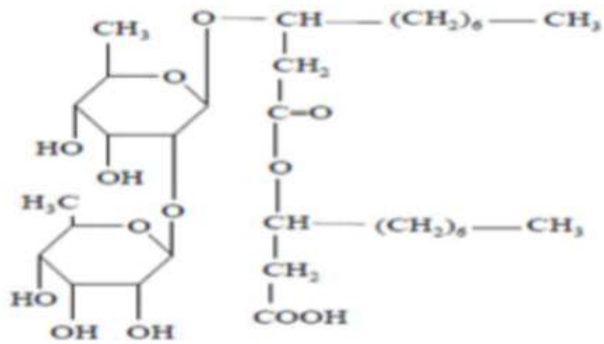


Figure 6: Chemical Structure of Sophorolipid

The peculiarities of Sophorolipid that make it superiority to synthetic surfactants and having wide range of applications these properties including sopherolipid could be produced in large quantities: the chemical characteristic of sopherolipideis stability in broad range of pHs, temperatures, and salinity(Kurtzman,2012; Pekin *et al*,2005), decline foaming and having the properties of detergent (Hirata *et al*,2009) augment surfactants activisms through synergism between acidic and lactonic forms (Chandran and Das,2011) good surface tension activity and displayed easily biodegradable,(Onwosi, Odibo,2012) based on renewable resources easy and simplified product recovery (Hassan *et al*,2016)], these properties Given the increasing interest in SLP biosurfactant and their highly attractive characteristics,

Sophorolipid Could produce by several microorganisms in yeast species particulrarely,like *orulopsis magnolia*, partiandida batistae, Rhodotorula bogoriensis, Candida floricola, C. riodocensis, C.rugosa, C.kuoi, C.tropicalis, Cryptococcus sp., Cyberlindnera samutprakarnensis, Pichia anomala PY1, odotorula muciliginosa, Torulopsis gropengiesseri, Torulopsis petrophilum, and Wickerhamiella domercqiae Y2A,. Among these species Starmerella bombicola ATCC is the beast example of SLP producing yeast, the highest yield and productivity.They are produced as a mix of structurally related molecules, reaching up to fourteen different kind and associated isomers.(Kurtzman 2012; Pekin *et al*,2005; Hirata *et al*,2009; Chandran. and Das,2011; Bajaj and Annapure,2015).

***Biosynthesis of Sophorplipids***

The biosynthesis of SLP occurs in the stationary phase of cell growth, under nitrogen limiting conditions total phosphate exhaustion, and dissociated from cellular growth(Espuny *et al* 1996) The biosynthesis process of sophorolipid in *Candida bombicola* begins with a hydroxylation of the fatty acid as hydrophobic carbon sources, such as the alkanes, alcohol and esters of fatty acids present in the medium. This fatty acid can be of numerous

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forms, in the case the form is n-alkanes, alcohol, aldehyde, triglycerides or esters of fatty acids, it will be metabolized until its correspondent fatty acid, and if it isn't present, the fatty acid will be formed from the acetyl-CoA. it will be metabolized through the β- oxidation and use for the cellular maintenance instead of the SLP synthesis when lower glucose concentration(Franzetti *et al*,2009). The activation process of the fatty acids occur through hydroxylation of carbon terminal or subterminal, this process is mediated by the enzyme CYP52M1 (cytochrome P450 monooxygenase belonging to the CYP52 family) NADPH depending, bonded to the cellular membrane, leading to the formation fatty acid with activated hydroxilated. It can be metabolized through β-oxidation or act as precursor for the synthesis of SLP (de Oliveira *et al*,2014, Van Bogaert *et al*,2011) The enzyme CYP52M1 is expressed exclusively in the stationary phase and possibly potentialized by a damage resistant protein (DAP1) which stabilizes and regulates the CYP450 protein and participates in the metabolism of the lipids and sterols (Ciesielska *et al*,2013) In the next two stages, two molecules of glucose will be linked to the activated fatty acid. The reactions require the glucose is activated in the form of UDP-glucose (Uridine diphosphate glucose) which acts as a donor of glucosyl grouping.synthesis processe required presence of two enzymes Glucosyltransferase I (UgtA1).and Glucosyltransferase II (UgtA2). '. Both enzymes UgtA1 and UgtA2 are expressed in high amounts in the early of stationary phase.

The whole steps of sophrolipid biosynthesis were summarized schematically in The figure 7 shows the biosynthesis of the SLP. In the presence of hydrophobic carbon sources, such as the alkanes, alcohol, fatty acids and esters of fatty acids,(Ciesielska *et al*,2014,; Van Bogaert *et al*,2007).

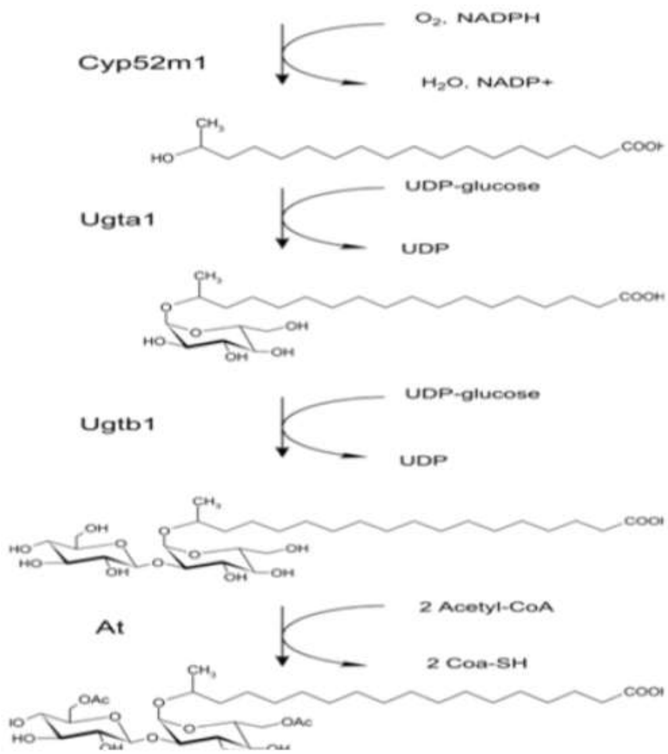


Figure 7: diagram of biosynthesis of the sophrolipid in presence of hydrophobic carbon sources cited from (de Oliveira *et al*, 2014).

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***Application of Sophorolipid***

The applications of Sophorolipid depended on the structural characteristics, and it is directly related to the composition of their acidic and lactonic forms. These applications are highlighted in agriculture, food, cosmetic, bioremediation and biomedicine with antimicrobial activity (Freitas *et al*, 2018)

Lactonic forms are more hydrophobic, and have been reported to have better biocide activities (Glover *et al*,1999), anticancer and antibacterial(de Oliveira *et al*,2014), On other hand, acidic forms are better foaming agents, have higher water solubility (Paulino *et al*,2016) and have been reported wide application on food industry, bioremediation and cosmetics (François *et al*,2012) the composition of the SLP mixture of the acidic or lactonic forms depends on many different factors, such as: strain of producer microorganism, composition of medium - carbon sources (Paulino *et al*, 2016) nitrogen and salt sources (Ribeiro *et al*, 2013, Daverey and. Pakshirajan, 2010) environmental conditions (temperature, pH, agitation, aeration and period of culture) (Casas and. García-Ochoa 1999, Otto *et al*,1999)

In agriculture, SLP can be used for phytopathogens control (biocidal properties) and as adjuvant in formulation of herbicides. SLP show antifungal activity against plant pathogenic fungi, including *Phytophthora* sp. and *Pythium* sp. Damping-off is a soil borne root disease caused by these phytopathogens (Yoo *et al*, 2005)

SLP biosurfactant have been produced and commercially applied as active ingredient in cosmetics products for body and skin applications. They are used as emulsifiers, foaming agents, solubilizers, wetting agents and detergents (Lourith, and Kanlayavattanakul,2009) Among of several expected characteristics for cosmetics application, SLP show low cytotoxicity towards human keratinocytes and fibroblasts (Ankulkar and Chavan,2019).

In the food industries the emulsifying property of SLP can be used for improve the physical properties (volume, texture, viscosity, stability and general eating characteristics) of products of vegetable fat emulsion-based or starch-based and animal food products (Allingham, 1971) SLP added to wheat flour or a product containing it, modifies quality of final product. Production of bread from the wheat flour containing the SLP promotes better volume, appearance and shelflife of bread (Akira, S. and Akira, 1986).

The antimicrobial activity of SLP is due to their biological activity through certain mechanisms that involve destabilization and alteration of the permeability of the cellular membrane and rupture of cells causing extrusion of the cell contents (Shah,2005) Besides antibacterial agent, SLP also act as antialgal (Paulino,*et al*,2016), antifungal, antimycoplasma and antiviral agents (Van Bogaert *et al*,2007).

## IV. CONCLUSION

Despite numerous advantages and applications of glycolipid with different it types (rhamnolipids, trehalolipids, sophorolipids) but the production still very expensive compared to the synthetic surfactants, in order to disentangling this problem by using inexpensive material as substrates of cultural media, incubation at optimization condition (pH, temperature, source of nitrogen and carbon) which conduced to maximum production of Biosurfactants:

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