

Biosurfactant production by fungi as a sustainable alternative

Produção de biossurfactantes por fungos como uma alternativa sustentável

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ABSTRACT: A wide variety of bacteria is far more exploited than fungi as biosurfactants (BS) or bioemulsifiers (BE), using renewable sources. BS are considered to be environmentally safe and offer advantages over synthetic surfactants. However, the BS yield depends largely on the metabolic pathways of the microorganisms and the nutritional medium. The production of BS or BE uses several cultural conditions, in which a small change in carbon and nitrogen sources affects the quantity of BS or BE produced. The type and quantity of microbial BS or BE produced depend mainly on the producer organism, and factors such as carbon and nitrogen sources, trace elements, temperature and aeration. The diversity of BS or BE makes it interesting to apply them in the pharmaceutical and cosmetics industries, agriculture, public health, food processes, detergents, when treating oily residues, environmental pollution control and bioremediation. Thus, this paper reviews and addresses the biotechnological potential of yeasts and filamentous fungi for producing, characterizing and applying BS or BE.

KEYWORDS: surface active compounds; agroindustrial substrates; fungi; amphiphilic molecules.

RESUMO: Uma grande variedade de espécies bacterianas é bem mais explorada que os fungos como agentes biossurfactantes (BS) ou bioemulsificantes (BE), usando fontes renováveis. Os BS são considerados ecologicamente seguros e oferecem vantagens sobre os surfactantes sintéticos. Entretanto o rendimento de BS depende grandemente das vias metabólicas dos micro-organismos e do meio nutricional. A produção de BS ou BE utiliza várias condições culturais, em que uma pequena alteração nas fontes de carbono e nitrogênio afeta a produção de BS. O tipo e a quantidade de BS ou BE microbianos produzidos dependem principalmente do organismo produtor e de fatores como fontes de carbono e nitrogênio, oligoelementos, temperatura e aeração. A diversidade de BS ou BE torna-os interessantes para aplicação nos campos farmacêutico, cosmético, da agricultura, da saúde pública, em processos alimentares, detergentes, no tratamento de resíduos oleosos, no controle de poluição ambiental e na biorremediação. Assim, a presente revisão aborda o potencial biotecnológico de leveduras e fungos filamentosos para produção, caracterização e aplicações de BS ou BE.

PALAVRAS-CHAVE: compostos de superfícies ativas; substratos agroindustriais; fungos; moléculas anfífilas.

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INTRODUCTION

Surfactants are chemical compounds, characterized by the presence of amphipathic molecules consisting of hydrophobic and hydrophilic moieties, which are partitioned preferentially at the interface between fluid phases with different degrees of polarity and hydrogen bonds. Thus, they reduce surface and interfacial tension, and form microemulsions, in which hydrocarbons can solubilize. The polar portion (head) may be ionic (cationic or anionic), nonionic and amphoteric, while the apolar portion often consists of hydrocarbons and can be solubilized in water (CALVO et al., 2009). Therefore, biosurfactants (BS) or bioemulsifiers (BE) are considered the most versatile chemical groups of amphipathic molecules possessing hydrophobic and hydrophilic domains used in various industrial processes. As they have to compete in the market, however, it is essential to produce BS or BE that are eco-friendly and to do so economically (DESAI; BANAT, 1997; PRADHAN, 2017; BHATTACHARYA et al., 2017).

BS and BE are amphipathic compounds produced by various microorganisms, and have unique properties, e.g., they are lipophilic and hydrophilic. Their extremities have opposing polarities, represented by an apolar and a polar head. BS or BE accumulate in the air-water and oil-water interfaces and surface (MUTHUSAMY et al., 2008). The main properties of these compounds are: they reduce surface and interfacial tensions; they have hydrophilicity actions; they are ecologically correct and non-toxic; they retain their functionality under extreme conditions of pH and temperature, stability, wettability and dispersion (KOSARIC, 2001). In this context, the large chemical diversity and diverse properties demonstrate that BS can be applied across a wide range of activities, such as biodegradation, emulsification, de-emulsification, and solubilization. They can be produced from renewable sources, and they both remain effective under extreme conditions of pH, salinity and temperature. The BS and BE were more advantageous than the chemical surfactants. The microbial BS produced in cold habitats provide a perspective on the most promising future applications (BANAT et al., 2000; PERFUMO et al., 2018).

The BS or BE are classified according to their chemical composition, molecular weight, physicochemical properties, mode of action and microbial origin. For example, those of low molecular weight are fatty acids, glycolipids, cyclic and acyclic lipopeptides, and those of high molecular weight are polysaccharides amphipathic, proteins, lipopolysaccharides, lipoproteins, polymeric and vesicles and microbial cells with surfactant activity, which are classified as particulate biosurfactants. Thus, low molecular weight BS are efficient at reducing surface and interfacial tensions, while high molecular weight BE are more effective in stabilizing oil-in-water emulsions (CALVO et al., 2009; DESAI; BANAT, 1997; RAJESH et al., 2017).

In the present study, it was found that BS or BE were produced by bacteria (KOMA et al., 2001; KREPSKY et al., 2007;

RAJESH et al., 2017; BOUASSIDA et al., 2018), followed by yeasts (SARUBBO et al., 2001; 2006; 2007; VANCE-HARROP et al., 2003; AMARAL et al., 2008; KATEMAI, 2012), and rarely by filamentous fungi (BATRAKOV et al., 2001; 2003; KATEMAI et al., 2008; KIRAN et al., 2009; SILVA et al., 2014; 2015; PELE et al., 2018).

Natural BS or BE produced by bacteria have been exploited a lot — those produced by yeast a little less, while research studies on filamentous fungi are rare. However, the few filamentous fungi exploited have shown potential to produce BS or BE, with higher yields when compared to those from yeasts, but mainly when compared to those from bacteria (BHARDWAJ et al., 2013). Therefore, the release of high amounts of BS produced by filamentous fungi has been attributed to cell wall stiffness, as suggested by KIM et al. (2002; 2006).

Thus, this paper reviews and addresses the biotechnological potential of filamentous fungi for producing BS or BE, considering the high industrial potential of these organisms, as described by CASTIGLIONI et al. (2009), PELE et al. (2018), and SILVA et al. (2014). In addition, using filamentous fungi meets the demands of society for concerns about the environment to be taken into account. This is also reflected in the new legislation and prompts the search for products obtained via microbial, toxicity-free alternatives, rather than depending on commercial surfactants synthesized from petroleum.

Therefore, this paper generates perspectives on how to face the challenges, especially the need to increase knowledge about biochemical pathways of BS or BE production and to assess the biotechnological potential of fungi (yeasts and filamentous fungi) for producing BS or BE. They are a versatile alternative, and it includes the sustainable use of agricultural by-products in the search for new surfactants with wide applicability and which cost less to produce is included. Therefore, this paper indicates the main challenges and the need to increase knowledge about the biochemical pathways of BS production, associated with the biotechnological potential of yeasts and filamentous fungi.

Thus, a versatile and sustainable alternative is presented, considering the high enzymatic capacity of using agricultural and industrial by-products to produce new BS or BE, besides which molecules can be applied widely and reduce production costs. Table 1 shows the studies performed with yeasts, the genera and species most studied, as well as the class of BS, and which biosurfactant is synthesized.

It is observed that most yeasts of the genus *Candida* are the most investigated among different types of BS or BE that have the potential to be produced economically. It is possible that the broader use of yeast to produce these substances with great success is related mainly to its GRAS status (generally considered as safe). Microorganisms that have a GRAS status do not present a risk of inducing toxicity and pathogenic reactions (FONTES et al., 2008). In addition, the versatility of the metabolic pathways of yeast, especially *Yarrowia lipolytica*, and the perfect stage of *Candida lipolytica*, both yeasts considered to have unconventional

metabolism, is highlighted in view of its growth in a wide variety of substrates with different chemical natures to produce BS and/or in bioremediation (HUA et al., 2003; LANCIOTTI et al., 2005; AMARAL et al., 2010; CAMPOS-TAKAKI et al., 2010; RUFINO et al., 2014; AGHAJANI et al., 2018).

It should be noted that filamentous fungi (Table 2) are less exploited than yeasts, due to their slower growth.

However, they are excellent producers of BS or BE, as well as emulsifiers, with stable emulsions, and have an excellent capacity to reduce stress. Moreover, they promote the dispersion of hydrophobic compounds, which enables them to be applied in different sectors (SILVA et al., 2014; SOUZA AF et al., 2016; SOUZA PM et al., 2016; 2017b; PELE et al., 2018).

Table 1. Types of biosurfactants or bioemulsifiers produced by yeasts according to the classification.

Biosurfactant	Microorganism	References
Glycolipids	<i>Candida bombicola</i>	Solaiman et al. (2004)
	<i>Candida bombicola</i>	Roelants et al. (2013)
	<i>Candida bogoriensis</i>	Kitamoto et al. (2001)
	<i>Candida sphaerica</i> UCPO995	Sobrinho et al. (2008)
	<i>Candida glabrata</i>	Andrade et al. (2015)
	<i>Candida ishiwadae</i>	Thanomsab et al. (2004)
	<i>Candida batistae</i>	Konishi et al. (2008)
	<i>Pseudozyma fusiformata</i>	Morita et al. (2007)
Sophorolipids	<i>Wickerhamomyces anomalus</i> CCMA 0358	Souza KST et al. (2017)
	<i>Pseudozyma aphidis</i>	Rau et al. (2005)
	<i>Candida bombicola</i>	Casas; Ochoa (1999)
	<i>Candida bombicola</i>	Cavaleiro; Cooper (2003)
	<i>Torulopsis petrophilum</i>	Cooper; Paddock (1983)
	<i>Candida (Torulopsis) apicola</i>	Hommel et al. (1994)
	<i>Candida bogoriensis</i>	Tulloch et al. (1968)
	<i>Candida antarctica</i>	Kim et al. (2002)
	<i>Pseudozyma rugulosa</i>	Fukuoka et al. (2007a; 2007b)
	<i>Candida</i> sp. SY16	Kim et al. (2006)
<i>Kurtzmanomyces</i> sp.	Kakugawa et al. (2002)	
Complex Carbohydrates/protein/lipids	<i>Candida lipolytica</i> UCPO988	Sarubbo et al. (2007)
	<i>Candida lipolytica</i> IA1055	Sarubbo et al. (2001)
	<i>Yarrowia lipolytica</i> NCIM 3589	Zinjarde et al. (1997)
	<i>Debaryomyces polymorphus</i>	Singh; Desai (1989)
	<i>Candida tropicalis</i>	Singh; Desai (1989)
	<i>Candida ingens</i>	Amézcuca-Vega et al. (2007)
	<i>Candida utilis</i>	Shepherd et al. (1995)
	<i>Candida valida</i>	Shepherd et al. (1995)
Complex Carbohydrate /protein	<i>Candida boleticota</i>	Mossa et al. (2006)
	<i>Candida lipolytica</i> ATCC8662	Cirigliano; Carman (1984; 1985)
	<i>Saccharomyces cerevisiae</i>	Cameron et al. (1988); Bahia et al. (2018)
	<i>Kluyveromyces marxianus</i>	Lukondeh et al. (2003)
	<i>Candida utilis</i>	Sheperd et al. (1995)
	<i>Candida ingens</i>	Amézcuca-Vega et al. (2007)
Lipopeptide	<i>Rhodotorula glutinis</i>	Yoon; Rhee (1983)
	<i>Candida glabrata</i>	Morita et al. (2007)

Source: adapted from: Amaral et al. (2008; 2010); Campos-Takaki et al. (2010); Katemai (2012); Bhardwaj et al. (2013).

Influence of physico-chemical parameters on the production of biosurfactants or bioemulsifiers

The BS or BE synthesis may be spontaneous or induced by the presence of lipophilic compounds, temperature variations, pH, agitation speed, stress and low concentrations of the nitrogen source. Probably the main source for synthesis of BS or BE is the carbohydrate in the culture medium. The flow of this source regulates both the glycolytic and lipogenic pathways that act to form the hydrophilic portion (head) and the lipid part (tail), depending on the nature of the substrates (SYLDATK; WAGNER 1987; FONTES et al., 2008; 2012) (Fig. 1). However, the carbon sources that influence the production of BS or BE by different strains of microorganisms have been the subject of several studies. Although the production of BS or BE occurs in the presence of water-soluble carbon sources, such as sugars, several studies show that the highest BS or BE production is obtained when hydrophobic substrates are added (KALYANI et al., 2014).

It is observed in Figure 1 that, when a hydrocarbon is used as a carbon source, microbial metabolism mainly uses the lipolytic pathway and gluconeogenesis (formation of glucose from precursors other than hexoses) that can be used in the production of fatty acids or saccharides. Thus, the production of saccharides is activated by the gluconeogenesis pathway. Oxidation of fatty acids occurs via β -oxidation with the formation of acetyl coenzyme A (acetyl-CoA) or propionyl coenzyme A (propionyl-CoA), in the case of odd-chain fatty acids. From the formation of acetyl-CoA, the reactions involved in the synthesis of polysaccharide precursors, such as glucose 6-phosphate, are essentially the inverse of those involved in glycolysis. However, the reactions catalyzed by pyruvate kinase and phosphofructokinase-1 are irreversible; therefore, other enzymes, which are unique to gluconeogenesis, are required to bypass such reactions. The main reactions are shown in Figure 2, until the formation of glucose 6-phosphate, as the main precursor of the polysaccharides, disaccharides to be formed to produce the hydrophilic portion of the glycolipids (SYLDATK; WAGNER, 1987; OCHSNER et al., 1994; FONTES et al., 2008; 2012).

The biosynthesis of the emulsifier can take place in the synthesis of the head — hydrophilic portion (carbohydrate) and the hydrophobic part (lipids), whereas the lipid chain length will depend on the carbonic substrate present in the medium (Fig. 2). The authors described in this study that the carbohydrate and lipid syntheses depend on the chemical nature of the substrates used (SYLDATK; WAGNER, 1987; FONTES et al., 2008; 2012).

The effective cost of biosurfactant or bioemulsifier productions

The literature affirms the economic stand point of BS or BE is not yet competitive with the synthetic surfactants obtained from petroleum (RUFINO et al., 2011).

Table 3 describes the renewable substrates used to BS or BE productions. However, the use of these biomolecules BS and BE is limited since the cost of production is high, and the production potential is low (PACWA-PLOCINICZAK et al., 2011; LUNA et al., 2009; 2013).

In order to raise the productivity of BS or BE, several investigations described the successful of these active molecules production depends on the use of renewable substrates from biotechnological processes, as it cuts total costs by around 50%. Despite of that, the purification process is considering another obstacle to producing these compounds from microbial origin (BANAT et al., 2010; 2014; ROCHA E SILVA et al., 2014).

BS or BE are considered extracellular secondary metabolites or associated with the cell membrane, the structure of which depends on the ratio of carbon and nitrogen sources. In this case, the BS or BE can be replaced by synthetic surfactants if the cost of the raw material reduces the cost of production (SILVA et al., 2014; ROCHA E SILVA et al., 2014).

Methods of extraction and characterization of biosurfactants or emulsifiers

The methods for extracting BS are variable and depend on the structure and chemical composition of the BS (MUTHUSAMY

Table 2. Types of biosurfactants or bioemulsifiers produced by filamentous fungi.

Biosurfactant	Microorganism	Reference
Lipopeptide	<i>Penicillium chrysogenum</i> SNP5	Gautam et al. (2014)
Glycolipid	<i>Aspergillus niger</i>	Kannahi; Sherley (2012)
Complex Carbohydrate/protein/lipid	<i>Cunninghamella echinulata</i>	Silva et al. (2014)
	<i>Fusarium</i> sp.	Qazi et al. (2014)
*Not identified	<i>Fusarium, Penicillium</i> and <i>Trichoderma</i>	Castillo-Méndez et al. (2017)
	<i>Aspergillus</i> spp.	Cavalcanti et al. (2017)
	<i>Aspergillus niger</i>	Silva et al. (2015)
	<i>Aspergillus fumigatus</i>	Castiglioni et al. (2009)
	<i>Rhizopus arrhizus</i>	Pele et al. (2018)

et al., 2008), as shown in Table 4. The most used methods are precipitation (acidic, ammonium sulphate) and extraction with organic solvents (methanol, chloroform, ethanol and their associations) (LUNA et al, 2009, 2013; CHEN, 2012; LIMA et al., 2017).

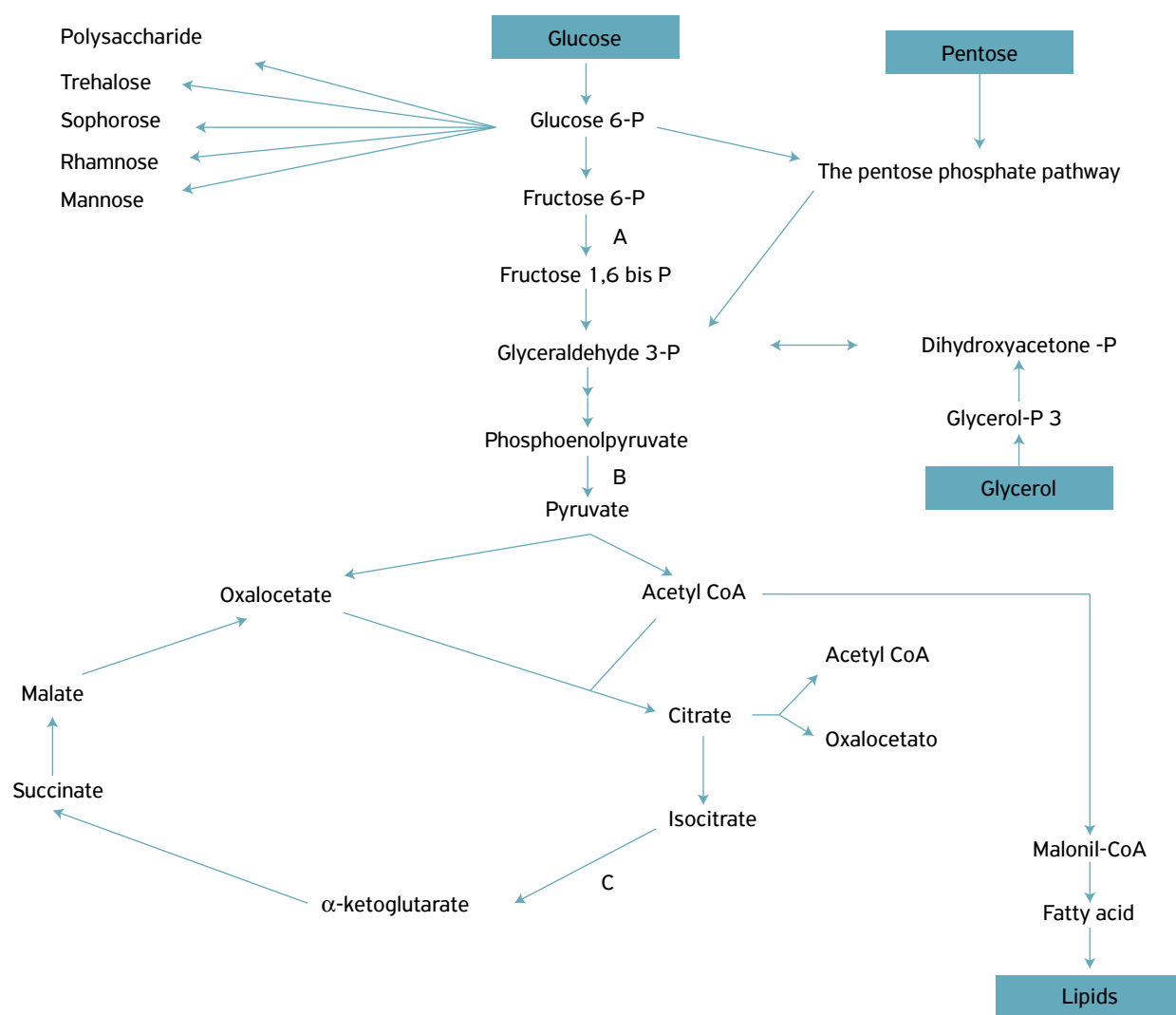
Precipitation is the most cited technique in the literature, and acidification, using hydrochloric acid (HCL), is common for extracting crude BS. This method consists of acidifying the cell free metabolic liquid to a pH 2–3 value. Another method to isolate BS is using ammonium sulphate precipitation, which follows a protocol similar to that one of acid precipitation, with the differential of the need for sample dialysis to remove salt in the final process (ANTUNES et al., 2013). Organic solvents such as ethanol and acetone are also commonly used to extract BS and are still a method that favors the reuse of solvents (SILVA et al., 2014). On the other hand, extraction with organic solvents favors the high yield of biosurfactants. However, it is a

time-consuming and costly process (MUTHUSAMY et al., 2008; ANDRADE et al., 2015).

The main methods to characterize surfactants, according to LIMA et al. (2017), feature:

- the ability to reduce surface and interfacial tension due to the formation of a molecular film;
- the ability to form stable macro and microemulsions of hydrocarbons in water or water in hydrocarbons;
- the formation of micelles;
- their antimicrobial properties.

Some authors describe the most important properties evaluated in the search for new and powerful BS for industrial application, for example: being able to reduce surface and interfacial tension, a critical micellar concentration (CMC), the ability to form emulsions and to maintain the stability of them (MUTHUSAMY et al., 2008; PACWA-PLOCINICZAK, et al., 2011; JAMAL et al., 2012).



Acetyl-CoA: acetyl coenzyme A; Malonil-CoA: malonil coenzyme A.

Figure 1. Intermediate metabolism related to the production of biosurfactant or bioemulsifier, using hydrophilic and hydrophobic sources as substrates, according to Fontes et al. (2008).

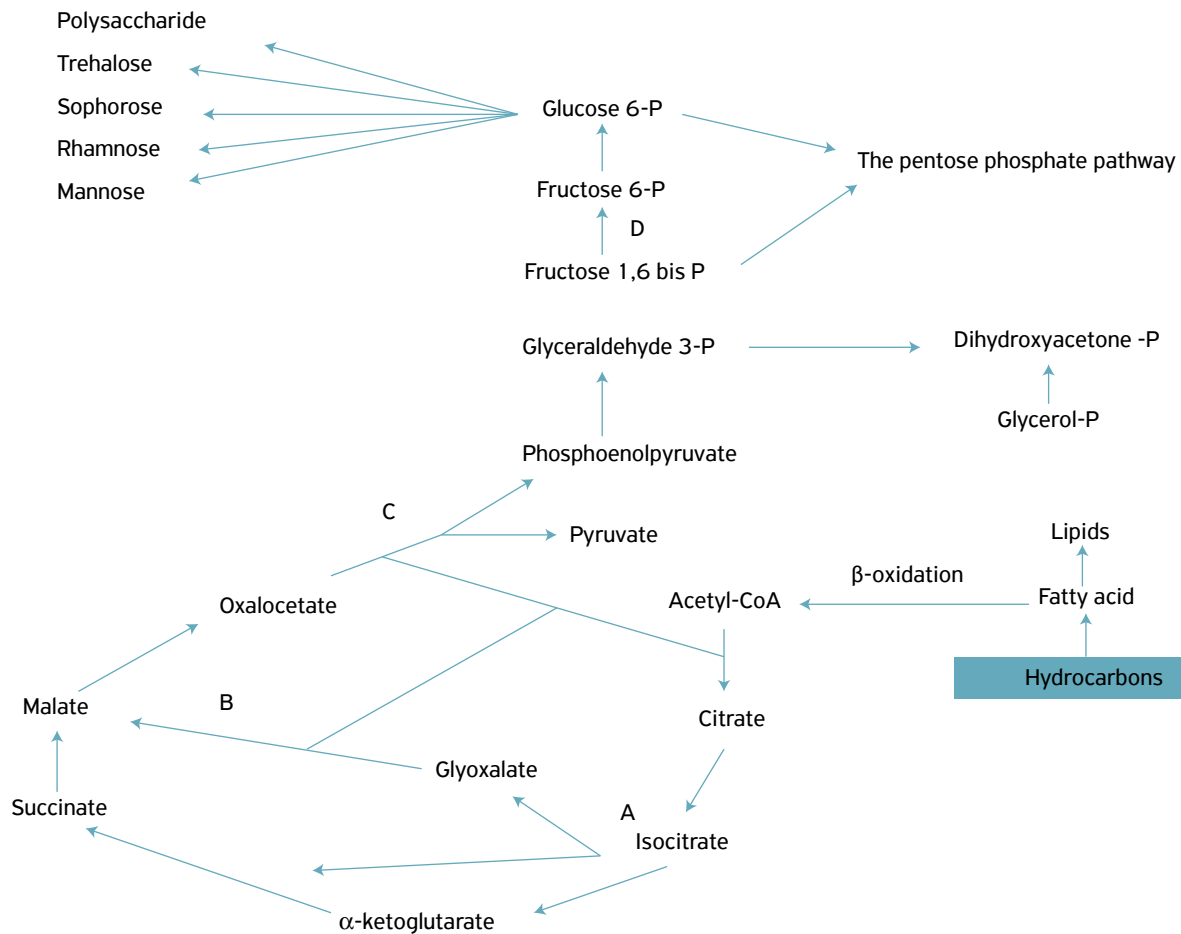


Figure 2. Intermediate metabolism related to the production of biosurfactant or bioemulsifier using hydrocarbons as substrates, according to Fontes et al. (2008).

Table 3. Renewable substrates as renewable sources for biosurfactant or bioemulsifier productions by yeasts or filamentous fungi.

Microorganism	Renewable substrates	Reference
<i>Candida lipolytica</i>	Canola oil/glucose	Sarubbo et al. (2007)
<i>Candida lipolytica</i>	Industrial hydrophobic waste	Rufino et al. (2014)
<i>Candida lipolytica</i>	Molasses	Karatay; Dönmez (2010)
<i>Candida lipolytica</i>	Waste soybean oil and corn steep liquor	Souza PM et al. (2016)
<i>Candida sphaerica</i>	Groundnut oil	Sobrinho et al. (2008)
<i>Cryptococcus curvatus</i>	Acetate	Gong et al. (2015)
<i>Candida utilis</i>	Corn oil	Shepherd et al. (1995)
<i>Candida glabrata</i>	Cotton seed oil/glucose	Sarubbo et al. (2006)
<i>Candida glabrata</i>	Corn steep liquor and whey	Lima et al. (2016; 2017)
<i>Candida glabrata</i>	Corn steep liquor, whey and cassava waste water	Silva et al. (2017)
<i>Yarrowia lipolytica</i>	Industrial glycerol	Papanikolaou et al. (2002)
<i>Yarrowia lipolytica</i>	Crude glycerin and cashew apple juice	Fontes et al. (2012)
<i>Rhodotorula glutinis</i>	Crude glycerol/Tween 20	Saenge et al. (2011)
<i>Rhodotorula graminis</i>	Corn steep solids/yeast extract	Galafassi et al.(2012)
<i>Ustilago maydis</i>	Crude glycerol	Liu et al. (2011)
<i>Penicillium chrysogenum</i> SNP5	Wheat bran, grease waste	Gautam et al. (2014)
<i>Cunninghamella echinulata</i> UCP	Soybean waste oil and corn steep liquor	Silva et al. (2014)
<i>Cunninghamella phaeospora</i> UCP1303	Post-fry soybean oil and corn steep liquor	Lins et al. (2017)
<i>Cunninghamella bertholletiae</i>	Waste soy oil and corn steep liquor	Souza PM et al. (2017)
<i>Fusarium</i> sp.	Sucrose and yeast extract	Qazi et al. (2014)
<i>Aspergillus fumigatus</i>	Glycerol on solid state fermentation	Castiglioni et al. (2009)
<i>Rhizopus arrhizus</i>	Soybean post-frying oil/sodium glutamate	Pele et al. (2018)

Applications of biosurfactants

The surface-active compounds produced by microorganisms have the potential to be applied, based on their functional properties, which include: emulsification, separation, wetting, solubilization, demulsification, inhibition of corrosion, and reduction of liquid viscosity and of surface tension. Thus, biosurfactants divide the interface between fluids with different degrees of polarity and hydrogen bonds such as air/water or oil/interfacial water. Therefore, due to these properties, biosurfactants are able to diminish the surface and interfacial tension and to form microemulsions, in which the hydrocarbons can be solubilized in water, or water emulsions can be formed using hydrocarbons. Therefore, BS of fungal origin have wide application and can be used in several industrial sectors that use chemical surfactants. Such industries include: petroleum, pharmaceuticals, cosmetics, agriculture with regard to formulating herbicides and pesticides, and the production of personal hygiene products and food processing, as described by NITSCHKE; PASTORE (2002), confirmed by the literature (Table 1), and presented in Table 5 (BANAT et al., 2000; BHARDWAJ et al., 2013; LIMA et al., 2016; 2017; SOUZA AF et al., 2016; SOUZA KST et al., 2017; AGHAJANI et al., 2018).

CONCLUSION

BS synthesized by yeast are the most studied, while there are not many studies on BS production by filamentous fungi. However, microorganisms isolated from soils, mangrove sediments and contaminated areas demonstrate excellent production potential, as well as the metabolic ability to use renewable substrates to produce high value surfactants and BE.

The components of culture media, especially carbon sources, for the production of BS are divided into three categories: carbohydrates, hydrocarbons and vegetable oils. BS can be extracted, recovered and characterized by simple and low cost techniques. Therefore, using BS produced by fungi from renewable sources becomes a versatile and sustainable alternative that should be encouraged, in view of society's demands for ecologically safe products obtained through green technologies.

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Table 5. Functions and applications of biosurfactants.

Functions	Fields of applications
Emulsification and dispersants	Cosmetics, paints, bioremediation, oils and food
Solubilizer	Pharmaceutical and hygiene products
Wetting and penetrating agents	Pharmaceutical, textile and paint products
Detergents	Cleaning products, agriculture
Foaming agent	Hygiene products, cosmetics and flotation of ores
Thickening agents	Paints and food
Metal sequestrants	Mining
Vesicle formers	Cosmetics and drug delivery system
Microbial grow factor	Treatment of oily residues
Demulsifiers	Waste treatment, oil recovery
Viscosity reducers	Transportation in pipelines, pipelines
Dispersants	Coal-water, lime-water mixtures
Fungicide	Biological control of phytopathogens
Recovery agent	Tertiary oil recovery (MEOR)

MEOR: microbial enhanced oil recovery.
Source: Nitschke; Pastore (2002).

Table 4. Extraction methods related to the properties of biosurfactants

Extraction methods	Properties	Vantages
Acid precipitation	Insoluble in low pH	Low cost, recuperation of crude biosurfactant
Precipitation with ammonium sulphate	Higher content of protein	Eficient for polymeric biosurfactant
Precipitation with ethanol/acetone	Soluble in organic solvent	Eficient for polymeric biosurfactant
Extraction with organic solvent	Soluble in organic solvent	Recuperation of crude biosurfactant, partial purification

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