BIOSURAFACTANTS FROM *CANDIDA* SP. – PROMISING BIOCOMPOUNDS FOR HUMAN HEALTH AND FOOD INDUSTRY

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ABSTRACT

Biosurfactants are amphiphilic molecules produced by microorganisms, with low toxicity and high specificity, characteristics which recommend them as ecological alternatives for chemical surfactants in a wide range of applications. The *Candida* genus comprises numerous species able to produce biosurfactants using non-conventional carbon sources such as industrial and household wastes. This production strategy has a double benefit: assures the recovery of potential polluting compounds from the environment transforming them in useful biocompounds for human health and economy.

The present review deals with some of the main classes of biosurfactants produced by various *Candida* species, the substrates used for their synthesis and the practical applications of the obtained biosurfactants in biomedicine and food industry.

Keywords: *Candida*, biosurfactants, wastes, biomedicine, food industry.

REZUMAT

Biosurfactanții sunt molecule amfifile, produse de microorganisme, cu toxicitate scăzută și specificitate ridicată, caracteristici care le recomandă ca alternative ecologice pentru surfactanții chimici într-o gamă largă de aplicații. Genul *Candida* cuprinde numeroase specii capabile să producă biosurfactanți folosind surse neconvenționale de carbon, cum ar fi deșeurile industriale și menajere. Această strategie de producție are un dublu beneficiu: asigură recuperarea compușilor potențial poluanți din mediul înconjurător, care sunt astfel transformați în biocompuși utili pentru sănătatea umană și economie.

Prezentul articol trece în revistă principalele clase de biosurfactanți produși de diferite specii aparținând genului *Candida*, substraturile folosite pentru sinteza acestora și aplicațiile lor practice în domeniul biomedical și în industria alimentară.

Cuvinte-cheie: *Candida,* biosurfactanți, deșeuri, biomedicină, industrie alimentară.

INTRODUCTION

The biosurfactants are amphiphilic compounds with a large range of biotechnological applications due to their ability to increase the solubility of the hydrophobic compounds by reducing the surface tension at their interface with the immiscible aqueous media [1]. The biosurfactants are produced by microorganisms (fungi, yeasts and bacteria) and represent an ecological alternative to synthetic surfactants due to their specific activity in extreme environmental conditions (temperature, pH and salinity), low toxicity, high biodegradability and foaming ability [2].

Although there are many studies concerning biosurfactant production at high rates using various bacteria genera (*Pseudomonas*, *Bacillus*, *Acinetobacter, Rodococcus, Lactobacillus*) [3-4], during the last decades the attention of the scientific community shifted towards the study of biosurfactants produced by yeast species.

Numerous *Candida* species are important sources of biosurfactans: *Pseudozyma* (*Candida*) *antarctica*, *Candida* (*Starmerella, Torulopsis*) *bombicola*, *Yarrowia* (*Candida*) *lipolytica*, *Candida guilliermondii*, *Candida glabrata*, *Candida rugosa*, *Candida tropicalis* and *Wickerhamiella* (anamorph *Candida) domercqiae* var. *sophorolipid.*

The present review focuses on the main aspects regarding the biosurfactants produced by *Candida* species: their classification, the wastes most frequently used as substrates for their synthesis and practical applications of biosurfactants in biomedicine and food industry.

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MAIN CLASSES of BIOSURFACTANTS PRODUCED by *Candida* sp.

The biosurfactants are extracellularly or cell-membrane bound molecules produced by microorganisms, comprising a hydrophilic moiety represented by a peptide, amino acids or a carbohydrate (mono-, di- or polysaccharide) which enables the interaction with the aqueous phase, and a hydrophobic moiety consisting of fatty acids or acetyl groups which confers them affinity for hydrophobic substrates. The biosurfactants produced by *Candida* sp. are represented by low-molecular-mass (glycolipids and phospholipids) as well as high-mass biosurfactants (polymeric surfactants) [5].

The glycolipids, sophorolipids and mannosylerytrithol lipids (MELs), are the most studied class of biosurfactants and comprise carbohydrates and aliphatic/hydroxy aliphatic acids linked to an ether/ester group. The sophorolipids are produced by several *Candida* species*: C. batistae, C. floricola, C. riodocensis, C. rugosa, C. kuoi, C. stellata, C. tropicalis,* C. *bombicola* [6]. MELs are produced by species from *Pseudozyma* genus, especially *C. (P.) antarctica*. MELs have a hydrophilic moiety represented by 4-O-β-D mannopyranosyl-meso-erythritol and a hydrophobic moiety composed of fatty acids with 7 to 12 carbon atoms and/or acetyl groups [5, 7].

The polymeric surfactants have high molecular mass and usually contain amphipathic polysaccharides, proteins and lipoproteins [8], the most studied being the mannan-lipid-proteins produced by *C. tropicalis* and *Candida albicans* [9] and the liposan produced by *Y. (C.) lipolytica*.

Fatty acids are produced by *Candida ingens* in order to facilitate the interaction between cells and hydrocarbons for their assimilation and biodegradation [2].

SUBSTRATES USED for BIOSURFACTANT SYNTHESIS in *Candida* sp.

The studies regarding biosurfactant production are, in general, focused on their practical applications. Therefore, the quantity and quality of the product obtained after cultivation represent important criteria depending on: the type of organism producing the biosurfactant, the nature and the rate between the carbon and nitrogen substrates, the concentration of microelements, the pH, temperature and aeration of cultivation medium.

Despite the numerous advantages of the biosurfactants, their widespread use is still limited due to their relatively high production costs. A growing number of studies are conducted for increasing the efficiency of the process [10], including the use of cheap substrates such as industrial, agro-industrial or household wastes followed by the development of an efficient bioprocess based on optimization of the cultivation conditions, the recovery and purification of the final products.

Agro-industrial wastes

The agro-industrial wastes are the result of the forest industry, agriculture and food industry, their long-term storage being difficult and regulated by environmental protection laws. They contain high amount of carbohydrates and lipids representing a rich carbon substrate for microbial growth [11]. Thus, *C. bombicola* biosurfactants were obtained using a mix of corn steep liquor, molasses and soybean waste frying oil [12], respectively, using soy molasses. Cassava wastewater was used as pre-inoculum medium for obtaining biosurfactants from *Pseudozyma* (*Candida*) *tsukubaensis,* while holocellulose from rice straw was reported as source for synthesis of sophorolipids in *W. (C.) domercqiae* var. *sophorolipid.*

Vegetable oils and oil wastes

Vegetable oils as well as the raw waste remaining after their processing, represent important substrates for biosurfactant production due to their high content in free fatty acids with 16 to 18 carbon atoms, mono- di- and triacylglycerols, proteins, glycolipids, phosphatides and unsaturated fatty acids [13]. According to Kitamoto *et al.* mannosylerythritol lipids were obtained from *C. antarctica* T-34 and *C. antarctica* KCTC 7804 using soybean oil, respectively, glycerol and oleic acid [14]. Sophorolipids with high antimicrobial activity were obtained for *C. antarctica* ATCC 22214 using glucose and canola oil. *C. lipolytica* UCP 0988 produced biosurfactants in presence of ground nut oil or soybean oil refinery residue [15].

Fried oil

Billions of liters of fried oil result each year from the activity that occurs only in restaurants.

Oil frying and heating are associated with modifications of the chemical structure through hydrolysis, polymerisation and thermal oxidation, the changes depending on the temperature, surface, number of heating cycles and oil composition in fatty acids [16]. Moreover, the storage and removal of these wastes are difficult. Therefore, the possibility of using them as an alternative source for the production of various biocompounds such as biosurfactants raised a growing interest.

A study conducted by Fleurackers used fried oil to produce sophorolipids from *C. bombicola* ATCC 22214 [17]. T

he strain *C. glabrata* CMGB32 was able to produce biosurfactants in the presence of fried sunflower oil, after 168 hours of incubation [18]. Other works recommended the use of a mix of vegetable oils or vegetable oils and glucose in order to reach significant biosurfactant synthesis in *C. glabrata* [19].

Animal fat and tallow

Animal fat and tallow are obtained in large amounts from meat processing. Since the exclusive usage of animal fat seems to inhibit yeast growth, adding glucose augments the growth rate and the amount of biosurfactant produced. Animal fat, glucose and corn steep liquor were used to obtain 120 g/L of sophorolipid in 68 h from *C. bombicola* [20].

Dairy industry wastes

The main residues resulting from dairy industry are whey (containing 75 % lactose as dry matter and proteins), buttermilk and their derivatives.

Some of the whey is re-used for the manufacture of beverages, the rest being considered unusable waste. Daniel *et al.* [21] developed a two-stage biotechnological process for obtaining sophorolipids using whey: (1) whey deproteinisation results in production of lactose used as a substrate for the growth of *Cryptococcus curvatus*, while the biomass is homogenized at high pressure to obtain crude cell extract; (2) the extract is further used as a substrate for growing *C. bombicola* which produces sophorolipids [22]. *C. bombicola* was also able to synthesize biosurfactants in presence of glucose and whey, supplemented with yeast extract and oleic acid [23].

BIOMEDICAL APPLICATIONS

Biosurfactants have a broad spectrum of possible biomedical applications being used for optimizing the methods of sanitizing hospital environments or in the treatment of the most dangerous diseases (Table 1). Although there are a lot of studies and clinical trials on cancer treatment and prophylaxis, focused both on the causes of malignant tumors appearance and on the discovery of compounds to replace the currently used cytostatic, the researchers could not develop an efficient treatment for this disease considered the most prevalent of the 21st century.

Some chemical compounds derived from plants can be successfully used in cancer therapy [24]. Despite the good results obtained, this solution is not economically feasible since plants require large surface of lands, enough water supplies or advanced genetic engineering techniques that are too complex or not legally permitted. To overcome these shortcomings, the researchers focused on the discovery of microbial antitumor compounds, such as biosurfactans. Due to their amphiphilic nature, the biosurfactants showed impressive antimicrobial, antiviral, antifungal activity. Recently, Fan *et al.* described the potential applications of MELs in biomedicine as inducing agents in apoptosis and differentiation of B16 melanoma cells [25]. The study proposed that MEL-A mechanism of action is based on the self-assembling properties that affect the cytomembranes and facilitate the interaction with extracellular proteins activating a signaling cascade that ends with endoplasmic reticulum stress, cell apoptosis and finally death of the cancerous cell.

Another interesting research direction is using the antimicrobial and antiadhesion activity of biosurfactants for fighting against antibiotic resistant pathogenic microorganisms. One of the most amazing process in microbiology is cell to cell adhesion and formation of biofilms that are highly resistant to antibiotics or environmental challenges. In biomedicine, biofilm represents a real threat to human health. Although in hospitals the disinfection of surfaces is done in a strict and controlled manner, infections occur quite frequently. Almost all kinds of surfaces can become suitable for biofilm development: central venous catheters, urinary catheters, heart valves, intrauterine devices, contact lenses or voice prostheses were colonized by biofilms. Coating medical surfaces with antimicrobial agents, including yeast biosurfactants, might represent a successful strategy for preventing biofilm formation. Thus, adsorption of the biosurfactant produced by *C. lypolytica* on the surface of the abiotic surfaces might interfere with the adhesion mechanism of some pathogenic microorganisms by modifying the hydrophobicity of the surface [26].

IMPORTANCE in FOOD INDUSTRY

The emulsion is defined as a heterogeneous system consisting of an immiscible liquid dispersed in another liquid and is characterized by a minimal stability. Various surfactants such as lecithin, gum Arabic, yolk and proteins from milk are used for stabilization of the emulsions in food industry in beverages, dressings and sauces [8].

In present, their use is limited, on one hand, by the new cooking technologies such as warming in the microwave and, on the other hand, by the need for large quantities of products for coverage of global food requirement. One of the strategies aimed to overcome this inconvenient consists is obtaining natural

Table 1. Biomedical applications of *Candida* sp. produced biosurfactants

Biosurfactants	Applications	Yeast species
Sophorolipids	• Cytotoxic activity against cancerous cell lines (H7402, KYSE 109, KYSE 450); • Antimicrobial activity against Gram negative bacteria (causing gastroenteritis and urinary tract infections), Gram positive bacteria (*Staphylococcus aureus, Bacillus subtilis*) and pathogenic yeasts; • Can be used as ingredients for hygienic and cosmetic products; • Spermicidal and anti-viral activity; • Anti-inflammatory and anti-sepsis properties; • Promote activity of some antibiotics (tetracycline); • Anti-aging agents that can decrease the elastase activity responsible for the appearance of wrinkles	*C. (T.) bombicola* [6, 25, 28]
	• Anti-cellulitis agents that stimulate the leptin synthesis through adipocytes; • Activate macrophages and act as desquamating agent	*W.(C.) domercqiae* var. *sophorolipid* [6, 28]
Mannosylery-trithol-lipids	• Antimicrobial action against Gram positive bacteria; • Inhibit the growth of human leukemia cells; • Improvement of DNA transfection by cationic liposomes; • Inhibit mouse melanoma cells by inducing apoptosis; • Induce the neurite outgrowth of rat pheochromocytoma PC12 cells; • Cell activation, stimulate the papilla cells; • Moisturizing activity for skin care products	*C.(P.) antarctica* [28-30]
Lunasan	• Inhibit the adhesion of *Streptococcus epidermis*, *Staphylococcus aureus*, *Candida albicans* and *Streptococcus oralis* on plastic tissue culture plates	*C. sphaerica* [31]
Rufisan	• Antiadhesive activity against *Streptococcus agalactiae*, *Streptococcus mutans* and *Staphylococcus aureus*	*C. lipolytica* [32]

surfactants in larger quantities from genetically modified organisms (GMOs) [8, 27]. This is not fully accepted due to the controversy regarding the possible adverse effects of GMOs on human health and environment conservation [8].

Therefore, the attention of researchers focused on biosurfactants as an interesting, natural alternative to chemical surfactants. The biosurfactants have similar structure to the chemical surfactants, can participate in the formation of stable emulsions and act as de-emulsifying agents depending on to their molecular weight.

The biosurfactants unanimously accepted in foods are generally recognized as safe (GRAS) and are produced by various yeast species. Their role is to improve the texture of food, to extend the shelf life of food products, to prevent the damage of fruits and vegetables during transportation (Table 2). Additionally, biosurfactants produced by *Candida valida, Candida utilis* and *Hansenula anomala* have successfully replaced gum Arabic and carboxymethyl cellulose [8].

The biosurfactants can also control the texture of food and the validity of products based on fats. Adding sophorolipids in flour improves the quality and prolong the shelf life of bread and improve the texture and viscosity of foods based on vegetable oils [6]. Due to their antimicrobial and antifungal action, the sophorolipids can also be used to keep fruits and vegetables fresh during transportation by inhibiting fungi proliferation and spore germination and preventing biofilm formation by Gram positive bacteria [6].

CONCLUSION

The study of biosurfactants represents an active and continuously growing field of scientific research. *Candida* sp. produced biosurfactants have numerous biomedical applications in domains of high interest, being used as antiviral and anticarcinogenic agents, in the development of immunomodulating molecules or in viral therapies.

Moreover, *Candida* sp. are able to use low cost compounds as substrates for synthesis of biosurfactants used in food preservation and processing.

The variety and importance of applications of biosurfactants from *Candida* sp. open the way for further extensive work concerning their structure, genetics and production strategies.

Conflict of interests: The authors have no conflict of interest to declare.

Table 2. *Candida* biosurfactants for food industry

Biosurfactants	Applications	Yeast species
Sophorolipids	• Improve food texture and viscosity; • Prolong shelf life of the bread; • Keep fruits and vegetables fresh during transportation; • Inhibit weeds growth	*C. (T.) apicola* *C. (T.) bombicola* *Y. (C.) lipolytica* W. *(C.) domercqiae* var. *sophorolipid* [5, 6, 8]
Mannosylery-trithol-lipids	• Surface tension reduction; • Antimicrobial activity against Gram positive bacteria	*C.(P.) antarctica* [33]
Carbohydrate proteins-lipids complex	• Emulsifying agent in salad dressing formulations	*C. tropicalis* *Y. (C.) lipolytica* *C. utilis* [34]
Lipomannan	• Increasing antimicrobial and anti-adhesive activities against food pathogens	*C. tropicalis* [34]
Liposan	• Emulsifying activity against edible oil	*Y. (C.) lipolytica* [8]

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