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Review

Candida –produced biosurfactants– beneficial agents for environmental remediation biotechnologies

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Abstract Although the *Candida* genus is extensively studied mainly due to its pathogenicity and virulence, the genus comprises numerous species and strains isolated from industrial or natural environments (soil, plants, a.s.o.). Due to their specific metabolic abilities, these strains are able to synthesize compounds with high economic value such as lipids, lipases, fatty acids and biosurfactants using low cost substrates including industrial and household wastes. The biosurfactants represent an advantageous ecologic alternative for synthetic surfactants for a wide range of biotechnological and industrial applications. Bioremediation of polluted soils, including those used in agriculture, represent a challenging domain in which the *Candida*-produced biosurfactants can be successfully used for in situ as well as ex situ technologies. The biosurfactants are also important biocontrol agents for crop protection. Therefore, during the last decades many studies were developed concerning the classification, gene regulation and optimization of parameters for obtaining biosurfactants from *Candida* sp.

Keywords *Candida*, biosurfactants, structure, genetics, substrates, bioremediation, agriculture, biocontrol

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Introduction

Biosurfactants, natural emulsifiers produced by a wide range of microorganisms, are an interesting research direction since the 1960s. Due to their amphiphilic nature, these compounds are successfully used in fields related with foaming, dispersion and emulsification of hydrophobic substrates (R.S. GANGULY & al. [1]).

Although there are some synthetic surfactants that can be easily produced, biosurfactants have the advantage to be biodegradables, nontoxic for the environment and stable at extreme environmental conditions such as: high or low temperatures, extreme pH variation and high salinity (S. SHEHKAR & al. [2]). In present, the biosurfactants are considered the "green'' alternative for the chemical surfactants in agriculture, cosmetics, petroleum industry, food, biomedical field and environment protection.

The present mini-review on *Candida*-produced biosurfactants is a comprehensive study comprising some of the most important and actual data regarding their structure, genetics, production strategies and modern applications in environmental remediation biotechnologies: bioremediation and improvement of agricultural soils as well as biocontrol.

Classification, structure and genetics

The biosurfactants are surface-active biomolecules comprising in their structure peptide, mono-, di- or polysaccharide residues conferring hydrophilic properties and saturated or unsaturated, linear or branched fatty acids representing the hydrophobic part. Depending on the chemical structure and origin of the hydrophobic and / or hydrophilic part, the *Candida*-produced biosurfactants can be classified in glycolipids, polymeric biosurfactants and fatty acids (Table 1).

The sophorolipids have a hydrophilic component represented by a sophorose dimer (2'-O-β-D-glucopyranosyl-β-D-glycopyranose) linked by a β-glycosidic bond to a fatty acid residue with 16 or 18 carbon atoms usually hydroxylated and placed at the carboxyl end. The sophorose dimer has two glucose units linked β 1-2 presenting acetylated hydroxyl groups in the 6 and 6' positions (N.G.KARANTH & al. [3]). Sophorolipids can have either lactonic or acid form depending on the culture media, pH value or the cultivation method, and are produced as a mixture of several chemical compounds differentiated by the fatty acid chain length or by the saturation and acetylation degree of the glucidic part (M.R. DE OLIVEIRA & al. [4]). The cluster involved in

Table 1. Main classes of *Candida* produced biosurfactants

Biosurfactants		***Candida* species**
Glycolipids	Sophorolipids	*Candida (Pseudozyma, Torulopsis) apicola* *Candida (Torulopsis) bombicola* *Yarrowia (Candida) lipolytica* *Wickerhamiella (Candida) domercqiae* var. *sophorolipid*
	Mannosylerytrithol lipids	*Candida (Pseudozyma) antarctica*
Polymeric surfactants	Carbohydrate-protein complex	*Candida lypolitica* *Candida lipolytica*
	Carbohydrate-protein-lipid complex	*Candida lipolytica* *Candida tropicalis* *Yarrowia (Candida) lipolytica*
	Mannan-lipid-proteins	*Candida albicans* *Candida tropicalis*
	Lipomannan	*Candida tropicalis*
	Liposan	*Yarrowia* (*Candida*) *lipolytica*
Fatty acids		*Candidaingens*

sophorolipid synthesis in *C. bombicola* (T. MATSUZAWA & al. [5]) contains five genes encoding: a cytochrome P450 monooxygenase, two glucosyltransferases, an acetyltransferase and a transporter. A mutation in the gene *MFE2* (enoyl-CoA hydratase/3-hidroxyacyl-CoA dehydrogenase) is responsible for shifting the metabolism from β-oxidation towards sophorolipid production. In synthesis of the lactonic form is also involved an extracellular lipase (K. CIESIELSKA & al. [6]).

The mannosylerytrithol lipids (MELs) have a hydrophilic moiety represented by 4-O-β-D manno-pyranosyl-meso-erythritol and a hydrophobic moiety composed of fatty acids with 7 to 12 carbon atoms and/or acetyl groups (T. MORITA & al. [7]). MELs are synthetized as a mixture of MEL-A, MEL-B and MEL-C differentiated by the nature of the hydrophobic component. *C.(P.) antarctica* produces 70% MEL-A, while *P. hubeiensis* preferentially produce MEL-C (D. KITAMOTO & al. [8]). MELs have self-assembling properties: MEL-A can form sponge phases, MEL-B and MEL-C can form large unilamellar vesicles. The biosynthesis pathway of MELs is similar with the β-oxidation from mammalian peroxisomes. MELs metabolism in *C. (P.) antarctica* involve genes for erythritol/mannosetransferase (*PaEMT1*), acyl transferase (*PaMAC1* and *PaMAC2*), acetyltransferase (*PaMAT1*) and a putative transporter (*PaMMF1*) (T. MORITA & al. [9]). Liposan is an extracellular compound produced by *C. (Y.) lipolytica* cells grown on hexadecane, contains 83% carbohydrate and 17% proteins, and has high stability at pH and temperature variations (M.A.Z. COELHO & al. [10]). When grown on glucose, *C. (Y.) lipolytica* can synthesize yansan (J.M. CAMPOS & al. [11]). The biosurfactants produced by *C. tropicalis* and *C. albicans* are mannan-lipid-proteins containing hydroxyl, amino, olefin and carboxyl groups (B. PADMAPRIYA & S. SUGANTHI [12]).

The fatty acids produced by *C. ingens* are able to emulsify alkanes, forming microdoplets (S. SHEKHAR & al. [2]).

Production strategies

The strategies developed for improving biosurfactant production are focused on using a high range of carbon and nitrogen sources, including low cost carbon substrates and anorganic or organic nitrogen compounds (Table 2). Petroleum and biofuel industries by-products and wastes are successfully used for biosurfactant synthesis in *Candida* sp. In the yeast cells, the *n*-alkanes with C_{12} to C_{16} carbon chain are degraded mainly through the mono-terminal oxidation pathway, biosurfactant synthesis being possibly linked to lipase and lipid production (O. CSUTAK & al. [18]), while glycerol metabolism is coupled with fermentation.

Candida biosurfactants are also obtained using agro-industrial wastes: molasses, corn steep liquor or cassava wastewater rich in carbohydrates, macro and microelements. Molasses, the main co-product from sugar refining, can be used for animal feed as well as for citric acid, ethanol and biosurfactant production. Lignocellulosic waste from the forest industry and agriculture contains a large amount of organic carbon and can be used for synthesis of ethanol, organic acids and biosurfactants (M. MITACHE & al. [26]).

Vegetable oils (canola, olive, rapeseed, palm, soybean oils) and their by-products are substrates for obtaining biosurfactants. The most used is the olive oil mill effluent containing a large amount of sugars, nitrogen compounds, organic acids and polyphenols. Using ground nut oil, R. RUFINO & al. [14] have isolated a new *C. lipolytica* biosurfactant with lipopeptidic structure and enhanced emulsifying activity at high salinity. During frying, vegetable oils suffer chemical transformations forming compounds with high polluting potential. Besides being very toxic, the environment polluted with these compounds are rather difficult to remediate. D.K.F. SANTOS & al. [27] obtained a *C. lipolytica* glycolipid with enhanced oil displacement activity for motor oil recovering using animal fat and corn steep liquor.

Bioremediation and improvement of agricultural soil quality

Agriculture is the main food source of mankind. Whether we are talking about agricultural products consumed raw or refined or about agricultural products used as animal feed, agricultural products are part of the human diet. The massive growth of population increased the need for food leading to the development of new approaches and strategies aimed to augment the productivity in the food industry. Conventional agriculture has been replaced by modern agriculture which has many advantages but also has the major inconvenient of affecting the soil quality.Bioremediation represents the removal of contaminants from the environment being also defined as a mechanism by which the natural process of biodegradation is accelerated. The bioremediation technologies are *in situ* and *ex situ* and can involve using biosurfactants in order to

Table 2. Most frequent used carbon and nitrogen sources for production of biosurfactants

Yeast species	Carbon source	Nitrogen source	Reference
C. apicola *C. batistae* *C. bombicola*	glucose + alkanes *n*-alkanes	yeast extract, peptone, urea NH_4Cl, $(NH_4)_2SO_4$	P.F.F. AMARAL & al. [13]
C. (Y.) lipolytica	*n*-hexadecane	yeast extract, $(NH_4)_2SO_4$	P.F.F. AMARAL & al. [13]
	soybean oil refinery residue	yeast extract, glutamic acid	R. RUFINO & al. [14]
	ground nut-oil	yeast extract, NH_4NO_3	R. RUFINO & al. [15]
C. glabrata	fried sunflower oil glycerol + glucose	yeast extract, peptone, NH4NO3	O. CSUTAK & al. [16]
C. (P.) antarctica	*n*-octadecane glycerol, oleicacid	yeastextract, $NaNO_3$	D. KITAMOTO & al. [17]
C. tropicalis	glucose, petroleum *n*-hexadecane fried sunflower oil	yeast extract, peptone NH_4Cl, $(NH_4)_2SO_4$	P.F.F. AMARAL & al. [13]; O. CSUTAK & al. [18]
C. tropicalis *C. albicans*	glucosepetroleum *n*-hexadecane, diesel, glycerol	yeast extract, peptone NH_4Cl	B. PADMAPRYIA & S. SUGANTHI [12] F.R. ACCORSINI & al. [19]
C. rugosa	*n*-alkanes, petroleum	yeast extract, peptone	O. CSUTAK & al. [20]
C. sphaerica	ground nut oil refinery residue, corn steep liquor	water based medium	M.J. CHAPRÃO & al. [21]
C. (P.)tsukubaensis	fermented cassava waste	yeast extract, urea	A.E.C. FAI & al. [22]
W. (*C.*) *domercqiae*	holocellulose oleic acid	yeast extract	X.G. LIU & al. [23]
C. bombicola	corn steep liquor molasses, soybean frying oil	water based medium	J.M. LUNA & al. [24]
	soy molasses	yeast extract, urea	D.K. SOLAIMAN & al. [25]

enhance desorption, to solubilize the hydrophobic compounds and to remove heavy metals from soil (R. RUFINO & al. [14]).

For the *in situ* technologies, the population of indigenous microorganisms is stimulated in order to enhance the rate of degradation of the pollutants by changing certain factors such as moisture content, temperature, pH or concentrations of inorganic compounds (M. BUSTAMANTE & al. [28]). The biological treatment of polluted soil using biostimulation or bioaugmentation can include biosurfactants. Biostimulation refers to stimulation of the indigenous microbes to assimilate the pollutant as carbon source, while bioaugmentation is more complex, involving addition of certain microbial species in the contaminated area in order to achieve controlled and predictable biodegradation. For example, *C. tropicalis* SK21 biosurfactant was efficient in remediation of soil polluted with petroleum using bioaugmentation (M.Y. FAN & al. [29]).

The *ex situ* technologies take place in a specialized environment. Soil washing is based on the amphiphilic nature of biosurfactants. B.G. KANG & al. [30] showed that sophorolipids had a higher efficiency compared to non-ionic surfactants (Tween and Span 80/60/20 20/80/85)

in bioremediation of soil contaminated with 2-methylnapthalene. Clean up combined technology (V. KILDISAS & al. [31] and E. BASKYS & al. [32]) has two stages: first, the biosurfactants are used for loosing tight fractions of pollution agent adhered to soil particles; then, biodegradation occurs using the enzymatic equipment of bacteria or yeasts.

The time elapsed between pollution and the moment when bioremediation techniques are introduced is very important. Adsorption or covalent bonding takes place between the hydrophobic organic compounds (HOC) and the organic soil matter. The first connection established is represented by adsorption of the pollutants on the surface of soil particles. The process can be reversible or irreversible depending on exposure time. Biosurfactants can be used to transfer HOCs from the non-aqueous phase (soil sorbed phase) to the aqueous phase for removing crude oil from contaminated soil (M. BUSTAMANTE & al. [28]).

Slurry is a liquid contaminant of dark color, has a bad smell and contains organic and inorganic compounds, mainly heavy metals. Although certain heavy metals are micronutrients involved in plant metabolism, an augmented concentration due to excessive use of fungicides might be harmful for plants. Extraction of heavy metals from soil can occur through ion exchange, precipitation and counterion binding (J.F. PENG & al. [33]). The ion exchange method is based on the ability of the anionic biosurfactant negatively charged to form a strong ionic bond when it encounters a cationic metal (positively charged) bound to soil particle. Thus, the bond between the cationic metal and the soil particle is broken and the metal can be removed. The biosurfactants can also associate and form micelles, an electronegative charged complex, which binds cationic metal. R. RUFINO & al. [14] used a permeable barrier represented by soil or mixture of soils containing *Y. (C.) lipolytica* biosurfactants for heavy metal removal from slurry generated within a landfill.

Biocontrol

One of the most important sources of agricultural soil pollution is the intensive use of pesticides and synthetic fungicides which accumulate in the soil causing abnormal growth of plants. Since the complete removal of these substances from use is not possible due to the huge demand of agricultural products in a very short time, there is an increased interest in the possibility if limiting their toxic action through alternative technologies, such as biocontrol.

Biocontrol involves using microorganisms as natural antagonists of plant pathogens or producing biocompounds able to replace the pesticides or similar chemical products. An example is the replacement of surfactants from pesticide composition with biosurfactants. Surfactants are designed to function as adjuvants due to their emulsifying and dispersing action aimed to augment the action of pesticides. The main disadvantage of using pesticides is represented by their accumulation in the soil resulting in heavy metal pollution with negative impact on the plant growth. On the contrary, the biosurfactants do not have toxic effect; they have a high degradability and have similar action to synthetic surfactants. The potential role of biosurfactants in the production of pesticides is to engage the elimination of pathogens, to inhibit the growth of weeds and to activate the bioremediation processes by reducing the toxicity of certain compounds.

Sophorolipids were integrated in the composition of herbicides in order to increase the adherence to the surface of weeds. They successfully replaced polyethoxylated tallow amines (POEA), surfactants derived from oil, a real source of pollution. The lactone form produced by *C. bombicola* and the acid form produced by *Candida kuoi* were successfully added to lemongrass oil based herbicides to form a stable emulsion with increased biocontrol action (M.R. DE OLIVIERA & al. [4]). Also, the use of sophorolipids in combination with the lemongrass oil (LGO) herbicide has a stronger effect than POEA-LGO complex. Biosurfactants beneficial role in biocontrol is highlighted in relation to microbial growth considering that their presence facilitates interaction between the microbial cells and different substrates, including, hydrocarbons.

On the other hand, many biosurfactants have antimicrobial activities that can be used in order to enhance the antifungal and antiviral abilities of microorganisms from the surface of the plants. The biosurfactants may contribute to the yeast-mediated induced systemic resistance aimed to control phytopathogens and promote crop yield. Thus, G. LEE & al. [34] indicated the biosurfactant produced by *Pseudozyma* spp. as possible factor in the lipid-mediated pepper plants systemic defense against *Xanthomonas axonopodis* infection.

Conclusions

The genus *Candida* comprises versatile species depending on the environment from which the strains are isolated. The biosurfactants produced by *Candida* species able to degrade low cost substrates, represent a growing field of research. Although progresses have been made concerning the metabolic pathways of various substrates in yeast cells, the genetics of biosurfactant synthesis is complex and still poorly understood. During the last decades,

technologies have been developed using biosurfactants from *Candida* for bioremediation of polluted soils. Furthermore, the ability of these compounds to act as antimicrobial agents opened the way for their use as ecological replacements of pesticides and fungicides.

Conflict of interest disclosure

There are no known conflicts of interest in the publication of this article. The manuscript was read and approved by all authors.

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