Rev. Biol. Biomed. Sci. 2020 3 (1) 1-9
DOI: 10.31178/rbbs.2020.3.1.1

The yeasts – versatile antimicrobial agents

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Received: 17 January 2020 / **Revised**: 17 April 2020 / **Accepted**: 23 April 2020 / **Available online**: 7 April 2020

Abstract Antagonistic yeasts present antimicrobial activity against a wide range of microorganisms, from other yeast species or strains, to bacteria and fungi from natural habitats, industrial processes or immunocompromised patients. The diversity of antimicrobial mechanisms depends mainly on the yeast species and might be represented by the production of killer toxins, enzymes and various cellular compounds or the presence of substrate competition mechanisms. The growing interest developed during last decades for the identification and characterization of yeasts with antimicrobial abilities, is related to their applications in biotechnology. The studies focus not only on understanding the genetic background of the antagonistic potential, but also on its improvement for controlling microbial contamination in natural and industrial fermentations, fruit decay and proliferation of pathogenic strains or biofilm formation on biomedical devices.

Keywords: yeasts, antimicrobial, biocontrol, biomedical

Introduction

Yeasts are ubiquitous microorganisms present in nature (soil, air, water, plants, animals, human, extreme environments) as well as in products related to human activities (fermented foods, feeds, waste waters, polluted sites) (Garcis-Bejar et al., 2019). Numerous yeast species comprise strains with specific genetic background correlated with various characteristics, from antimicrobial activity to production of biocompounds of large interest for biotechnology, medicine or environment protection.

During last decades there is a growing interest in using yeasts as an ecological solution to chemical agents extensively used in biomedicine, biocontrol or bioremediation. This is based on some important characteristics of the yeasts: (i) yeast strains with antagonistic abilities can be isolated from most ecosystems (Liu et al., 2013); (ii) the molecules involved in antimicrobial activities (killer toxins, pulcherrimin, carotenoid pigments) are not allergenic (Charoco et al., 2015) most of them having practical applications in more than one domain (e. g. biocontrol and biomedicine; biocontrol and bioremediation) (Younis et al., 2017); and last, but not least, (iii) many yeast strains have the ability to grow on cheap substrates, including those with polluting potential, producing high biomass yields (Abeln and Chuck, 2019). Due to their intensive use in production of fermented foods and beverages, some yeast species are considered as GRAS (Generally Regarded as Safe). Many yeasts described so far do not have pathogenic potential and have a high metabolic versatility which recommends them for a large range of applications in industry.

The present review deals with the main aspects and the newest information concerning the mechanisms of the antimicrobial activity of the yeasts, its practical applications, as well as limitations and perspectives in various domains of modern biotechnology.

Killer yeasts

The killer phenotype of the yeasts was observed in the early 1960s at *Saccharomyces cerevisiae* strains able to excrete toxic proteins that produced the death of other microorganisms from the environment. Killer yeasts belong mainly to *Saccharomyces*, *Hanseniaspora*, *Pichia*, *Zygosaccharomyces*, *Ustilago* and *Kluyveromyces* genera, and can be isolated from fruits and plants, fermented foods (beer, wine, diary), soil or water. Also, some *Candida* and *Issatchenkia* strains were found to have killer activity, although there is few data concerning the toxins produced. The killer yeast strains are important not only in food production and preservation but also in biomedicine. Therefore, the studies regarding the characteristics, mechanism of action and genetic regulation of the killer toxins are of great interest for the scientific community. The *S. cerevisiae* strains can produce four types of killer toxins: K1, K2, K28 and Klus, encoded by ds-RNA genomes from virus-like particles found in the cytoplasm.

The four killer phenotypes are classified based on their killing profile, cross-immunity and type of cell wall receptors recognized in the attacked cell. However, the K28 toxin is characteristic for the *S. cerevisiae* wine strain 28, and the Klus toxin was identified in the *S. cerevisiae* strains isolated from spontaneous fermentations of grapes from vineyards of the Ribera del Guadiana (Spain), showing amino acid sequence homolgy with a protein encoded by the chromosomal ORF *YFR020W* (Rodriguez-Cousino et al., 2011; Becker and Schmitt, 2017). Lately, two killer toxins possibly encoded by chromosomal genes were isolated from Cf8 and M12 *S. cerevisiae* strains from wineries in Argentina, and a Kx killer factor able to inhibit growth of all known *S. cerevisiae* killer types was isolated from spontaneous fermentations of fruits and berries from Lithuania (Melvydas et al., 2016).

Species from *Hanseniaspora*, *Zygosaccharomyces* and *Ustilago* genera also present virus-like particles responsable for producing killer toxins with similar structure and mechansim of action as *S. cerevisiae* (Csutak, 2014).

The *Pichia* genus comprises species presenting a killer phenotype of great interest, in this case, the killer toxins being encoded by nuclear genes. *Pichia anomala* (*Wickerhamomyces anomalus*, *Hansenula anomala*) killer toxin is a glicoprotein with high exo-β–1–3 glucanase activity, *Pichia kluyvery* produces a toxin with a similar mode of action as *S. cerevisiae* K1 toxin (forming ion-permeable channels in the cell membrane of the sensitive cell), while *Pichia membranefaciens* toxin (PMKT2) seems to be induced by the presence of sodium chloride in the environment (Marquina et al., 2002).

Kluyveromyces species have two cytoplasmic linear DNA plasmids encoding the killer phenotype, the *Kluyveromyces lactis* killer toxin causing cell cycle arrest in the G1 phase in the sensitive cells.

Due to the broad range of microorganisms attacked (yeasts from the same species or other species/genera, bacteria, fungi) and to their different mechanism of action, the killer toxins are regarded as an ecological option for biocontrol and therapeutic applications. Thus, *S. cerevisiae* toxins suppressed the growth *in vitro* of *Botrytis cinerea*, while *P. membranefaciens* was able to protect *Vitis vinifera* plants against the same fungal infection (Chen et al., 2018). The activity of *P. membranefaciens* (PMKT2) and *Ustilago maydis (KP6)* toxins against the growth of *Brettanomyces bruxellensis* and *Dekkera bruxellensis* in wine production was found to be in close correlation with their concentration (Mehlomakulu et al., 2015).

The *P. anomala* killer toxin (PiKt) showed high activity against numerous *Dekkera*/*Brettanomyces* strains involved in wine spoilage (Padilla et al., 2018). The exo-β-1-3 glucanases activity is responsible for the *P. anomala* antifungal activity against *B. cinerea* and *Penicillium expansum* on apples, *B. cinerea* on grapes, *Penicillium digitatum* on oranges and *Colletotrichum gloeosporioides* on papayas. This toxin produces emptied hyphae (*B. cinerea*), disruption of hyphal surfaces (*Botryodiplodia theobromae*) or growth inhibition (*Penicillium roqueforti*) (Muccilli and Restuccia, 2015).

The *Kluyveromyces phaffii* toxin (KpKt) is able to disrupt the integrity of the fungal cell wall, while the toxin produced by *Kluyveromyces wickerhamii* (KwKt) was effective against *B. bruxellensis* and *D. bruxellensis* had the ability to bind to the β-1-6-glucan receptors from the cell wall (Mehlomakulu et al., 2015).

Killer toxins are also intensively studied for their potential use in therapeutic applications. Thus, the possibility of successfully using *P. anomala* killer toxins for antimicrobial therapy, was demonstrated during experiments with anti-idiotypic antibodies for treatment of model candidiasis in rats (Passoth et al., 2006). Same toxins showed β-glucanase activity against *Candida* strains from mouth, bladder and skin infections. Pathogenic *Candida* strains were also inhibited by *Zygosaccharomyces bailii* toxin (zygocin). Bacterial pathogenic strains of *Escherichia coli*, *Enterococcus faecalis*, *Klebsiella* sp., *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Pseudomonas alcaligenes* were inhibited by *Pichia kundravzevii* killer toxin. Asynthetic decaPeptide derived from *P. anomala* killer toxin, named KP, had high antimicrobial activity against *Candida albicans*, *Cryptococcus neoformans* and *Malassezia pachydermatis*, while *Z. bailii* zygocin was active against *C. albicans*, *Candida glabrata*, *Candida krusei* and *Sporothrix schenckii* (Muccilli and Restuccia, 2015). Also, from the vaginal fluid of women infected with killer toxin sensitive microorganisms, human natural *Pa*KT-like Abs (*Pa*KTAbs – *P. anomala* Killer Toxin-Antibodies) were isolated with protective role against *Candida*, *Cryptococcus* and *Aspergillus* infections (Polonelli et al., 2011).

Substrate competition

Competition for the nutrients and/or for space is one of the more effective mechanisms of antimicrobial action developed by few yeast species. In some cases, the antagonistic yeast is better adapted for surviving in certain environmental conditions compared to the competitor, has a better ability to colonize the surfaces or releases compounds that might act as chelators of various substrates.

Yeasts belonging to *S. cerevisiae* are well known for being effective in depleting sugars in fermented products. When inoculated during the first days of wine fermentation, *S. cerevisiae* has high antimicrobial activity reducing the number of other yeast species (*Hanseniaspora uvarum*, *Candida zemplinina* and *Toluraspora delbrueckii*) present at the end of the process (Lleixa et al., 2016). Sugar competition was also involved in antifungal activity of *Sporobolomyces roseus*, *Rhodosporidium toruloides* and *Cryptococcus humicola* against *Botrytis cinerea* and, respectively, the activity of

Pichia guilliermondii against *Ceratocystis paradoxa*. On the other hand, *P. expansum* growth was inhibited due to nitrogen source competition in presence of *P. guilliermondii* and *Candida sake* (Muccilli and Restuccia, 2015).

In the case of *P. guilliermondii*, carbon and nitrogen substrate competition seems to be enhanced by the ability of the yeast cells to attach to the hyphae of *P. digitatum*, *B. cinerea* and *Colletotichum capsici* (Papon et al., 2013). Also, *P. guilliermondii* colonize rapidly the wounds from the surface of fruits stimulating synthesis of enzymes (peroxidase, polyphenoloxidase, superoxide dismutase, catalase, phenylalanine ammonia-lyase) which correlates with an increasing of the plant defense mechanism (Zhao et al., 2008).

One of the best studied yeast species with applications in biocontrol is *Metschnikowia pulcherrima*. The antimicrobial mechanism of action is based on the competition for the iron ions from the environment (Saravanakumar et al., 2008). *M. pulcherrima* cells release the pulcherriminic acid (resulted from cyclodileucine, an intermediate in the L-leucine biosynthetic pathway) which chelates the iron Fe^{3+} forming the pigment pulcherrimin ($C_{12}H_{22}$ $Fe_2N_20_4{}^{2+}$). The ability of *M. pulcherrima* of depleting iron from the growth media is correlated with the availability of Fe^{3+} and the ability of the competitive microorganisms to chelate Fe^{3+} (Sipiczki, 2006). Whole genome sequencing revealed that the gene *SNF2* regulates pulcherriminic acid biosynthesis and, thus, the antifungal activity in *M. pulcherrima* (Gore-Lloyd et al., 2018).

M. pulcherrima strains were very effective against *B. cinerea*, *P. expansum*, *Alternaria* sp., *Monilia* sp., *Aspergillus carbonarius* and *Aspergillus niger*, the pulcherrimin pigment affecting the fungal mycelium, the sporulation and formation of germination tubes (Piano et al., 1997; Spadaro et al., 2002; Bleve et al., 2006). Moreover, *M. pulcherrima* strains isolated from fruits were able to inhibit the growth of *Candida* pathogenic strains from human infections, the activity being augmented by the presence of sodium bicarbonate and calcium chloride (Csutak et al., 2013). In fact, using mixtures of yeasts inoculum and various chemicals (sodium bicarbonate, calcium chloride, ethanol or fungicides) in small concentrations is a successful approach in biocontrol applications (Karabulut et al., 2003).

Pulcherrimin production was also reported for other yeast species. Thus, Krause et al. (2018) described the structure and functions of the four genes (*PUL1-4*) forming a PULcherrimin (PUL) gene cluster present in *K. lactis*, *Kluyveromyces aestuarii*, *Metschnikowia fructicola* and *Zygotorulaspora mraki*. According to their study, *PUL1* and *PUL2* genes are involved in pulcherrimin biosynthesis, *PUL3* encodes a membrane bound protein required for iron uptake from the medium using pulcherrimin and bringing the pigment-iron complexes into the cells (siderophore-like transporter mechanism), while *PUL4* is involved in pulcherrimin biosynthesis as well as in *PUL3* positive regulation.

Red yeasts

The "red yeasts" represented by species belonging to the *Phaffia, Rhodotorula, Sporobolomyces* and *Sporidiobolus* genera, are able to synthesize carotenoid pigments, forming pink, orange or red colonies. The carotenoids are classified in hydrocarbon carotenes (β-carotene, torulene) and oxygenated xanthophylls (torularhodin and astaxanthin). In present, there are known over 600 carotenoids produced by plants and microorganisms. The demand for these "natural" carotenoids is growing, being used as precursors of vitamin A, in removing the oxygen radicals, as food colorants and antimicrobial agents.

Rhodotorula sp. is able to produce relative high amounts of β-carotene, torulene and torularhodin using simple growth media, even industrial wastes (hydrocarbons, vegetal oils, glycerol). Carotenoid biosynthesis in *Rhodotorula* yeasts has been described. Whole genome sequencing studies of the strain *Rhodotorula mucilaginosa* RIT389 revealed the existence of a cluster consisting in three closely located genes encoding phytoene synthase (*crtB*), lycopene cyclase (*crtY*), and phytoene desaturase (*crtI*). The gene for geranyl pyrophosphate synthase has a separate location, while the genes *crtX* for carotenoid oxygenase and *crtBY* for phytoene synthase/lycopene cyclase, are located in close proximity and are convergently transcribed (Tang et al., 2019). Landolfo et al. (2018) showed that in the strain *R. mucilaginosa* C2.5 t1, the genes for phytoene desaturase (*CAR1*), phytoene synthase/lycopene cyclase (*CAR2*) and carotenoid dioxygenase (*CAR0*) are clustered in a genome region (CAR cluster) of approximately 10kb, and also, that the induction of genes involved in the early steps of carotenoid synthesis depends on the accumulation carotenoid pigment in the cells.

The carotenoid pigments extracted from *Rhodotorula glutinis* strains isolated from fruits, plants and soil had strong antibacterial effect against *Staphylococcus aureus*, *Bacillus subtilis, Bacillus cereus*, *E. coli* and *Salmonella enteritidis* (Keceli et al., 2013). Also, the pigment extracted from *Sporobolomyces* sp. had inhibitory activity against human pathogenic strains of *E. coli* and *S. aureus* (Manimala and Murugesan, 2014).

R. glutinis is also an effective biocontrol agent. The yeast cells were able to colonize rapidly the wounded fruits and inhibited the growth of *B. cinerea* and *P. expansum* on strawberries and apples by affecting spore germination (Zhang et al., 2007; Zhang et al., 2009).

The rhodotorulic acid produced by *Rhodotorula* species is a hydroxamate-type siderophore with high affinity for the Fe^{3+} ions from the environment. The antifungal effect of the rhodotorulic acid against *P. expansum* and *B. cinerea* constists in a delay of fungal installation in the host, respectively, inhibition of conidial growth, and is correlated with the iron concentration in the medium, the optimal activity being observed at Fe^{3+} concentrations up to 2 µM (Ferramola et al., 2013).

Due to its importance and wide range of action, there are many studies regarding the possibility of enhancing the antimicrobial activity of *Rhodotorula* sp. For exemple, addition of 0.5% chitin in the growth media, augmented the effect of *R. glutinis* and *R. mucilaginosa* against *P. expansum* and *Rhizopus* sp. from peaches (Zhang et al., 2016). The antifungal activity of *R. mucilaginosa* against *P. expansum* was enhanced in combination with low concentrations of phytic acid which reduced the levels of patulin (Yang et al., 2015) and by heat shock treatments which determined a better yeast colonization on the surface of fruits (Cheng et al., 2016).

Production of enzymes and molecules with antimicrobial activity

1. Volatile compounds

Many yeast species produce a high number of volatile compounds (VOCs) with low molecular weight and high volatility, responsible mainly for the aroma of various final products. The VOCs are extensively used in the production of wine, fermented foods and feeds or in the flavor industry (for various cosmetics, chemicals or pharmaceuticals). Therefore, there are many studies dealing with the characterization of the yeast VOCs and their metabolic pathways. For example, *S. cerevisiae* strains involved in winemaking are able to produce 257 volatile metabolites represented by acetals, acids, alcohols, aldehydes, ketones, terpenic compounds, esters, ethers, furan-type compounds, hydrocarbons, pyrans, pyrazines and S-compounds related to different metabolic pathways. Thus, the aliphatic acids with short carbon chain result from fermentative metabolism, the medium-carbon-chain fatty are intermediates in fatty acid biosynthesis while the higher alcohols are produced by the Ehrlich pathway and/or by the pyruvate pathway (Alves et al., 2015).

Some VOCs, such as esters, alcohols and sulfides, have antimicrobial activity against a wide range of phytopathogenic fungi. Since VOCs are considered as ecofriendly compounds, they might represent an interesting alternative in biocontrol for preventing plants and fruits decay. One of the yeast species producing VOCs with antifungal activity is *Candida sake*. The strains of *C. sake* are able to synthesize alcohols, from which 3,7-Dimethyl-6-octen-1-ol (citronellol) and phenylethyl alcohol with antifungal activity against dermatophytes and *Aspergillus* sp., and phenylethyl alcohol effective against *Muscodor albus* (Arrarte et al., 2017). *C. sake* also produces esters (ethyl hexanoate, 3-methylbutyl hexanoate and 2-phenylethyl acetate) with antifungal activity.

Other *Candida* species producing VOCs are *Candida intermedia* and *Candida friedrichii*. *C. intermedia* along with *Sporidiobolus pararoseus*, *W. anomalus* (*H. anomala*, *P. anomala*) and *M. pulcherrima* VOCs inhibited spore germination and mycelial growth of *B. cinerea* (Arrarte et al., 2017). The compounds synthesized by *C. friedrichii* as well as *C. intermedia*, *Cyberlindnera jadinii* and *Lachancea thermotolerans* determined the reduction of vegetative growth, sporulation and production of ochratoxin A (OTA) by *Aspergillus carbonarius* and *Aspergillus ochraceus* (Arrarte et al., 2017; Farbo et al., 2018). In this case, the main compound of yeast VOCs was 2-phenylethanol, an alcohol with high antifungal activity. For example, the 2-phenylethanol from *S. cerevisiae* was observed to control *Sclerotinia sclerotiorum* growth *in vitro* and in bean seeds and the one produced by *Kloeckera apiculata* was active against *Penicillium italicum* mold in citrus fruit. The *P. anomala* (*W. anomalus*) 2-phenylethanol inhibited spore germination and aflatoxin B1 production by *Aspergillus flavus* in correlation with down regulation of aflatoxin biosynthesis genes and an altered expression of chromatin modifying genes (Hua et al., 2014). Also, the VOCs from *P. anomala* (*W. anomalus*), *Pichia kluyveri* and *H. uvarum* were described as active against *A. ochraceus* growth and OTA synthesis during processing of *Coffea arabica* (Farbo et al., 2018).

The increase of ethyl acetate concentration as a result of glucose metabolism in *P. anomala* (*W. anomalus*) cells, determined the decrease of *Penicillium roqueforti* growth (Passoth et al., 2006). Ethyl acetate, acetate, acetic acid and hydrogen sulfide produced by *H. uvarum*, *S. cerevisae*, and *Meyerozyma* (*Candida*, *Pichia*) *guilliermondii* were also responsible for inhibiting *B. cinerea* development on *Vitis vinifera* berries (Cordero-Bueso et al., 2017).

Less studied yeast species such as *Aureobasidium pullulans* or *Galactomyces candidum* also showed high rates of *P. expansum* and *B. cinerea* inhibition using VOCs (Chen et al., 2018).

2. Cell wall- lytic enzymes and proteases

Cell wall-lytic enzymes (chitinases, β-glucanases) and proteases with antimicrobial activity are produced by yeast species belonging mainly to *Aerobasidium*, *Pichia*, *Hanseniaspora*, *Metschnikowia* and *Rhodotorula* genera. The antimicrobial activity of the chitinases is based on their ability to hydrolyze the β-(1-4) linkages in the chitin and is related to the fact that filamentous fungi present more than 20% chitin in their cell wall and hyphae (Hartl et al., 2012). The β-glucanases involved in antimicrobial activity are mostly exo-β-1-3-glucanases that break β-1-3-glucans, while the proteases are involved in various physiological and metabolic functions from cell division and signal transduction to apoptosis.

A. pullulans is one of the species able to produce chitinases, CMCase (carboxymethyl cellulase), pectinases and β-1,3-glucanases (responsible for hydrolyzing the laminarin in fungal cell wall) (Chen et al., 2018). Some strains produced an extracellular alkaline serine protease of 415 amino-acids (M=42.9 kDa) encoded by the gene *ALP5*, which inhibited the mycelia growth of *Monilia* *laxa* on plums, peaches and stone fruits, and *B. cinerea* and *P. expansum* on apples and pomme fruits (Zhang et al., 2012).

M. pulcherrima and *M. fructicola* strains were able to secrete high levels of chitinases in presence of cell wall fractions of *B. cinerea*, respectively, *Monilinia fructicola*. The *M. fructicola* chitinase is encoded by *MfChi* gene, has 365 amino and a molecular weight of 40.9 kDa, and a recombinant protein obtained in *Pichia pastoris* cells showed high impact on fungi spore germination and germ tube length (Banani et al., 2015).

R. mucilaginosa produced lytic enzymes active against *Colletotrichum gloeosporioides* from papaya, while *Rhodotorula minuta* and *S. cerevisiae* were able to produce β-1-3-glucanases. Sometimes, the ability of yeast species to act as antimicrobial agents is based on the synthesis of more than one type of lytic enzymes (chitinases, β-1,3 and β-1,6-glucanases, proteases or mannanases) which perform thus a more efficient attack against the cell wall of phytopathogenic fungi. For exemple, multiple lytic enzymes were described as responsible for the activity of *R. mucilaginosa* and *Candida famata* against *C. gloeosporioides* producing postharvest anthracnose in papaya fruits (Muccilli and Restuccia, 2015; Ferazz et al., 2016).

Pichia guilliermondii strains revealed chitinase and β-1,3-glucanase activity against *B. cinerea* when grown in presence of fungal cell wall and sucrose or glucose (Ferazz et al., 2016). *In vitro* studies using a recombinant *P. guilliermondii* β-1,3-glucanase and *E. coli* host cells, showed that the main mechanism of action relies in reduction of *B. cinerea* germ tube length (Papon et al., 2013). Also, several strains of *P. guilliermondii* were described as biocontrol agents against *P. expansum*, *P. italicum*, *P. digitatum*, *B. cinerea*, *C. capsici*, *Rhizopus stolonifer*, *Rhizopus nigricans* and *Botryodiplodia theobromae*. *P. guilliermondii* and *P. membranefaciens* also had β-1,3-glucanase and chitinase activities against *R. stolonifer* on harvested nectarine and peach fruits (Fan et al., 2002).

3. Biosurfactants

The biosurfactants are amphiphilic compounds produced by different microbial strains in order to increase the solubility of hydrophobic compounds. Such compounds are widely used in bioremediation, agriculture, food and pharmaceutical industry mainly due to their safety characteristics. The biosurfactants have low toxicity, high biodegradability and high stability in stress conditions induced by variation of physical parameters such as pH, temperature, ionic strength (Fakruddin 2012). These unique metabolic molecules produced by a wide range of microorganism present both hydrophilic and hydrophobic moieties being able to reduce surface/interfacial tension. Many yeasts species such as *Yarrowia lipolytica, Candida apicola, C. tropicalis, Starmerella bombicola, Pseudozyma antarctica*, are able to synthetize biosurfactants as an efficient mechanism for adapting to growth conditions. Although at the beginning biosurfactants were mainly used in bioremediation processes, recently they became an important area of interest for biomedical purpose due to their ability to act as anti-adhesive and antimicrobial agents.

Sophorolipids (SLP), a major class of biosurfactants, are produced as a mixture of structurally related molecules (lactonic and acidic form) synthetized by several yeast species (*C. bombicola* (de Rientso et al., 2015), *Candida. batistae* (Konishi et al., 2008), *Candida riodocensis* (Kurtzman et al., 2010), *Candida kuoi* (Kurtzman et al., 2012), *P. anomala* (Thaniyavarn et al., 2008), *Cyberlindnera samutprakarnensis* (Poomtien et al., 2013) and *Wickerhamiella domercqiae* (Chen et al., 2006)). The lactonic form of SLP exhibit biocidal activities (being intensively studied for their biomedical potential) while the acidic form is a better foaming agent (with potential use in food and cosmetic industries). The biocidal activity of SLP is due to their property to destabilize and to alter the permeability of the cellular membrane of microbial cells. Depending on the chemical structure of the wall of the pathogenic microorganisms and the composition of the obtained sophorolipids mixture, different degrees of inhibition were observed (de Oliveira et al., 2015; Archana et al., 2019). The acetylated lactonic form of SLP inhibits growth of Gram positive bacteria such as *B. subtilis, S. epidermidis, Streptococcus faecium and Propionibacterium acnes*. Lauryl alcohol-derived sophorolipids inhibit growth of pathogenic Gram negative bacteria (*E. coli*, *P. aeruginosa*) by causing the cells shrinking or by inducing irregularities on the cell surface, and of Gram positive bacteria (*S. aureus* and *B. subtilis*) by destabilizing the bacterial wall (Dengle-Pulate et al., 2014). Other studies have shown that this type of SLP can be used as adjuvants in antibiotics treatment by enhancing their activity. SLP also increase antifungal activity of polyhexamethylene biguanide, a polymer used as disinfectant for preventing *Trichophyton rubrum*, *Trichophyton mentagrophytes* and *Tinea pedis* infections (Sanada et al., 2014).

Mannosylerythritol lipids (MELs) represent a different class of biosurfactants produced by *Ustilago* and *Pseudozyma* strains. Depending on the number of acetyl groups attached to the hydrophilic moiety MELs were separated into 4 main categories. Of these, MELs A and B exhibits high antibacterial activity against a variety of Gram positive bacteria but week activity against Gram negative bacteria. A study conducted by Nashida et al., (2018) showed that different MELs with 4-O-β-D-mannopyranosyl-D-erythritol structures obtained at lab scale exhibit high antimicrobial activity against multidrug-resistant bacteria such as methicillin-resistant *S. aureus* and vancomycin resistant *Enterococci* (*E.faecalis* and *E. faecium*).

A worldwide challenge is represented by the pathogenic microbial biofilm that is a consortium of microbial cells preserved by a polymeric matrix which acting as a barrier against chemical antimicrobial drugs and the protective mechanisms of the host immune systems (Paraszkiewicz et al., 2019). The formation of biofilms usually starts as soon as a biomedical device (catheters, prosthesis) is implanted in the body of a patient exposing him to the risk of getting a difficult to treat infection (Epstain et al., 2011; Satpute et al., 2016). Several studies have reported that biosurfactants can be very effective as biofilm formation inhibitors or as agents for controlling/disruption of already existent biofilms (Banat et al., 2014).

Although the mechanism of anti-adhesive action of biosurfactants is not well understood it seems that the adsorption of biosurfactants on the abiotic surface changes its hydrophobicity interfering thus with the early stages of biofilm formation. Biosurfactants produced by *Candida sphaerica*, lunasan (Luna et al., 2011), showed antimicrobial activity against *Streptococcus oralis*, *Staphylococcus epidermidis* and *Candida albicans* and antiadhesive activity against *P. aeruginosa*, *Streptococcus agalactiae* and *Streptococcus sanguis*. Also rufisan, a biosurfactant produced by *Y. lipolytica*, is an effective anti-adhesive biocompound against *Streptococcus mutans*, *S. aureus* and *S. agalactiae* (Rufino et al., 2013).

Present limitations and future prospective

The interest in using yeasts as antimicrobial agents for biocontrol and biomedical applications implies a deeper knowledge on the limitations and perspectives of the processes.

In present, a number of compounds comprising yeasts with antifungal activity are produced in order to control different phytopathogens, such as gray mold caused by *B. cinerea*: Shemer (based on *M. fructicola*), produced by Bayer/Koppert Biological Systems (Neteherlands, Germany), Candifruit (based on *C. sake*) produced by IRTA (Spain), Boni protect/Botector (based on *A. pullulans*) produced by Bio-Ferm/Manica (Austria) or Nexy (based on *Candida oleophila*) produced by Lasaffre (France) (Romanazzi et al., 2016).

Although using yeast as biocontrol agents might represent an ecological solution for the fungicides, there are still some problems to be solved. For instance, in order to be efficient, the biocontrol agent must be applied as soon as possible after fruits harvesting, on fruits with no pre-existing infections, preserved in a proper environment. Also, the concentration of the agents and their storage are important factors. The antagonistic agents act on the surface of the plants/fruits in a microenvironment, in correlation with other microorganisms representing the native microbiota, their activity being influenced by the climatic conditions.

In order to augment the biocontrol effect of the yeasts, the surface of the plants/fruits can undergo thermal, chemical or radiation treatments and various compounds (salts, alcohols, surfactants) can be added in order to modify the pH and nutritional substrate of the infection site or to enhance the antimicrobial activity of the yeasts. Thus, sodium bicarbonate was used to enhance the antifungal activity of *Cryptococcus laurentii* and *Trichosopron pullulans* (Yao et al., 2004), sodium bicarbonate and calcium chloride augmented the antimicrobial activity *M. pulcherrima* against fungi as well as human pathogenic *Candida* strains (Csutak et al., 2013), while microwave treatements of the wounds on the surface of the fruits also had a positive influence on the antifungal activity of *M. pulcherrima* against *Penicillium citrinum* (Guo et al., 2016). Ochratoxin synthesis by *A. ochraceus* was highly reduced when the fungus was exposed to volatile compunds produced by *P. anomala*, *P. kluyveri* and *H. uvarum* grown on malt yeast glucose peptone medium (Masoud et al., 2005). The accurate identification of the volatile compounds responsible for the antimicrobial activity and the optimization of synthesis parameters are necessary in order to enhance the impact of these compounds on phytopathogens and also for obtaining artificial mixtures of volatile compounds with high efficiency and non-specific reduced (Farbo et al., 2018).

In what concerns the killer toxins, they are very sensitive to environmental conditions, most of them acting at acid pH values and approximately 20°C. Therefore, their use in medicine implies special requirements. However, addition of killer toxins to buffered solutions is used for treatment of skin and mucosal membranes candidiasis (Marquina et al., 2002). Due to their antigenic properties, the killer toxins cannot be used as antibiotics, but anti-idiotypic antibodies were obtained, presenting similar effect against *C. albicans* infections as *P. anomala* killer toxins. The growth media might also influence the production of killer toxins, the addition of glucose augmenting the production of killer toxins in *P. anomala* (Passoth et al., 2006).

On the other side, the production of carotenoids in “red yeasts” is influenced by the temperature and regulated by light and presence of metal ions, and can be improved by UV or chemical mutagenesis (Tang et al., 2019).

Conclusions

Antimicrobial activity against human or food borne pathogens is a remarkable feature of some yeasts recommending them for biocontrol or biomedical use. The diversity of mechanisms (killer toxin production, substrate competition, secondary metabolites production such as: carotenoids, pigments, biosurfactants, volatile compounds, enzymes) involved in the antimicrobial activity of yeasts represent an advantage for using these microorganisms in developing new bio-based products for food preservation, biocontrol or as new drugs for human infections treatment.

In present, the pollution level has reached highest rates. As a consequence, most of the legal provisions are directed towards finding ecological alternatives for the chemicals used in the numerous industrial processes.

Although there are many problems waiting to be solved prior using yeasts as antimicrobial agents, there are numerous yeast based products already commercialized and successfully used for food preservation or crops biocontrol. Another problem of humanity is the inability to combat infections caused by multidrug resistant microorganisms. Since a mechanism of antibiotic-resistant gene transfer between yeasts and other microorganisms has not yet been described, yeasts might represent the safest alternative for probiotic development or growth inhibition of human pathogens. Therefore, the study of yeasts with antimicrobial potential is and will remain a priority research direction.

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