The Eurasia Proceedings of Science, Technology, Engineering & Mathematics (EPSTEM)
ISSN: 2602-3199

The Eurasia Proceedings of Science, Technology, Engineering & Mathematics (EPSTEM), 2018

Volume 3, Pages 1-10

ICVALS 2018: International Conference on Veterinary, Agriculture and Life Science

Candida vanderwaltii CMGB-ST1 from Peony Petals – Identification and Biotechnological Potential

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Abstract: Yeasts are an important group of microorganisms with a wide range of biotechnological applications, able to synthetize various enzymes (lipases, proteases) and chemical compounds (pigments, flavor precursors). Many yeast strains are used for biocontrol in agriculture or for biomedical applications due to their ability to inhibit the growth of pathogenic microorganisms isolated from crops or nosocomial infections. *Candida vanderwaltii* is one of the yeast species isolated from natural environment with multiple, yet unexplored, applications. The present work deals with the identification and characterization of the new yeast strain CMGB-ST1 isolated from peony (*Paeonia lactiflora*) petals from Bucharest Botanical Garden (Romania). The conventional tests (classic, BIOLOG system) and PCR-RFLP analyses of the ITS1-5,8S-ITS2 region showed that CMGB-ST1 belongs to *Candida vanderwaltii* species. Further tests were performed for determination of lipase production on optimized media with trybutirin as sole carbon source supplemented with Tween 80. The strain *C. vanderwaltii* CMGB-ST1 showed good lipolytic activity forming clear halos after only three days of incubation. The mechanism of trybutyrin assimilation was studied using two different liquid media containing trybutirin and inorganic nitrogen source and trybutirin-glucose as carbon source and organic nitrogen source. The results showed that lipase synthesis during the first three days of monitoring is mainly influenced by the carbon source because adding glucose increases the growth rate of the yeast cells. *C. vanderwaltii* CMGB-ST1 had also high antimicrobial activity against pathogenic and industrial *Candida* strains in presence of low pH values. In conclusion, the newly characterized strain *C. vanderwaltii* CMGB-G1 isolated from peony petals proved important potential for biotechnological and biomedical applications.

Keywords: *Candida vanderwaltii*, Antimicrobial activity, Lipolytic activity

Introduction

The yeasts have an important biotechnological potential. Yeasts are eukaryotic microorganisms highly used in many areas related to human activity: for the production of fermented foods or synthesis of various compounds used at different stages of food processing (Love et al., 2018), in medicine for obtaining probiotic compounds (Arévalo-Villena, 2018) or for pharmaceutical use, in the chemical and cosmetic industry, in bioremediation and biocontrol studies (Cristobal-Sarramian and Atzmuller, 2018; Mattanovich et al., 2014).

At European level, a special emphasis is given to studies aimed to replacing biotechnological processes that have the secondary effect of polluting the environment with their ecological alternatives, such as the increase in agricultural soil pollution due to the excessive use of chemical pesticides, fungicides and herbicides.

Until recently, fungicide treatments have been the main technique used to control postharvesting disease of crops, fruits or vegetables. However, most of them are chemical compounds that can affect human health and represent a threat to the environment. Therefore, there is a growing interest for developing healthier and ecological alternatives for these treatments (Droby et al., 2009). The yeasts appear to be the most promising biocontrol agents due to their antimicrobial mechanisms which involve nutrient competition, direct interaction with the pathogens or induction of host defence. None of these mechanisms are as dangerous as using classical fungicide treatments. Also, yeasts do not produce allergenic spores or toxins, cannot colonize dried surfaces for long periods of time and also yeasts are capable of adapting to hostile environment, these characteristics are the most prevalent advantages as using yeasts as biocontrol agents. Moreover, yeasts cannot participate to the exchange of antibiotic resistance genes. The antimicrobial activity of yeasts is also important for developing biomedical approaches. Many studies are based on using yeast strains as producers of antimicrobial compounds used against colonization of the surgical instruments by pathogenic microorganisms (Rodrigues et al., 2006).

Since the ‘80s many yeast species with antimicrobial activity have been identified and characterized (Csutak 2014). Most of them have been isolated from the surface of the fruits or vegetables, *Metschinikowia pulcherrima* being one of the most known yeast species with biocontrol applications. Other yeast species with antimicrobial activity are: *Cryptococcus albidus*, *Cryptococcus laurentii*, *Debaryomyces hansenii*, *Pichia burtonii*, *Pichia guilliermondii*, *Pichia membranaefaciens*, *Rhodotorula glutinis* and last but not least *Saccharomyces cerevisiae* (Belda et al., 2017; Feraz et al., 2016; Bajaj et al., 2013). *Candida vanderwaltii* was isolated for the first time in 1966 from winery equipment (Kurtzman, 2011). Since then, only few studies have focused on determining the biotechnological potential of this species, and only one reported the antifungal activity of a *C. vanderwaltii* strain isolated from table grapes (Lima et al., 1997).

Our study focused on the preliminary identification and characterization of a new strain CMGB-ST1 isolated from peony (*Paeonia lactiflora*) petals. The biotechnological potential of this strain as biocontrol agent and as lipases producer was also investigated.

Method

Yeast strains and media

The yeast strain CMGB-ST1 was isolated from peony (*Paeonia lactiflora*) petals from Bucharest Botanical Garden (Romania) and maintained in a Revco Legaci^{TM} Refrigeration System (Copeland, U.K) at -70°C on Yeast Peptone Glucose (YPG) medium (0.5% yeast extract, 1% peptone, 0.2% glucose) supplemented with 20% glycerol. The new yeast strain was included in the Collection of the Microorganisms of the Department of Genetics from the Faculty of Biology, University of Bucharest, Romania. In this study there were also used reference yeast strains (*Candida albicans* ATCC 10231, *Candida parapsilosis* CBS604) and pathogenic / potential pathogenic yeast strain provided by the Collection of the Microorganisms of the Department of Genetics from the Faculty of Biology, University of Bucharest, Romania (CMGB): *Candida parapsilosis* CMGB79 *Candida tropicalis* CMGB165, *Candida tropicalis* CMGB114, *Candida krusei* CMGB 94, *Candida albicans* CMGB-Y1; *Candida parapsilosis* CMGB-Y3; *Candida tropicalis* CMGB-Y8, *Candida catenulata* CMGB-Y7, *Candida krusei* CMGB-Y8; *Yarrowia lipolytica* CMGB32 and *Saccharomyces cerevisiae* CMGB-RC.

Morpho-physiological tests

The aspect of the CMGB-ST1 colonies was observed using a stereomicroscope SZM-1 (Optika Microscopes, Italy) after 24 and 48 hours of growth on YPGA medium (YPG medium supplemented with 2% agar) at 28°C. The shape of the yeast cells and the budding type were observed using an optical microscope (MICROS, Austria) after 24 hours of incubation.

A series of conventional taxonomy tests were used in order to preliminary identify the newly isolated yeast strain CMGB-ST1 (Barnett et al., 1983). First, it was determined the ability of the yeast strain to grow on YPGA medium at different temperatures: 20, 28, 37 and 42°C. Also it was determined the ability of growth under

osmotic stress on YPGA medium supplemented with 50%- 60% glucose. Urease test was also performed using as positive reference the yeast strain *Yarrowia lipolytica* CMGB32 and as negative reference *Saccharomyces cerevisiae* CMGB-RC. A positive result is represented by the changing of the color of the medium from yellow/cream to pink. The results of the conventional taxonomy test were determined in all three cases after 24, 48, 72 hours and 1, 2, 3 weeks of incubation.

For more accurate physiological identification, we used the Biolog Microbial ID System according to the producers' indications. The results were recorded after 48 and 72 hours of incubation.

Genomic DNA isolation and purification

Genomic DNA isolation and purification was performed using a protocol described by Csutak et al., 2014. An overnight colony of CMGB-ST1 grown on YPGA medium was resuspended in 100µl of lithium chloride solution (0.8%LiCl and 1%SDS) and the spheroplasts were obtained after stirring and incubation for 15 minutes at 70°C. At the end of the incubation time, the suspension was cooled down until it reached the room temperature and 300µl of cold 100% ethanol were added. The suspension was centrifuged (5 minutes at 13000 rpm, room temperature) and the DNA represented by the sediment was washed with 70% ethanol. After centrifugation (5 minutes at 13000 rpm, room temperature) the sediment was dried for about 20 minutes and resuspended in 40 µL TER (Tris 25mM, EDTA 10mM, glucose 50mM, pH 8, RN-ase 40 µg/ml). After the final centrifugation (3 minutes, 13000 rpm, room temperature) the supernatant containing the genomic DNA was stored at 4°C for short period of time and after that at -20°C.

The quality of the DNA extract was determined by gel electrophoresis (0.8% agarose in 0.5X Tris-Borate-EDTA (TBE) and the concentration and purity of the extract was determined using a NanoVue Plus spectrophotometer.

Analysis of the ITS1-5,8S-ITS2 Amplicons

The analysis of the ITS1-5,8S-ITS2 region was performed according to Csutak et al. 2014. The genomic DNA previously isolated was amplified using the PCR program: initial denaturation 5 min at 94°C, 40 cycles which consist of 1 min at 94°C, 30 sec at 55°C and 2 min 72°C followed by a final extension 5 min at 72 °C. In a final 50 µl volume of reaction mix were added: 1 µl of genomic DNA, 25 µl of Thermo Scientific Dream Taq Green PCR Master Mix (2X) and 0,3 µl of each primer (ITS1:5'-TCCGTAGGTGAACCTGCGG-3' and ITS4: 5'-TCCTCCGCTTATTGATATGC-3') in a final concentration of 60pM. 6 µl of amplicons were further digested with 0,5 µl of *Cfo* I (5'-GCG/C-3'), *Hinf* I (5'-G/ANTC-3'), *Hae* III (5'-GG/CC-3'), *Msp* I (5'-C/CGG-3') endonucleases (10U/µl, Promega) in a 12 µl final volume of reaction and incubated 1.5 hours at 37°C. Digested fragments were visualized by gel electrophoresis using 1.7% agarose in TBE 0.5X. The size of the amplicons and restriction fragments was determined using Quantity One program (Bio-Rad).

Screening for Lipase Production

For the evaluation of lipase production, the yeast strain was grown on YPGA plates overnight and a loop of culture was placed on YPTA medium (0.7% Yeast Nitrogen Base with amino-acids, 0,5% ammonium sulphate $(NH_4)_2SO_4$ 0.5% tributyrin, 0.0125% Tween 80 and 2% agar, pH-6.8) and incubated at three different temperatures (20, 28, 37°C). A positive result appeared as a clear halo surrounding the yeast colony. For the qualitative evaluation of the lipolytic activity it was determined the ratio between the measured diameter of the halo and the diameter of the colony. When the ratio is equal to 1 then the yeast strain cannot hydrolyze the tributyrin and therefore cannot produce lipases. When the ratio is greater than 1 then the yeast strain is able to produce lipases and to hydrolyze tributyrin (Corbu et al, 2017).

Influence of the Growth Media on the Lipolytic Activity

In order to optimize the production of lipases by the yeast strain we determined the influence of the growth media on the lipolytic activity. Lipase production was determined using a technique based on evaluating the yeast growth on three liquid media: L-SC (yeast peptone 0.3%, peptone 0.5%, 0.1% glucose, 0.5% monopotassium phosphate pH-6) L-SC tributyrin (L-SC medium supplemented with 1% tributyrin pH-6) and

YPT (the liquid form of the medium used for lipase production screening). The cell suspension (final concentration of $1x10^6$ cells/ml) was incubated at 28°C and 150 rpm. The ability of the yeast strains to produce lipase was estimated by monitoring cell growth after 24, 48, 72 hours and 1 week with a Thoma counting chamber. Also, it was observed microscopically the aspect of the cells (Csutak et al., 2016).

Antimicrobial Activity

The antimicrobial activity was assessed using two different assays. The first assay was based on nutrient competition evaluating the antimicrobial effect of the yeast strain CMGB-ST1 against potential pathogenic *Candida* strains: *C. albicans* ATCC10231, *C. krusei* CMGB94, *C. parapsilosis* CMGB79, *C. parapsilosis* CBS604, *C. tropicalis* CMGB165, *C. tropicalis* CMGB 114, respectively, pathogenic *Candida* strains isolated from nosocomial infections*: C. albicans* Y13, *C. parapsilosis* Y3, *C. krusei* Y8, *C. catenulata* Y7. The *Candida* strains were grown in YPG broth for 20 hours, at 28°C and 150 rpm and 10^7 cells/ml cells were spread on Yeast Malt Extract Agar (YMA) plates (yeast extract 0.3%, D-glucose 1%, peptone 5%, malt extract 0.3%, agar 15%, pH 6). Then, CMGB-ST1 yeast strain was spotted on the Petri dishes and incubated at 28°C. The results were evaluated daily for 1 week and considered positive if a clear halo appeared surrounding the CMGB-ST1 yeast strain suggesting that this strain may have an advantage of using the substrate compared to the susceptible *Candida* strains.

The second assay evaluated the killer activity using a special medium (0.1M phosphate citrate buffer, 2% D-glucose, 1% yeast extract, 2% agar, 0.03% methylene blue pH 4.8) (Csutak et al., 2016). The Petri dishes with killer medium were inoculated with 10^7 cells/ml of potential sensitive *Candida* yeast strains: *C. albicans* ATCC10231, *C. krusei* CMGB94, *C. albicans* CMGB-Y13, *C. parapsilosis* CMGB-Y3 following the same protocol as previously. Thus, CMGB-ST1 yeast strain was tested for antimicrobial activity at low pH values. In this case, the positive result was considered the appearance of an inhibition halo or a zone with reduced growth of the sensitive strain surrounding the CMGB-ST1 spot. The results obtained from both assays were measured as size of the inhibition zone (Csutak et al., 2015).

Results and Discussion

Morpho-physiological and molecular identification

The taxonomical identification of a yeast strain begins with a general description of the morpho-physiological characteristics. After 48 hours of growth on YPGA medium the strain CMGB-ST1 formed white-cream smooth colonies of 1-2 mm. CMGB-ST1 cells were small and ellipsoidal (Fig.1A). The microscopical analysis revealed that CMGB-ST1 strain has multipolar budding (Fig.1B).

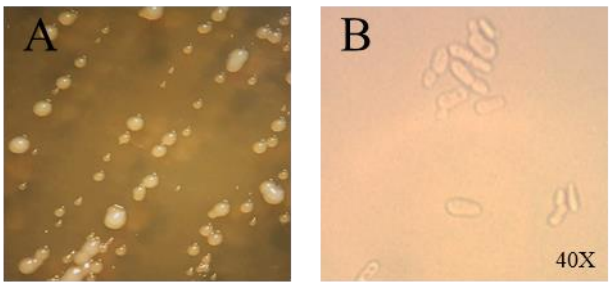

Figure 1. Colonies(A) and cells(B) of CMGB-ST1 strain

Further physiological analysis was performed for a preliminary identification of the strain. The CMGB-ST1 strain was isolated from the surface of peony petals, presenting thus an optimal growth temperature between 20-37°C (Table 1). According to Table 1, the strain CMGB-ST1 our strain showed high resistance to osmotic stress determined by high concentration of glucose in the growth medium, presenting a similar physiological profile described in Kurtzman et al., 2011 for *C. vanderwaltii* CBS 5524.

Table 1. Results of the conventional tests

Strain/species	Urease	Temperature				Osmotic stress growth	
		20°C	28°C	37°C	42°C	50% glucose	60% glucose
CMGB-ST1	-	+	+	+	-	+	+
***C. vanderwaltii* CBS 5524** (Kurtzman et al., 2011)	-	+	+	+/-	-	+	-

The urease test has a high taxonomical potential since only few yeast species are able to synthetize urease. The test revealed that CMGB-ST1 strain cannot produce urease since the color of the medium remained unchanged. This result matches previous results and indicate possible membership of the genera *Candida* and *Debaryomyces.*

In order to limit the number of species corresponding to the morphological and physiological profile obtained to this point we performed a physiological analysis using the Biolog Microbial ID System. According to Pincus et al., 2007, this system has an accuracy of 48.8% regarding taxonomical identification of yeast species. After 72 hours of incubation, the strain CMGB-ST1 showed high similarity with the reference strain *C. vanderwaltii*. The physiological characterization obtained using the Biolog Microbial ID system placed CMGB-ST1 close to *C. vanderwaltii* specie (Fig. 2).

In conclusion, the correlation of the results obtained from the classical tests with the phenotypic profile obtained from the Biolog Microbial ID system, allowed us to preliminary identify the strain CMGB-ST1 as belonging to *Candida vanderwaltii*.

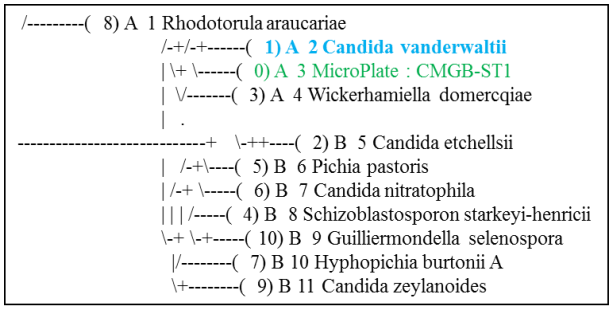

Figure 2. Phenotypic phylogeny (Biolog Microbial System)

In order to obtain a more accurate taxonomical classification, we performed PCR-RFLP analyses on ITS1-5,8S-ITS2 rDNA region. The genomic DNA isolated from CMGB-ST1 strain was amplified using ITS1 and ITS4 primers. The amplicon had 450 pb and was further digested with 4 endonucleases: *Cfo* I, *Hae* III, *Hinf* I and *Msp* I. The agarose gel electrophoresis (Fig. 3) revealed 3 restriction fragments for the amplicons digested with *Cfo* I ranging from 60 to 190 pb, 2 with *Hae* III and *Hinf* I and no fragments when the amplicons were digested with *Msp* I (Table 2).

We compared this pattern with those described for other *C. vanderwaltii* strains (Esteve-Zarzoso et al., 1999). Since there are only few studies concerning the PCR-RFLP analyses of this region in case of *C. vanderwaltii* we performed a theoretical digestion of a sequence included in the NCBI nucleotide database (EU 443388 accession number) using RestrictionMapper- free molecular biology resources. The pattern of restriction is highly similar for our strain and *C. vandervwaltii* CECT 11169, and the differences could be due to strain specificity or to the technique used for amplification. In terms of the differences between the *C. vanderwaltii* CMGB-ST1 restriction profile and the theoretical profile of the *C. vanderwaltii* CBS 5524 they are determined by the use of a partial sequence of the ITS1-5.8S-ITS2 region for the *C. vanderwaltii* CBS 5524.

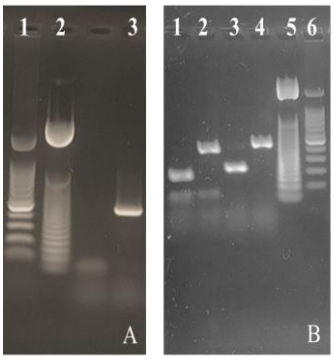

Figure 3. PCR-RFLP of the ITS1-5.8S-ITS2:
Legend: A-1/B-6 BenchTop 100- bp DNA Ladder (Promega); A-2/B-5 50 bp DNA Step ladder (Promega) A-3 undigested amplicons; B-1 *Cfo*I, B-2 *Hae* III; B-3-*Hinf* I; 4-*Msp* I

Table 2. Amplicon and restriction fragments size from ITS1-5.8S-ITS2

Strain/Specie	**Amplicon (pb)**	**Restriction fragments (pb)**			
		***Cfo*I**	***Hae*III**	***Hinf*I**	***Msp*I**
***C. vanderwaltii* CMGB-ST1**	450	60, 180, 190	60, 400	230, 235	450
C. vanderwaltii CECT 11169 (Esteve-Zarzoso et al., 1999)	480	100, 150, 225	480	240, 240	-
C. vanderwaltii CBS 5524 (EU 443388)	389	201, 119, 56,13	389	228, 161	389

Screening for the Biotechnological Potential of the *C. vanderwaltii* CMGB-ST1

Lipolytic Activity

The biotechnological potential of *C. vanderwaltii* is still poorly described. Therefore, we tested our strain for lipase production. The lipolytic activity was first evaluated by determining the ability of hydrolyzing tributyrin to glycerol and butyric acid when the strain is grown on a specific medium containing 0.5% tributyrin and 0.0125% Tween 80. Tributyrin is an ester composed by 3 molecules of butyric acid and 1 molecule of glycerol. The butyric acid, a short chain fatty acid, is known as exhibiting strong inhibitory effect on cells growth. This fatty acid has high probiotic importance being the main energy source consumed by colonocytes (Heidor et al., 2012). Also, some studies described the butyric acid as a valuable anticarcinogen drug being able to inhibit the evolution of several types of cancers (Kuefer et al., 2004). Therefore, tributyrin hidrolyses is extremely important for biomedicine.

Whereas the optimal temperature of growth in CMGB-ST1 was between 20 and 37 °C, the lipase screening test was performed at three different values corresponding to the temperature value most used in biotechnological processes (20°C), the optimal temperature for yeasts growth (28°C) and the temperature of the human body (37°C), this value being important for biomedical applications. Best results were obtained after 7 days of incubation at 28°C (Tabel 3, Fig.4). This temperature is the optimal temperature for yeast growth and the cells cells are not subject to thermal stress that may influence the production of lipases. At 20°C the ratio TH/CC remains constant after 4 and 7 days of incubation. This is very important for the biotechnological use of the yeast strain, the lipases production being constant in time. At 37°C it can be observed a slight increase in the ratio TH/CC proving that the lipase produced has also biomedical significance. Even if there are some difference between the sizes of the opacity halos at different temperature values, *C. vanderwaltii* CMGB-ST1 produced good level of lipase in all three cases, which reveals its biotechnological importance.

Table 3. Lipase production on YPTA

Strain	**Opacity halos as ratio TH/CC (mm)**					
	4 days			**7 days**		
	20°C	28°C	37°C	20°C	28°C	37°C
***C. vanderwaltii* CMGB-ST1**	1.75	2	1.71	1.83	2.8	2.4

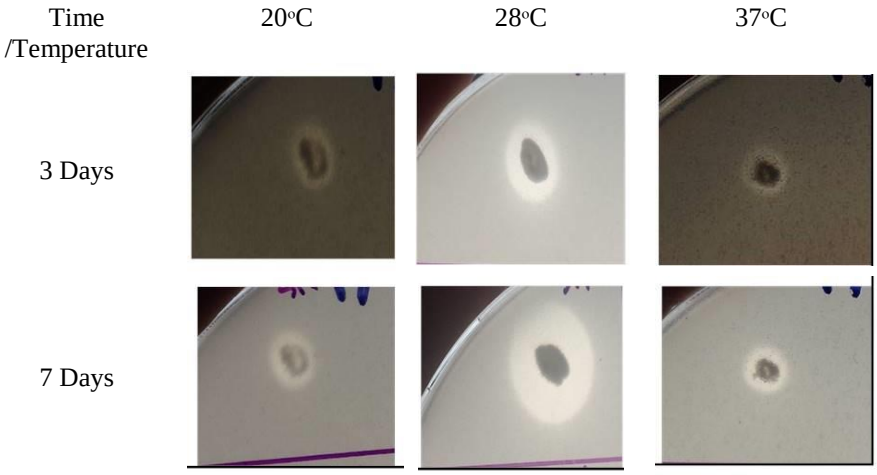

Figure 4. Aspect of the culture spot on YPTA

Since *C. vanderwaltii* CMGB-ST1 showed the best lipolytic activity at 28°C, we decided to investigate some factors that could influence lipase production, such as the nitrogen source and the carbon source. We determined the growth of the yeast strain using different media containing organic/ inorganic nitrogen source and glucose with/without tributyrin as carbon source (L-SC; L-SC tributyrin and YPT). In YPT, Tween was added in order to facilitate the hydrolysis of tributyrin, a chemical compound immiscible with water.

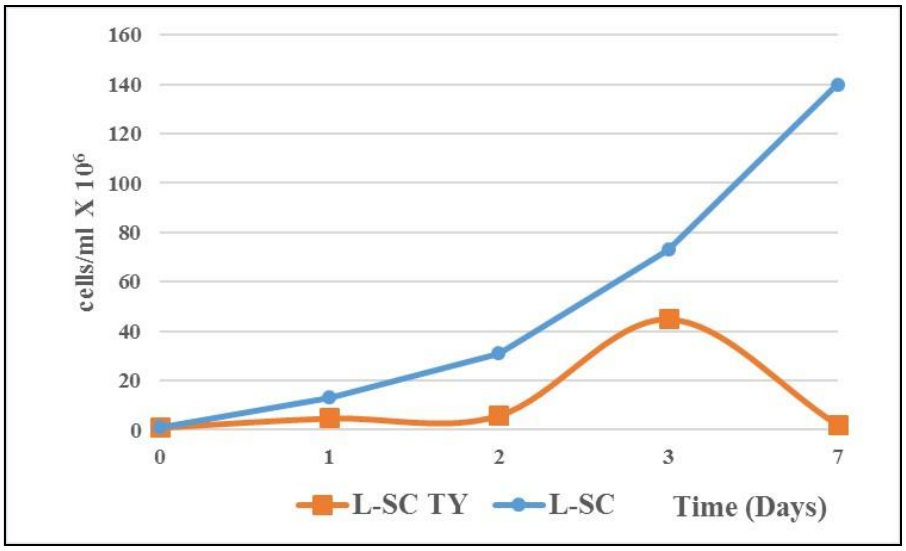

Figure 5. Cell growth on L-SC Tributyrin and L-SC

No growth was observed when CMGB-ST1 was grown on YPT even after 7 days. On the contrary, in the case of the other two media, results were interesting, and the growth dynamics was very different (Fig. 5). On L-SC medium the growth was exponential starting with the first day of incubation. On L-SC tributyrin medium the growth curve showed a lag phase during the first two days and incubation followed by an exponential growth starting with the third day. When the strain was cultivated on L-SC medium containing only glucose as unique carbon source, the growth was exponential. In the second case, the presence of tributyrin seemed to inhibit the cellular growth. It is possible that in this case the two compounds resulted from tributyrin hydrolysation might have acted as growth inhibitors. Regarding the nitrogen source, it seems that this does not have an essential role

in lipase production but further studies will be performed in this regard. The microscopical observation of the CMGB-ST1 strain grown on L-SC and L-SC-tributyrin did not indicate any significant changes in the cells.

Antagonistic Activity

The yeast antimicrobial activity can be determined mainly by: the competition for nutrients, production of compounds with antimicrobial properties such as killer toxins (Csutak et al., 2015; de Ingeniis et al., 2009), production of high quantities of ethanol or pH variations caused by organic acid production (Passoth and Schnürer, 2003; Muccilli and Restuccia, 2015). In this study we tested the antimicrobial activity of the strain using two approaches. In the first case, we used YMA, a complete medium with glucose and malt extract as carbon sources, frequently used for isolation and cultivation of a wide range of yeasts and moulds. If the strain *C. vanderwaltii* CMGB-ST1 had presented a higher assimilation ability of the nutrients from the YMA medium than the *Candida* cells, an inhibition zone would have occurred surrounding the CMGB-ST1 colony spot. The results observed after 4 and 7 days of incubation showed that *C. vanderwaltii* CMGB-ST1 strain did not inhibit *Candida* growth in the presence of YMA medium at pH=6.0.

In the second case, we used a special killer medium with a low pH value (4.8) and only glucose as carbon source. The strain *C. vanderwaltii* CMGB-ST1 showed antimicrobial activity against all four *Candida* strain after only 4 days of incubation (Tabel 4 and Fig. 6). One explanation could be that *C. wandervaltii* CMGB-ST1 is more effective as a biocontrol/ antimicrobial agent in presence of low pH values.

Potential sensitive strain	***C. albicans* ATCC 10231**		***C. albicans* CMGB- Y13**		***C. krusei* CMGB94**		***C. parapsilosis* CMGB-Y3**	
Time (days)	**4**	**7**	**4**	**7**	**4**	**7**	**4**	**7**
Size of the inhibition zone	+	+	+	+ +	+	+ +	+ +	+ +

Table 4. Antimicrobial activity of *C. vanderwaltii* CMGB-ST1
Legend: (+) inhibition zone 2-3 mm; (++) inhibition zone >4 mm

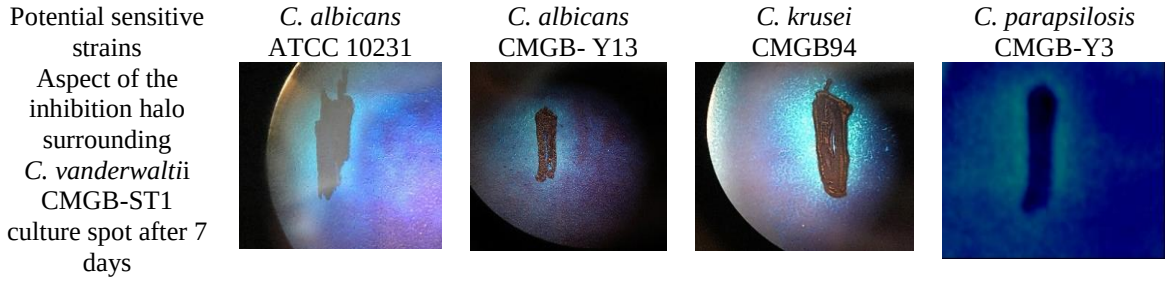

Figure 6. The aspect of the inhibition halo

The specific antimicrobial mechanism of action of *C. vanderwaltii* CMGB-ST1 remains to be studied but at this point the appearance of the inhibition halo on killer medium with low pH value may suggests a possible killer toxin production. This is very interesting, since *C. vanderwaltii* CMGB-ST1 might be efficient for protection of fruits and vegetable. According to Perez et al.(2015), yeasts with antimicrobial action at low pH values can represent an alternative for replacing traditional fungicides used for controling postharvesting disease in case of citric fruits. When citric fruits are wounded, the pH value decreases significant and the clasical fungicides become inefficient because they shift from their neutral form to the ionized form. This determines the reduction of fungicidal activity as the ionized form cannot penetrate through the membrane of the pathogens (Lopez et al., 2003).

In present, there is only one study regarding the antimicrobial activity of a *C. vanderwaltii* strain isolated from the surface of table grapes and tested on wounded fruits of strawberries, table grape berries and kiwifruits for antifungal activity against *Botrytis cinereea* and *Rhizopus stolonifer* (Lima et al., 1997).

Conclusion

The present work deals with the identification and characterization of a new yeast strain *C. vanderwaltii* CMGB-ST1 from peony flowers (Botanical Garden, Bucharest, Romania). The strain *C. vanderwaltii* CMGB-ST1 showed high potential as lipases producer and as biocontrol agent. To our knowledge, our study is the first describing the production of lipases from *C. vanderwaltii* using tributyrin as substrate. Lipase production was observed at three different temperature values (20°C, 28°C, 37°C), recommending *C. vanderwaltii* CMGB-ST1 for both biomedical and industrial applications. Moreover, *C. vanderwaltii* CMGB-ST1 shows killer activity against different pathogenic *Candida* strains indicating its potential use as antimicrobial agent against different types of human pathogens. Our future work will focus on highlighting the *C. vanderwaltii* CMGB-ST1potential as a biocontrol agent against plant pathogens and on improving its biotechnological potential.

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