Volume 6, Issue 3, 2016, 1308-1313 ISSN 2069-5837

Biointerface Research in Applied Chemistry

www.BiointerfaceResearch.com

Original Research Article Open Access Journal

Received: 25.04.2016 / Revised: 26.05.2016 / Accepted: 12.06.2016 / Published on-line: 15.06.2016

Production of biosurfactants by *Candida glabrata* CMGB35 and *Kluyveromyces* (*Nakaseomyces*) *delphensis* CMGB62 strains belonging to the *Nakaseomyces* clade

Ortansa Csutak [1,*], Alexandra Ghiţă [1], Tatiana Vassu [1]

[1]Department of Genetics, Faculty of Biology, University of Bucharest, 1-3 Aleea Portocalelor, Romania

*corresponding author e-mail address: ***cs_ortansa@yahoo.fr***

ABSTRACT

Biosurfactants represent one of the most interesting emerging research domains with high applicative potential as alternative technology for bioremediation of polluted environments. The data obtained for the ITS1-5.8S-ITS2 region allowed the molecular characterization of the strain *Candida glabrata* CMGB35. The ITS-PCR studies and sexual reproduction assays allowed the reclassification of *Candida glabrata* CMGB62 as *Kluyveromyces (Nakaseomyces) delphensis* CMGB62. The phylogenetic analysis confirmed the close evolutionary relation between *C. glabrata* CMGB35 and *K. (N.) delphensis* CMGB62, similar to the one described in *Nakaseomyces* clade studies. Biosurfactants were obtained at high rates for both strains on NH_4NO_3, yeast extract , 4% glycerol and 2% glucose. *K. (N.) delphensis* CMGB62 showed good ability to use glycerol and fried cooking sunflower oil for biosurfactant synthesis, a direct relation being observed between the delay of glycerol assimilation and the addition of glucose in the growth medium. According to our results, the carbon substrates seemed to have a higher impact than the nitrogen sources on biosurfactant production in the two strains. Two types of emulsions were observed against *n*-hexadecane and fried sunflower oil, probably due to the chemical composition of the emulsified substrate. The present work establishes the optimal growth parameters for biosurfactant production in *C. glabrata* and *K. (N.) delphensis* using carbon substrates wastes from industry (glycerol) and household (fried cooking sunflower oil).

Keywords: *Candida glabrata*, *Kluyveromyces (Nakaseomyces) delphensis, ITS-PCR, biosurfactants, glycerol, oil.*

1. INTRODUCTION

Biosurfactants are amphiphilic molecules produced by the microorganisms (bacteria, yeasts, fungi), with the ability to reduce the surface tension due their structure comprising hydrophobic and hydrophilic moieties. The best studied yeast biosurfactants are represented by sophorolipids produced by *Candida* and *Rhodotorula* species [1; 2], mannosylerytritol lipids from *Candida* (*Pseudozyma*) *antarctica* [3], liposan from *Yarrowia* (*Candida*) *lipolytica* [4], carbohydrate-protein complexes from *Rhodotorula glutinis* [5]. The mannoproteins extracted from the cell walls of *Kluyveromyces marxianus* have been mentioned in several studies [6], but there are only few data concerning the conditions required for their production [7]. Yeast biosurfactants are stable at various pH, salinity and temperatures values, having a high applicative potential in industry, biotechnology and therapeutics [8; 9; 10]. One of the advantages of using yeasts for obtaining biosurfactants, is their ability of assimilating renewable substrates as carbon and energy sources, such as industrial byproducts and wastes or petroleum hydrocarbons [11; 12].

The present study deals with the taxonomical identification and classification of two yeast strains *Candida glabrata* CMGB35 and *Kluyveromyces (Nakaseomyces) delphensis* CMGB62, and establishes the optimized parameters for biosurfactants production using as substrates compounds represented by industrial and household wastes.

2. EXPERIMENTAL SECTION

2.1. Yeast strains

The yeast strains *C. glabrata* CMGB35 and *C. glabrata* CMGB62 were previously identified using morpho-physiological methods, maintained in the Collection of Microorganisms of the Department of Genetics, Faculty of Biology, University of Bucharest, Romania (CMGB) and used as fresh cultures grown on Yeast Peptone Glucose agara (YPGA) medium (5 g/L yeast extract, 10 g/L peptone, 2 g/L glucose, 20 g/L agar-agar).

The reference yeast strains used during the study were: *C. albicans* ATCC10231, *C. boidinii* CMGB115, *C. guilliermondii* CMGB44, *C. krusei* CMGB94, *C. parapsilosis* CBS604, *C. rugosa* CMGB-CR6, *C. tropicalis* CMGB165, *Kluyveromyces lactis* CBS2359/152 (K^+R^+ a met^- [K1,K2] [2359]), *Kluyveromyces marxianus* CMGB159 and *Saccharomyces cerevisiae* D649 (MATa/MATα MAL2/ mal2 trp1/ TRP1 pet 6/ PET6 ade2/ADE2 ADE1/ade1 lys2/LYS2 HIS4/his4 LEU2/leu2).

2.2. Sexual reproduction assays

Two methods were used to determine the sexual reproduction type for the strains *C. glabrata* CMGB35 and *C. glabrata* CMGB62. For the first technique the two yeast strains and the reference strain *S. cerevisiae* D649 were grown for five days at room temperature (22°C), on Petri plates with carbonate medium (100 g/L $CaCO_3$, 10 g/L agar-agar), then the cells were stained with fuchsin and methylene blue according to [13]. The *S. cerevisiae* asci appeared coloured in blue and the ascospores in red.

In the second technique the two strains along with *K. lactis* CBS 2359/152 and *K marxianus* CMGB159 were grown for five days on YM agar (3 g/L yeast extract, 3 g/L malt extract, 5 g/L peptone, 10 g/L glucose, 20 g/L agar) at room temperature and observed at microscope. The form and number of ascospores were recorder [14].

2.3. ITS-PCR studies. Dendrogram construction

Genomic DNA was isolated according to [15] and amplified in a total volume of 50 µl using GoTaq Green Master Mix 2X (Promega), 2 µl genomic DNA, 1,2 µM of each primer ITS1 (5'TCCGTAGGTGAACCTGCGG) and ITS4 (5'TCCTCCGCTTATTGATATGC) and the the PCR program: initial denaturation 5 min at 94°C, 40 cycles of 1 min at 94°C, 30 sec at 55°C, 2 min at 72°C, and a final extension 5 min at 72°C. The amplicons were digested for 90 min with 0.5 µl of *Cfo* I (5'-GCG/C-3'), *Hae* III (5'-GG/CC-3'), *Hinf* I (5'-G/ANTC-3') and *Msp* I (5'-C/CGG-3') (10U/µl, Promega). After the gel electrophoresis in 1.5 % agarose and Tris-Borate-EDTA (TBE) 0.5X, the immages were computerized and the sizes of the amplicons and restriction fragments were determined using the program Quantity One (Bio-Rad).

The dendrogram was constructed using the profiles obtained with the endonuclease *Hinf* I and the unweighted pair group method with arithmetic mean (UPGMA) from the Quantity One program (Bio-Rad).

2.4. Biosurfactant production. Emulsification index

One colony from a 24 hours culture on YPG agar from *C. glabrata* CMGB35 and *C. glabrata* CMGB62 was incubated for 168 hours (seven days) at 28°C and 150 rpm on biosurfactant production media (BS) [16; 17] (1 g/L NH_4NO_3, 0.2 g/L KH_2PO_4, 0.2 g/L $MgSO_4 \times 7H_2O$, 3 g/L yeast extract) supplemented with 40 g/L glycerol (Sigma-Aldrich) (BS-Gly), 40 g/L glycerol and 20 g/L glucose (BS-Gly+Glc) or 40 g/L olive oil (Carl Roth) (BS-olive oil), respectively, on Yeast Peptone (YP) medium (10 g/L yeast extract, 10 g/L peptone) with 1% cooking sunflower fried oil commercially available.

The production of biosurfactant was determined after 72 hours (three days), respectively, 168 hours (seven days) according to [18] using the emulsification index (E_{24}) determined by adding equal volumes of crude biosurfactant (cell-free supernatant) and substrate (Table 2).

2.5. Interactions between the carbon substrates and yeast cells

After 168 hours cells from yeast cultures grown for biosurfactant production were observed at optic microscope (40X). The aspect of glycerol and oil droplets and presence of yeast cells were recorded.

3. RESULTS SECTION

The two yeast strains, CMGB35 and CMGB62, identified based on their morpho-physiological characteristics, as belonging to *C. glabrata* were subject to PCR-RFLP analysis of the ITS1-5.8S-ITS2 region (ITS-PCR). The data obtained for *C. glabrata* CMGB35, had some similarities with those from the other studies according to which a rather high degree of polymorphism was obtained for the same *C. glabrata* strain during different experiments [19; 20]. Therefore, for a more accurate identification of our strain, we looked for the restriction profile obtained with a rare-used restriction endonuclease: *Msp* I / *Hpa* II. Our results were highly similar to those reported by [21; 22] (Table 1). In conclusion, the molecular analysis confirmed the preliminary taxonomical classification of *C. glabrata* CMGB35.

Table 1. The size of the amplicons, number and sizeof restriction fragments for ITS1-5.8S-ITS2 region of the strains CMGB35 and CMGB62

Yeast strain	Amplicon (bp)	Fragment size (bp)			
		Cfo I	*Hae* III	*Hinf* I	*Msp* I
***C. glabrata* CMGB35**	830	390, 160	620, 210	360, 250	520, 310
***C. glabrata*/ *K. (N.) delphensis* CMGB62**	810	370, 130	330, 220,150, 110	370, 110	690, 110

The case of the strain *C. glabrata* CMGB62, was more complicated, since the ITS-PCR patterns were very different from the *C. glabrata* species, resemebling more to those mentioned for *Saccharomyces cerevisae* or *Kluyveromyces (Nakaseomyces) delphensis*. For a discriminatory characterization, we performed two techniques aimed to determine the sexual reproduction type using *C. glabrata* CMGB62, *C. glabrata* CMGB35 and the reference strains *S. cerevisiae* D649, *K. lactis* CBS 2359/152 and *K. marxianus* CMGB159. The first technique, using special growth media for the *Saccharomyces* genus, allowed to colour the asci and the ascospores for *S. cerevisiae* D649, without any results for the rest of the strains. In a second technique, the strains were grown on YMA medium and observed at microscope after five days. We could see the asci and 2, 3 ascospores for *C. glabrata* CMGB62, similar to the samples from *K. lactis* CBS 2359/152. The results suggested a possible identification of *C. glabrata* CMGB62 as *K. (N.) delphensis*.

Additionally, we decided to analyze the phylogenetic relation between *C. glabrata* CMGB62, now possibily *K. (N.) delphensis, C. glabrata* CMGB35 and other seven *Candida* strains, using the *Hinf* I digestion profiles of the ITS1-5.8S-ITS2 region (Figure 1). From the UPGMA dendrogram obtained with the Quantity One program (Figure 2), we can see that the two strains are closely related evolutionary, forming a separate branch. A comparative study with the results reported by [23; 24; 25], confirmed our previous data, allowing the reclassification of the strain *C. glabrata* CMGB62 as *K. (N.) delphensis* CMGB62. The accuracy of our work is also supported by the positions of *C.*

tropicalis CMGB165 and *C. albicans* ATCC10231 (Figure 2), corresponding to the data described by the authors mentioned above. Moreover, [26] describe *C. glabarata* as being more distant from *C. albicans* than other *Candida* species, and part of a newly-sequenced *Nakaseomyces* clade besides other 17 Saccharomycotina species.

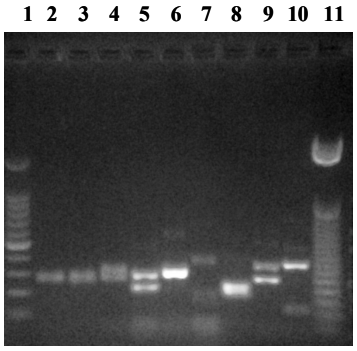

Figure 1. Gel electrophoresis of the restriction fragments obtained by digestion with *Hinf* I of the ITS1-5.8S-ITS2 regions for the strains: 1– 100 bp DNA Ladder (Promega); 2 – *C. albicans* ATCC10231; 3 – *C. parapsilosis* CBS604; 4 – *C. tropicalis* CMGB165; 5 – *C. krusei* CMGB94; 6 – *C. guilliermondii* CMGB44; 7 – *C. boidinii* CMGB115; 8 – *C. rugosa* CMGB-CR6; 9 – *C. glabrata* CMGB35; 10 - *K. (N.) delphensis* CMGB62; 11 – 50 bp DNA Step Ladder (Promega)

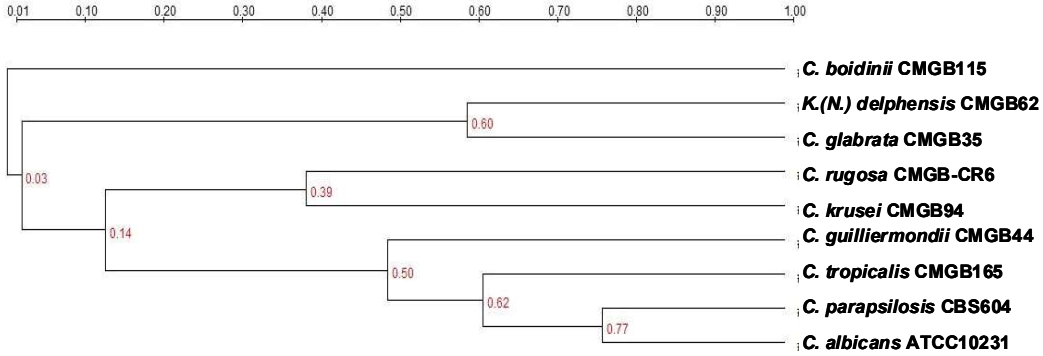

Figure 2. The UPGMA dendrogram showing the phylogenetic position of *C. glabrata* CMGB35, *K. (N.) delphensis* CMGB62 and seven *Candida* refrence strains

Biosurfactant production was evaluated using the emulsification activity of the crude biosurfactant. Two types of emulsions were observed against *n*-hexadecane and fried sunflower oil, when *C. glabrata* CMGB35 and *K. (N.) delphensis* CMGB62 were grown for 72 hours on BS-Gly+Glc medium (Figure 3). This is most probably due to the chemical composition of the substrate: long-chain carbon alkane (C_{16}), respetively, a mixture of fatty acids (approximately 30% oleic acid and 50-60% linoleic acid).

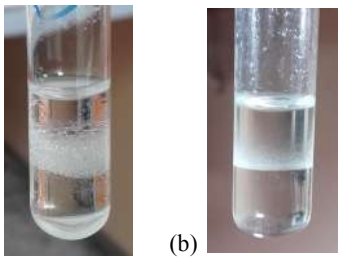

Figure 3. Types of emulsions observed for the crude biosurfactant from:
(a) *C. glabrata* CMGB35 against *n*-hexadecane;
(b) *K. (N.) delphensis* CMGB62 against fried sunflower oil

In general, the two strains showed similar levels of biosurfactant production, the lowest rates being obtained when BS-olive oil medium was used (Table 2). This indicates that, most likely, both strains use for biosurfactant synthesis preferably other fatty acids then the oleic acid (75% in the olive oil), acids which found in large amounts in the sunflower oil (60% linoleic acid compared to only 25-30% oleic acid). Moreover, similar work on *C. glabrata*, recommend the use of a mix between vegetable oils or vegetable fat waste and glucose in order to reach significant biosurfactant synthesis [17; 27].

Best rates of biosurfactants were obtained for *C. glabrata* CMGB35 on BS-Gly+Glc (Table 2), with an impressive augmentation of E_{24} against fried sunflower oil, from 6 to 46%, after 168 hours (Figure 5), and a relative constant high E_{24} against the *n*-hexadecane (around 25%) during the whole period of incubation (Figure 4). Glycerol in combination with glucose as carbon substrates, and NH_4NO_3 as nitrogen source, were also recommended for optimization of biosurfactant production within seven days (168 hours) for fungi strains isolated from coastal areas [28]. On glycerol, the production of biosurfactants in *C. glabrata* CMGB35 was reduced, without semnificative variations after the first 72 hours, as implied by the emulsification of fried sunflower oil. The results are similar to the one reported by [29] on *Candida* strains isolated from soil contaminated with petroleum oil hydrocarbons, where the biosurfactants obtained from glycerol

induced a drop of the surface tension after 24 hours of incubation, without any further variation.

Table 2. The emusifying activity of biosurfactants produced by *C. glabrata* CMGB35 and *K. (N.) delphensis* CMGB62

Strain	E_{24}% after	BS-Gly		BS-Gly+Glc		BS-olive oil	YP-fried sunflower oil
		n-hexadecane	fried sunflower oil	*n*-hexadecane	fried sunflower oil	*n*-hexadecane	*n*-hexadecane
***C. glabrata* CMGB35**	**72 h**	11	13	28	6	-	5
	168 h	5	15	21	46	15	17
***K.(N.) delphensis* CMGB62**	**72 h**	10	15	-	14	-	26
	168 h	5	54	29	32	5	28

The strain *K. (N.) delphensis* CMGB62 showed a higher ability to produce biosurfactants with emulsifying activity against various tested substrates compared to *C. glabrata* CMGB35 (Figure 4, Figure 5). According to the results (Table 2), *K. (N.) delphensis* CMGB62 prefered glycerol (Bs-Gly) for biosurfactant production, since the highest emulsification activity was obtained against the fried sunflower oil after 168 hours (54%) (Figure 5). The complete assimilation of glycerol is also supported by the microscopical observations, revealing numerous proliferating cells (Figure 6a). In contrast, it seems that in BS-Gly+Glc medium, glucose is used as first carbon source for growth and biosurfactant synthesis, the assimilation of glycerol being delayed, fact sustained by the presence of glycerol droplets surrounded by yeast cells even after 168 hours of incubation (Figure 6b). In the presence of YP-fried sunflower oil medium, the biosurfactant production seemed to be maintained at relative equal levels for the entire period of the experiment (E_{24}% values were 26 and 28 after 72, respectively, 168 hours) (Table 2), yeast cells being observed invading the oil droplets (Figure 6c). Until present, [7] mentioned lactose-based-medium for biosurfactant production in *K. marxianus*.

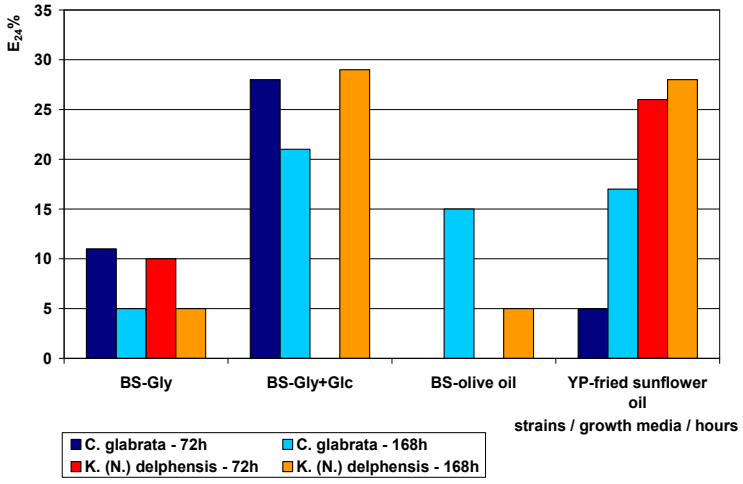

Figure 4. Emulsification of *n*-hexadecane by the biosurfactants produced after 72 and 168 hours of incubation on the tested media

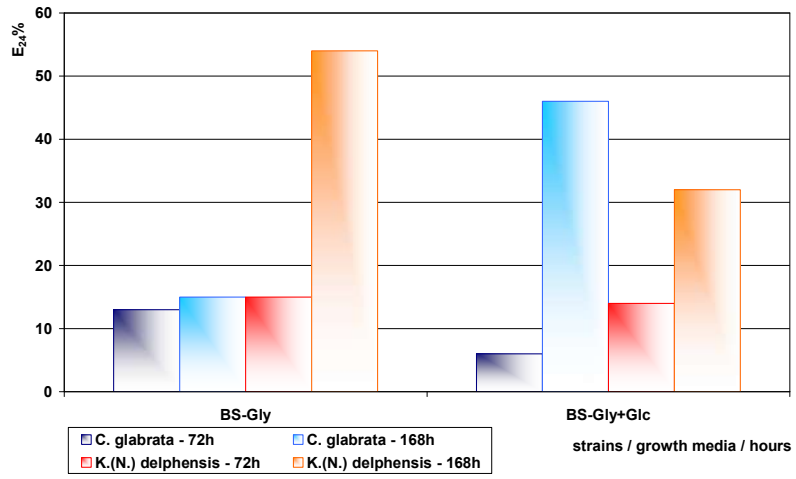

Figure 5. Emulsification of fried sunflower oil by the biosurfactants produced after 72 and 168 hours of incubation on BS-Gly and BS-Gly+Glc

Figure 6. Assimilation of carbon substrates by *K. (N.) delphensis* CMGB62 after 168 hors of incubation on: (a) BS-Gly, (b) BS-Gly+Glc, (c) YP-fried sunflower oil

Overall, in the case of *C. glabrata* CMGB35 and *K. (N.) delphensis* CMGB62, the carbon substrates represented by glycerol, glucose and fatty acids from the fried sunflower oil and the olive oil, seem to have a higher impact on biosurfactant production, rather than the nitrogen sources from the growth media, i.e. NH_4NO_3 for BS, respectively, peptone for YP medium.

4. CONCLUSIONS

Two yeast strains *C. glabrata* CMGB35 and *K. (N.) delphensis* CMGB62 were classified as belonging to the *Nakaseomyces* clade based on their ITS1-5.8S-ITS2 restriction profiles, their sexual reproduction type and phylogenetic similarities. The two strains showed good levels of biosurfactant production, with best rates in the presence of glycerol (54%) and a mix of glycerol and glucose (46%) for *K. (N.) delphensis* CMGB62, respectively, *C. glabrata* CMGB35. The results also suggest that other fatty acids (e.g. linoleic acid) than the oleic acid are preferably used for biosurfactant synthesis in the two strains. This is one of the few studies concerning biosurfactant production by *Kluyveromyces* species. The two strains show high potential for biosurfactant production using wastes as carbon substrates.

5. REFERENCES

[1] Chandran P., Das N., Characterization of sophorolipid biosurfactant produced by yeast species grown on diesel oil, *International Journal of Science Nature*, 2, 1, 63-71, **2011**.

[2] Romero M.C., Urrutia M.I., Reinoso E.H., Kiernan A.M., Effects of the sorption/desorption process on the fluoranthene degradation by wild strains of *Hansenulaangusta* and *Rhodotorula minuta, International Research Journal of Microbiology*, 2, 7, 230-236, **2011**.

[3] Morita T., Koike H., Koyama Y., Hagiwara H., Ito E., Fukuoka T., Imura T., Machida M., Kitamoto D., Genome sequence of the basidiomycetous yeast *Pseudozyma antarctica* T-34, a producer of the glycolipid biosurfactants mannosylerythritol lipids, *Genome A-ASM*, 1, 2, e00064-132013, **2013**.

[4] Cirigliano M.C., Carman G.M., Purification and characterization of liposan, a bioemulsifier from *Candida lipolytica. Applied and Environmental Microbiology*, 50, 4, 846–850, **1985**.

[5] Oloke, J. K., Glick, B. R., Production of bioemulsifier by an unusual isolate of salmon/red melanin containing *Rhodotorula glutinis, African Journal of Biotechnology* , 4, 2, 164-171, **2005**.

[6] Saharan B.S., Sahu R.K., Sharma D., A review on biosurfactants: fermentation, current developments and perspectives. *Genetic Engineering and Biotechnology Journal*, GEBJ-29, **2011**.

[7] Lukondeh T., Nicholas J. Ashbolt, Peter L. Rogers, Evaluation of *Kluyveromyces marxianus* FII 510700 grown on a lactose-based medium as a source of a natural bioemulsifier, *Journal of Industrial Microbiology* and *Biotechnology*, 30, 715–720, **2003**.

[8] Banat I.M., Franzetti A., Gandolfi I., Bestetti G., Martinotti M.G., Fracchia L., Smyth T. J.,Marchant R., Microbial biosurfactants production, applications and future potential. *Applied Microbiology and Biotechnology*, 87, 427–444, **2010**.

[9] Muthusamy K., Gopalakrishnan S., Ravi T.K., Sivachidambaram P., Biosurfactants: properties, commercial production and application. *Current Science India*, 94, 6,: 736-747, **2008**.

[10] Silva R.C.F.S., Almeida D.G., Rufino R.D., Luna J.M., Santos V.A., Sarubbo L.A., Applications of biosurfactants in the petroleum industry and the remediation of oil spills, *International Journal of Molecular Sciences*, 15, 12523-12542, **2014**.

[11] Amaral P.F.F., Coelho M.A.Z., Marrucho I.M., Coutinho J.A.P., Chapter 18: Biosurfactants from yeasts: characteristics, production and application, In Biosurfactants, *Advances in Experimental Medicine and Biology*, 672, Landes Bioscience, **2010**.

[12] Makkar R.S., Cameotra S.S, Banat I.M., Advances in utilization of renewable substrates for biosurfactant production, *AMB Express*, 1, 5, **2011**.

[13] Vassu T., Stoica I., Csutak O., Musat F., *Genetics of microorganisms and microbial genetic engineering. Theoretical aspects and laboratory techniques*, Petrion, **2001.**

[14] Kurtzman C.P., Fell J.W., Boekhout T., *The Yeasts. A Taxonomic Study*, 5th Edition. Elsevier, **2011**.

[15] Csutak O., Stoica I., Vassu T., Molecular identification and antimicrobial activity of two new *Kluyveromyces lodderae* and *Saccharomyces cerevisiae* strains, *Biointerface Research in Applied Chemistry*, 4, 873-878, **2014**.

[16] Sarubbo L.A., de Luna J.M., de Campos-Takaki G.M., Production and stability studies of the bioemulsifier obtained from a new strain of *Candida glabrata* UCP 1002, *Electronic Journal of Biotechnology*, 9, 4, 400-406, **2006**.

[17] de Luna J.M., Sarubbo L., Campos-Takaki G.M., A new biosurfactant produced *by Candida glabrata* UCP 1002: characteristics of stability and application in oil recovery, *Brazilian Archives of Biology and Technology*, 52, 4, 785–793, **2009**.

[18] Cooper D.G., Goldenberg B.G., Surface-active agents from two *Bacilllus* species, *Applied and Environmental Microbiology*, 53, 224-229, **1987**.

[19] Guillamon J. M., Sabate J., Barrio E., Cano J., Querol A., Rapid identification of wine yeast species based on RFLP analysis of the ribosomal internal transcribed spacer (ITS) region, *Archives of Microbiology*, 169, 5, 387–392, **1998**.

[20] Esteve-Zarzoso B., Belloch C., Uruburu F., Querol A., Identification of yeasts by RFLP analysis of the 5.8S rRNA gene and the two ribosomal internal transcribed spacers, *International Journal of Systematic Bacteriology*, 49, 329–337, **1999**.

[21] Mirhendi H., Makimura K., Khoramizadeh M., Yamaguchi H., A one-enzyme PCR-RFLP assay for identification of six medically important Candida species, *Japanese Journal of Medical Mycology*, 47, 225-229, **2006**.

[22] Mousavi S.A.A., Salari S., Rezaie S., Nejad N.S., Hadizadeh S., Kamyabi H., Aghasi H., Identification of *Candida* species isolated from oral colonization in Iranian HIV-positive patients, by PCR-RFLP method, *Jundishapur Journal of Microbiology*, 5, 1, 336-340, **2012**.

[23] Chen Y.C., Eisner J.D., Kattar M.M., Rassoulian-Barrett S.L., Lafe K., Yarfitz S.L., Limaye A.P., Cookson B.T., Identification of medically

important yeasts using PCR-based detection of DNA sequence polymorphisms in the Internal Transcribed Spacer 2 region of the rRNA genes, *Journal of Clinical Microbiology*, 38, 6, 2302–2310, **2000**.

[24] Wong S., Fares M.A., Zimmermann W., Butler G., Wolfe K.H., Evidence from comparative genomics for a complete sexual cycle in the 'asexual' pathogenic yeast *Candida glabrata, Genome Biology*, 4, R10, **2003**.

[25] Tsuyoshi N., Fudou R., Yamanaka S., Kozaki M., Tamang N., Thapa S., Tamang J.P., Identification of yeast strains isolated from marcha in Sikkim, a microbial starter for amylolytic fermentation, *International Journal of Food Microbiology*, 99, 135– 146, **2005**.

[26] Gabaldón T., Martin T., Marcet-Houben M., Durrens P., Bolotin-Fukuhara M., Lespinet O., Arnaise S., Boisnard S., Aguileta G., Atanasova R., et al., Comparative genomics of emerging pathogens in the *Candida glabrata* clade, *BMC Genomics*, 14, 623-630, **2013**.

[27] de Gusmão C.A.B., Rufino R.D., Sarubbo L.A., Laboratory production and characterization of a new biosurfactant from *Candida glabrata* UCP1002 cultivated in vegetable fat waste applied to the removal of hydrophobic contaminant, *World Journal of Microbiology & Biotechnology*, 26, 1683–1692, **2010**.

[28] Luepongpattana S., Jindamarakot S., Thaniyavarn S., Thaniyavarn J., Screening of biosurfactant production yeast and yeast-like fungi isolated from the coastal areas of Koh Si Chang, *The 26th Annual Meeting of the Thai Society for Biotechnology and International Conference*, Chang Rai, THAILAND, 26th-29th NOV, 468-477, **2014**.

[29] Accorsini F.R., Rossini Mutton M.J., Macedo Lemos E.G., Benincasa M., Biosurfactants production by yeasts using soybean oil and glycerol as low cost substrate, *Brazilian Journal of Microbiology*, 43, 1,116-125, **2012**.