Scientific Bulletin. Series F. Biotechnologies, Vol. XVII, 2013
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

SELECTION OF YEAST STRAINS WITH ENHANCED LIPOLYTIC ACTIVITY

Alexandra GHIORGHIȚĂ[1], Gheorghe CÂMPEANU[1], Vasilica MANEA[2], Angela CĂȘĂRICĂ[2], Călina Petruța CORNEA[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest/Center for Applied Biochemistry and Biotechnology, 59 Mărăști Blvd., 011464, District 1, Bucharest, Romania
[2]Institute for Chemical Pharmaceutical Research and Development ICCF Bucharest, 112 Calea Vitan str., 031299, District 3, Bucharest, Romania

Corresponding author email: alexandra_ghiorghita@yahoo.com

Abstract

In reaction to expanding markets and increasing demand for novel biocatalysts, commercial enzyme production has been continuingly growing during the last century. Although some enzymes are extracted from plants and animals, the major source of industrial enzymes consists in microorganisms. Obtainment of a new microbial enzyme begins with a screening of microorganisms for the desired activity, using specific methods of selection. Lipases or glycerol-ester hydrolases are carboxyl-esterases which catalyze hydrolytic cleaving of glycerol esters with fatty acids. Microorganisms are rich sources of lipases, especially yeast strains pertaining to the genus Candida. Yeast strains from the CMII-WFCC232 industrial interest strain collection of the National Institute for Chemical Pharmaceutical Research and Development ICCF Bucharest have been studied regarding their lipase production. Specific screening media have been used in order to stimulate lipolytic activity in the yeast strains. Viability and presence of opaque areas on Petri dishes containing fatty acid esters and lipase inducers as proof of enzyme activity have been determined. The strains which presented lyoplytic activity have later on been cultivated on specific liquid medium and enzymatic activity has been determined.

Keywords: *lipase, screening, transesterification, yeast.*

INTRODUCTION

Lipases (triacylglycerol hydrolases, EC 3.1.1.3) are an important group of water soluble enzymes which have as main biological function the interfacial hydrolytic catalysis of lipids (Aehle, 2007).

Due to their versatility, they present vast biotechnological relevance and applications in fats and oils industries, food and feed processing, synthesis and production of fine chemicals, detergents and various degreasing products, cosmetics and pharmaceuticals, bakery and brewing, biofuels and waste treatment (Falch, 1991; Kazlauskas et al., 1998; Villeneuve et al., 2000; Sharma et al., 2001; Babu and Rao, 2007; Hasan et al., 2006; Hasan et al., 2009; Balakrishnan et al., 2011; Kishore et al., 2011).

Enzyme catalysis using lipases has been intensely researched since the reaction takes place at regular pressure and 30-40^{o}C, achieving the necessary energy, and reducing at the same time the emergence of unwanted secondary compounds.

At low water content, theses enzymes are also able to catalyze synthesis reactions, achieving esterification, alcoholysis, interesterification, transesterification, acidolysis, aminolysis, thiotransesterification, oximolysis, in addition to their hydrolytic activity on triglycerides (Villeneuve et al., 2000; Joseph et al., 2008).

Lipases can be found in different sources, such as plants and animals, but the most abundant source is represented by microorganisms (Haki et al., 2003; Gupta et al., 2004; Rahnman et al., 2006)

Due to their wide use as biocatalysts for numerous and also novel biotechnological applications (Jaeger and Eggert, 2002), there is a continuous search for new lipase sources and intensive studies are performed to better understand their structural biochemical characteristics and mechanisms (Sirisha et al., 2010; Fickers et al., 2011; Feng et al., 2012).

The production of the extracellular lipase is affected by various factors, such as: temperature, pH, composition of the medium, agitation and aeration (Chen et al. 1999; Gupta et al. 2004; Alonso et al. 2005).
The aim of the present paper was to obtain new microbial enzymes as a result of a screening performed on lipase producing yeasts pertaining to the CMII-WFCC232 industrial interest strain collection of the National Institute for Chemical Pharmaceutical Research and Development ICCF Bucharest, registered in the World Federation of Culture Collections Directory, no. 232.

MATERIALS AND METHODS

The yeast strains that have been selected to undergo the screening for lipase production belong to the Industrial Importance Microorganism Collection (IIMC) pertaining to the National Institute for Chemical Pharmaceutical Research and Development ICCF Bucharest, registered in the World Federation of Culture Collections Directory no. 232. 16 yeast strains were taken from the vegetative stock and grown on specific media, with lipase activity inducers (Table 1).

Table 1. Strains that have been studied for lipase producing potential from the ICCF collection

Strain name	Registration
Candida arborea – CBS* 64	ICCF 193
Candida glabrata	ICCF 182
Candida famata	ICCF 181
Yarrowia lipolytica – ATCC** 16618	ICCF 214
Yarrowia lipolytica	ICCF 215
Candida boidinii-CMBG*** 221	ICCF 26
Candida parafinica	ICCF 190
Candida parafinica	ICCF 184
Candida albicans – ATCC 10231	ICCF 91
Candida utilis-CMGB 237	ICCF 191
Candida guillermondii – CMGB 229	ICCF 183
Pichia pastoris – CMGB 267	ICCF 189
Candida sp.	ICCF 315
Hansenula polymorfa-CMGB 257	ICCF 218
Hansenula subpelliculosa-CMGB 261	ICCF 187
Hansenula anomala-CMGB 243	ICCF 217

The yeast strains were grown on a specific selection medium, containing cattle suet. The cattle suet is melted down and poured on the Petri dishes, in a shallow layer, 2-3 mm thick. Once it has solidified, the specific lipase synthesis culture medium is poured on top.
The cultures are than passed on the screening medium in the form of vertical strips, using the loop.
The lipase screening medium with cattle suet contains (%g/V) : peptone 1.0; sodium chloride 0.5; calcium chloride 0.01; Tween 80 1.0; agar 2.0. The pH was corrected to 5.5 and sterilization of the medium was at 120^{o}C for 15 min.
The microorganism strains which showed synthesis of extracellular lipases have later on been grown on liquid culture medium, in order to determine enzyme activity.
The preinoculum obtained in agar YPG medium, containing (%g/V) : yeast extract 0.5; peptone 0.5; glucose 2.0; agar 2.0; pH 5.5, 120^{o}C for 15 min sterilization, after cultivation for 72 hours at 28^{o}C was used for inoculation of 50 mL liquid inoculum medium in 500 mL Erlenmeyer flasks.
The culture resulted after 24 hours at 28^{o}C (the inoculum) was used for inoculation (2% vol/vol) of 50 mL of fermentation medium in 500 mL Erlenmeyer flasks.
The inoculum and fermentation media were the same: glucose 2.0; yeast extract 1.0; peptone 0.5; ammonium sulfate 0.1; Tween 80 1.0; dipotassium phosphate 0.5; pH 7, 120^{o}C for 15 min sterilization.
The fermentation was conducted at 28^{o}C, with shaking at 250 rpm, and samples were taken every 4 hours.
PH, biomass accumulation, glucose consumption and enzyme activity were determined for each sample.
The microbial growth was determined spectrophotometrically, measuring the values of optic density at 540 nm, 1:50 dilution ratio.
Lipolytic activity from the supernatant (after biomass removal by centrifugation at 6000 rpm, for 20 min, at 4^{o}C) was determined using the titrimetric Willstätter method, using olive oil as a substrate, according to Iordachescu and Dumitru (1980). Superior fatty acids resulted from triglyceride hydrolysis, in the presence of microbial lipase, were extracted with organic solvents and titrimetrically dosed with a sodium hydroxide alcoholic solution. One lipase unit, UL_{FIP} is the mass of the enzyme, which, under standard conditions, hydrolyses the vegetal oil and leads to the release of a microequivalent of carboxyl group per minute.

RESULTS AND DISCUSSIONS

The yeasts strains have been observed on the cattle suet medium for lipase activity. The lipases synthesized by the microorganisms act on the lipids in the cattle suet, leading to soap formation (Figure 1).

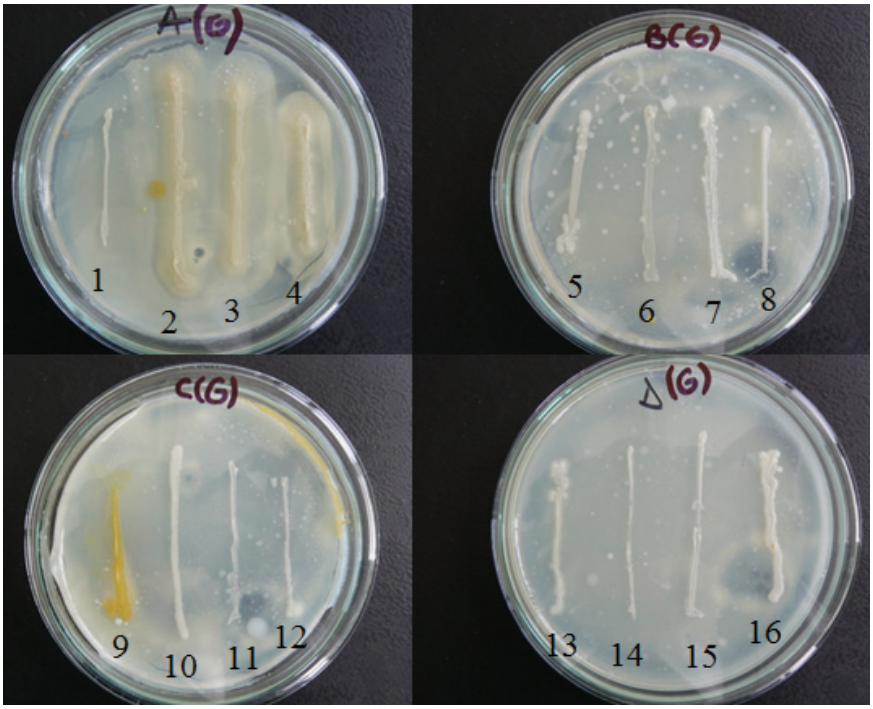

Figure 1. Lipase activity on culture medium containing cattle suet

Where:
1 = *Candida albicans* – ICCF 91
2 = *Candida parafinica* – ICCF 184
3 = *Yarrowia lipolytica* – ICCF 215
4 = *Yarrowia lipolytica* – ICCF 214
5 = *Candida parafinica* – ICCF 190
6 = *Candida boidinii* – ICCF 26
7 = *Candida famata* – ICCF 193
8 = *Candida glabrata* – ICCF 194
9 = *Hansenula anomala* ICCF 217
10 = *Hansenula subpeliculosa* ICCF 187
11 = *Hansenula polymorfa* ICCF 218
12 = *Candida arborea* ICCF 193
13 = *Candida sp.* – ICCF 315
14 = *Pichia pastoris* – ICCF 189
15 = *Candida utilis* – ICCF 191
16 = *Candida guillermondii* –ICCF 183

From the observation of the yeast strains, it can be seen that three of them showed lipolytic activity, namely *Candida paraffinica* – ICCF 184, *Yarrowia lipolytica* – ICCF 215, *Yarrowia lipolytica* – ICCF 214, and they have been further on grown on liquid fermentation medium, to determine enzyme activity.

For each of the three strains, samples were collected every 4 hours and pH, cell growth, glucose consumption and enzyme activity were determined.

The variation of the parameters and lipase yield is presented below (Figures 2 to 4).

Lipase activity was determined beginning with 16 hours of fermentation until the end of the process.

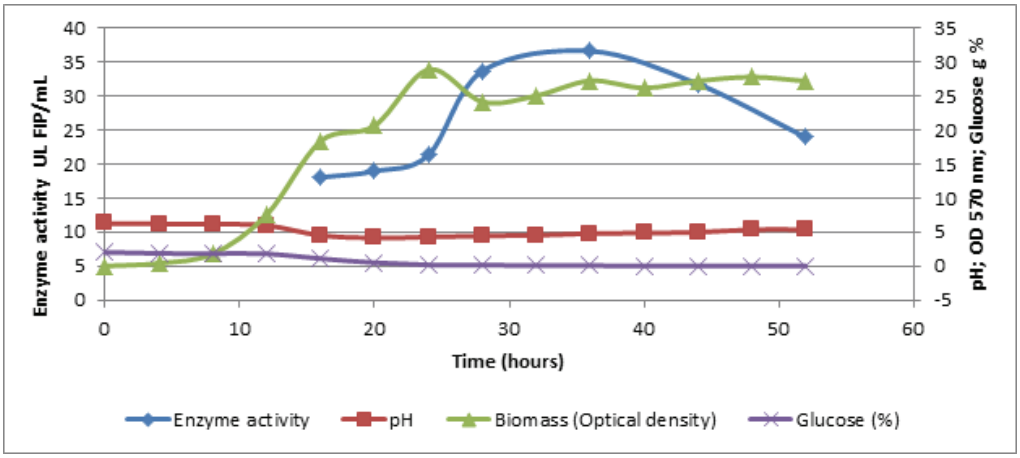

Figure 2. Variation of the main parameters during fermentation for *Yarrowia lipolytica* ICCF 214

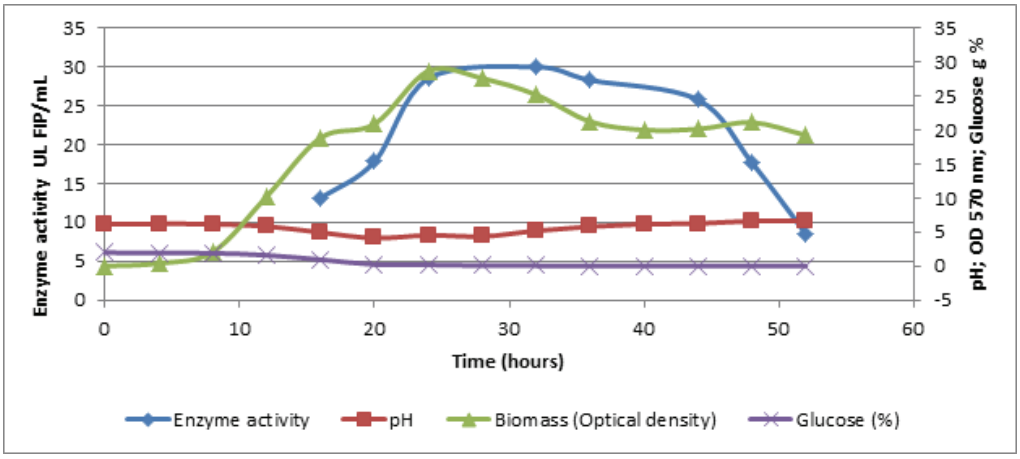

Figure 3. Variation of the main parameters during fermentation for *Yarrowia lipolytica* ICCF 215

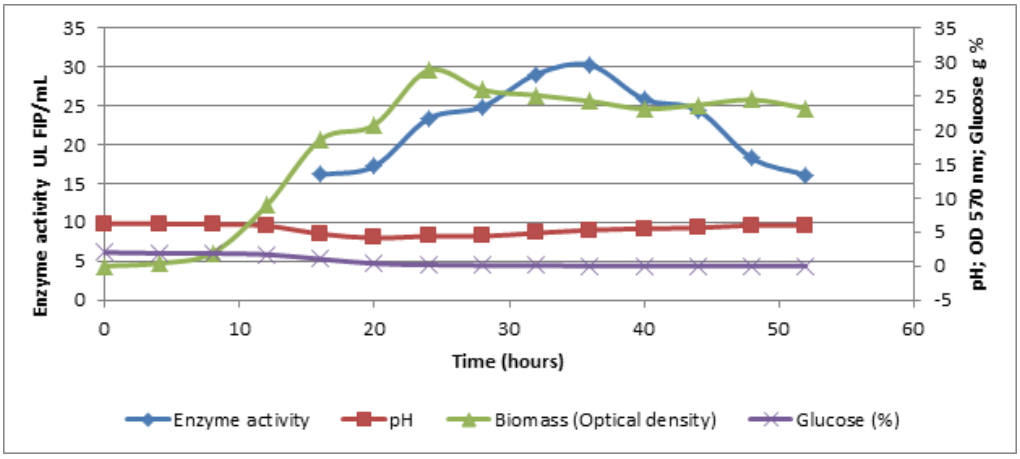

Figure 4. Variation of the main parameters during fermentation for *Candida paraffinica* ICCF 184

For all of the samples, the pH values varied during fermentation, registering a decrease from 6 to 4, between 12 and 20 process hours, coinciding with the beginning of the enzyme production.
At 24 hours, all three strains reached a maximum value for biomass growth, after which it decreased after glucose depletion.
The glucose was entirely consumed after 36 hours of fermentation.
The biosynthesis kinetics was not associated to microbial cell growth, the moment of lipase production being associated with the stationary phase.
All of the strains presented high lipolytic activities from the beginning to the end of the fermentation, with maximum values between 32 and 36 hours of cultivation. The highest activity showed *Yarrowia lipolytica* ICCF 214, with a value of 36.6 UL_{FIP}/mL.

CONCLUSIONS

Out of the 16 yeast strains selected from the Industrial Importance Microorganism Collection (IIMC), three presented lipolytic activity on cattle suet medium, namely *Candida paraffinica* – ICCF 184, *Yarrowia lipolytica* – ICCF 215, *Yarrowia lipolytica* – ICCF 214, and they were selected for fermentation on liquid medium.

During fermentation pH, biomass, glucose and enzyme activity levels were monitored for all three strains.

All of them showed high enzymatic activities, throughout the fermentation, but *Yarrowia lipolytica* ICCF 214 reached the highest value, of 36.6 UL_{FIP}/mL, at 36 hours fermentation.

Our results are better than those presented by other authors (Brígidaa et al., 2007; Kebabci et al., 2012), the level of lipase activity for *Yarrowia lipolytica* being higher than the ones presented by the literature.

Due to its enhanced lipase production potential, this strain will be used in biodiesel production through biocatalytic methods.

ACKNOWLEDGEMENTS

National Institute for Chemical Pharmaceutical Research and Development ICCF Bucharest for setting at our disposal their Industrial Importance Microorganism Collection (IIMC), for the performance of the screening tests.
The researches performed throughout this paper have been developed during the preparation of a PhD project, under the POSDRU/107/ 1.5/S/76888 programme from the University of Agronomical Sciences and Veterinary Medicine Bucharest, Romania.

REFERENCES

Aehle W., 2007, Industrial enzymes. In: Aehle W. ed. Enzymes in Industry, Production and Applications. Wiley; 2007: pp. 99-262
Alonso, F.O.M., Oliveira, E.B.L., Dellamora-Ortiz, G.M. and Pereira-Meirelles, F.V. (2005) Improvement of lipase production at different stirring speeds and oxygen levels. Braz
Babu IS, Rao GH., 2007 Lipase production by *Yarrowia lipolytica* NCIM 3589 in solid state fermentation using mixed substrate. Res J Microbiol 2: 469-474.
Balakrishnan, P., et.al., 2011, Production of lipase enzyme from Lactobacillus spp. And its application in the degradation of meat, World Applied Sciences Journal, 12 (10), 1798-1802
Brígidaa A. I. S., Amarala P. F., Gonçalvesb L. R., Coelhoa M. A. Z., 2007, Characterization of an extracellular lipase from Yarrowia lipolytica, Proceedings of European Congress of Chemical Engineering (ECCE-6), Copenhagen, 16-20 September 2007
Chen, J-Y., Wen, C-M. and Chen, T-L. (1999) Effect of oxygen transfer on lipase production by Acinetobacter radioresistens. Biotechnol Bioeng 62, 311–316.
Falch, E.A., 1991, Industrial enzymes-developments in production and application, Biotechnology Advances, 9 (4), 643-658
Feng Wei, Sun Xiuchan and Ji Peijun, Activation mechanism of *Yarrowia lipolytica* lipase immobilized on carbon nanotubes, *Soft Matter*, 2012,8, 7143-7150
Fickers P, Marty A, Nicaud JM., 2011, The lipases from *Yarrowia lipolytica*: genetics, production, regulation, biochemical characterization and biotechnological applications, Biotechnol Adv. 2011 Nov-Dec; 29 (6) :632-44. doi:10.1016/j.biotechadv.2011. 04.005. Epub 2011 Apr 28
Gupta, R., Gupta, N. and Rathi, P. (2004) Bacterial lipases: an overview of production, purification and biochemical properties. Appl Microbiol Biotechnol 64, 763–781
Hasan, F., Shah, A.A., and Hameed A., 2006, Industrial applications of microbial lipases, Enzyme and Microbial Technology, 39 (2), 235-251
Hasan, F., Shah, A.A., and Hameed A., 2009, Methods for detection and characterization of lipase: A comprehensive review, Biotechnology Advances, 27, 782-798
Iordachescu D., Dumitru I. F., 1980, Biochimie practica. Ed. Univ. Bucuresti, partea I, pp. 126–127
J Chem Eng 22, 9–18.

Jaeger KE, Eggert T., 2002, Lipases for biotechnology. Curr Opin Biotechnol 13: 390-397

Joseph B, Ramteke PW, Th omas G. Cold active microbial lipases: some hot issues and recent developments. Biotechnol Adv 26: 457-470, 2008

Kazlauskas, R.J., and Bornscheuer, U.T., 1998, Biotransformation with lipases, Biotechnology, New York: VCH., 8:37-192

Kebabci Ozgur, Cihangir Nilufer, 2012, Comparison of three Yarrowia lipolytica strains for lipase production: NBRC 1658, IFO 1195, and a local strain, Turk J Biol 36, 15-24

Kishore, J.P., Manoj, K. Z. C., and Raghunath, T. M., 2011, Lipase biodiversity, Indian Journal of Science and Technol-ogy, 4 (8), 971-982 Advances, 19, 627-662

Sharma, R., Christi, Y., and Benerjee, U. C., 2001, Production purification characterization and applications of lipases, Biotechnology

Sirisha E., Rajasekar N. and Narasu M. Lakshmi, 2010, Isolation and Optimization of Lipase Producing Bacteria from Oil Contaminated Soils, Advances in Biological Research 4 (5) : 249-252

Villeneuve, P., Muderwha, J. M., Garille, J., and Hass, M. J., 2000, Customizing lipases for biocatalysis: A survey of chemical, physical and molecular biological approaches, Journal of Molecular Catalysis B: Enzymatic, 9, 11.