SELECTION OF YEAST STRAINS WITH ENHANCED LIPOLYTIC ACTIVITY

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Abstract

In reaction to expanding markets and increasing demand for novel biocatalysts, commercial enzyme production has been continuously growing during the last century. Although some enzymes are extracted from plants and animals, the major source of industrial enzymes consists in microorganisms. Obtaining 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 lipolytic 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°C, achieving the necessary energy, and reducing at the same time the emergence of unwanted secondary compounds.

At low water content, these enzymes are also able to catalyze synthesis reactions, achieving esterification, alcoholysis, interesterification, transesterification, acidolysis, aminolysis, thio transesterification, 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 (Srirsha 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>
<thead>
<tr>
<th>Strain name</th>
<th>Registration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida arboarea – CBS* 64</td>
<td>ICCF 193</td>
</tr>
<tr>
<td>Candida glabrata</td>
<td>ICCF 182</td>
</tr>
<tr>
<td>Candida famata</td>
<td>ICCF 181</td>
</tr>
<tr>
<td>Yarrowia lipolytica – ATCC** 16618</td>
<td>ICCF 214</td>
</tr>
<tr>
<td>Yarrowia lipolytica</td>
<td>ICCF 215</td>
</tr>
<tr>
<td>Candida boidinii-CMGB*** 221</td>
<td>ICCF 26</td>
</tr>
<tr>
<td>Candida parafinica</td>
<td>ICCF 190</td>
</tr>
<tr>
<td>Candida parafinica</td>
<td>ICCF 184</td>
</tr>
<tr>
<td>Candida albicans – ATCC 10231</td>
<td>ICCF 91</td>
</tr>
<tr>
<td>Candida utilis-CMGB 237</td>
<td>ICCF 191</td>
</tr>
<tr>
<td>Candida guillermontii – CMGB 229</td>
<td>ICCF 183</td>
</tr>
<tr>
<td>Pichia pastoris – CMGB 267</td>
<td>ICCF 189</td>
</tr>
<tr>
<td>Candida sp.</td>
<td>ICCF 315</td>
</tr>
<tr>
<td>Hansenula polymorpha-CMGB 257</td>
<td>ICCF 218</td>
</tr>
<tr>
<td>Hansenula subpelliculosa-CMGB 261</td>
<td>ICCF 187</td>
</tr>
<tr>
<td>Hansenula anomala-CMGB 243</td>
<td>ICCF 217</td>
</tr>
</tbody>
</table>

The yeasts 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): 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°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): yeast extract 0.5; peptone 0.5; glucose 2.0; agar 2.0; pH 5.5, 120°C for 15 min sterilization, after cultivation for 72 hours at 28°C was used for inoculation of 50 mL liquid inoculum medium in 500 mL Erlenmeyer flasks.

The culture resulted after 24 hours at 28°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°C for 15 min sterilization.

The fermentation was conducted at 28°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°C) was determined using the titrimetric Willstätter method, using olive oil as a substrate, according to Iordachescu and Dumitruc (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, ULFIP 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).

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.

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

Figure 1. Lipase activity on culture medium containing cattle suet
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\text{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\text{FIP}/mL, at 36 hours fermentation. Our results are better than those presented by other authors (Brigidaa et al., 2007; Kebabi 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

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