Influence of some nutritional factors on lipase production by *Yarrowia lipolytica*

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ELENA SASARMAN[1)], CAMELIA DIGUTA[1)], STEFANA JURCOANE[1)], IRINA LUPESCU[2)], DIANA GROPOSILA-CONSTANTINESCU[2] LUMINITA TCACENCO[3)]

1) Centre of Microbial Biotechnology, Marasti, No. 59, 001464, Bucharest, Romania

2) Faculty of Biotechnology, University of Agriculture and Veterinary Medicine of Bucharest, Marasti, No. 59, 001464, Bucharest, Romania

3) National Institute of Research and Development for Biological Sciences, Splaiul Independentei, No. 296, 060031, Bucharest, Romania

Abstract

The production of extracellular lipase in submerged fermentation of selected strain of Yarrowia lipolytica has been investigated.Several compounds has been added to the culture medium in order to asses their efficiency as inducers of lipase biosynthesis consecutive optimization of carbon and nitrogen sources and mineral salts resulted in the increase in lipase activity.

Olive or sunflower oils uses as inducers or carbon sources increase lipase production. We replaced gradually glucose as carbon source with sunflower oil (at 1.5% to 3.0 %). obtaining the good results when we used the last concentration.

The production of lipase production by the fungus was stimulated in media containing nitrogen in the form of protein and ammonia phosphate.The optimum rate between protein source (bactopepton and yeast extract) and inorganic nitrogen source $(NH_4)_2HPO_4$ was 0.5:0.5:0.5, respectively. Lipase activity was the highest at this rate, 20.93 U/ mL^{-1} respectively.

Kewwords: lipase, *Yarrowia lipolytica*, inducers, nitrogen sources, mineral ions

Introduction

Lipases (triacylglycerol acylhydrolases, E.C. 3.1.1.3) are able to catalyze the hydrolysis of triacylglycerols to glycerol and free fatty acids on an oil-water interface. In the recent years, the large market potential for lipases developed due to the important role of these enzymes in chemical and pharmaceutical industries (as digestive enzymes and as glyceride-hydrolysing enzymes) [6;10], food additives (flavor modifying enzymes) [13], detergent industry or biodegradation of plastics (polyhydroxyalcanoate and polycaprolactone) [8; 9, 11].

Production of lipase by *Yarrowia lipolytica* can be optimized by designing the growth medium or conditions which have a positive effect on the synthesis of this enzyme. Lipase production is influenced not only by the carbon and nitrogen sources [2; 4; 8; 13; 14], the culture pH [7; 13], the growth temperature [7; 8; 13], stirring speeds [1], the dissolved oxygen concentration [1; 10]. It is well known fact that the production of extracellular lipases by many microorganisms can be improved significantly by addition of the inducers. Frequently, olive oil in combination with glucose was used as the carbon source and inducer for the production of lipase [10]. The others inducers could be use for improving of the lipase production: triglycerides (sunflower oil, tributyrin) , fatty acids (oleic acid) and several surfactants (Tween 80, Triton X-100, gum arabic, polyethylene glycol 200) [5; 13].

Literature date shows that lipase production was enhanced several fold when the medium was supplemented with Ca^{2+}, Mg^{2+}, Na^{+}, Co^{2+}, Cu^{2+}, Fe^{2+}, K^{+}, Mn^{2+}, Mo^{2+}, and Zn^{2+} [13; 14]. These inorganic ions could be activating or inhibiting lipase production. Many authors consider that exclusion of the magnesium ions from the medium determinate the reduction in lipase production [13; 14].

The goal of our research was to investigate the influence of different nutritional factors (inducers, nitrogen source) on lipase production by *Yarrowia lipolytica*. The influence of inorganic ions on lipase biosynthesis has been studied.

Materials and Methods

Micro-organism

The strain *Yarrowia lipolytica* (from Faculty of Biology - University of Bucharest) was used for lipase production.

Growth Medium and Growth Condition

The culture was maintained on YPG slants having the composition (%): glucose 2.0, peptone 2.0 yeast extract 1,0 and agar agar 2.0. The pH of the medium was adjusted at 6.0 and culture was incubated to 30^0C for 48h. Sub culturing was carried out once in every two weeks and the culture was stored at 4^0C.

Inoculum Preparation

The yeast *Yarrowia* strain was cultivated in a YPG liquid medium. The cell was cultivated in this medium at 30^0C on a shaker at 200 rpm for 24h.

Culture media

The medium for lipase production was YPG liquid medium enriched with mineral salts in different concentration and olive or sunflower oils used as inducer of lipase production and as carbon source (table 1).

Table 1. Composition of culture media (%) a view to establish the optima formula for lipase production

Culture media	I.	II.	III.	IV.	V.	VI.	VII.	VIII.	IX.	X.
Glucose	-	2,0	1,0	-	1,5	-	1,5	-	-	-
Olive oil	1,0	0,1	0,1	1,0	0,1	1,5	-	-	-	-
Sunflower oil	-	-	-	-	-	-	0.1	1.5	2.0	3.0
Bactopepton	0,64	2,0	1,0	1,0	0,5	0,5	0,5	0,5	0,5	0,5
Yeast extract	0,1	1,0	0,1	0,1	0,5	0,5	0,5	0,5	0,5	0,5
$NaNO_3$	0,1	-	0,1	0,1	-	-	-	-	-	-
KH_2PO_4	0,1	0,25	0,05	0,05	0,25	0,25	0,25	0,25	0,25	0,25
$(NH_4)_2HPO_4$	-	0,50	-	-	0,5	0,5	0,5	0,5	0,5	0,5
$MgSO_4 \cdot 7H_2O$	0,05	0,1	0,05	0,05	0,1	0,1	0,1	0,1	0,1	0.1

Fermentation was carried out in the 500 ml Erlenmeyer flasks containing 30 ml of culture medium; at pH=6,0; temperature 30^0C, for 48-72 hours. The stirring rate of 240 rpm was maintained. The culture media was inoculated with 5% inoculum

Optimization of Medium Parameters.

The strategyadopted was to optimize one particular parameter at a time and then include it at its optimum value in the next optimization step, if found beneficial. The parameters optimized were: (1) inducers and carbon sources, (2) nitrogen level and (3) mineral salts.

Lipase assay.

Lipase activity was determined by a titrimetric assay with NaOH 0.1N [9; 10] using emulsified olive oil as the substrate.

On unite of lipase activity was defined as the amount of enzyme that released 1μmol equivalent of carboxyl groups of fatty acids under analysis conditions (temperature = 37^0C, pH=7, reaction time = 60 minutes).

Bimass estimation

Biomass concentration was determined measuring the optical density (D.O. = 570 nm) of the fermentation medium by spectrophotometer (Helios γ Thermo Electron Corporation - USA).

Results and Discussions

Optimization of Production Media.

Depending of environmental and nutritional conditions *Yarrowia lipolytica,*a dimorphic nonconventional yeast with biotechnological potential, can grow in the form of yeast, pseudohyphe and true hyphe. pH was the most

important factor regulating the dimorphic transition. Mycelium formation is usually maximal at pH near neutrality and decreased as pH was lowered to become null at pH 3.0 (12)

The morphological analysis carried out at the end of fermentation process reveled that yeast cells were predominant, about 70 % and pseudohyphe only 30%. When it used sunflower oil as carbon source and inducer (3%), the cells contained lipidic inclusions.

It is a well known fact that importance aspect of biosynthesis process represented the relationship between biomass concentration and final metabolite accumulation. Table 2, gives changes of pH and lipase activity depending on various carbon and nitrogen sources and mineral salts. Biomass concentration at the end of fermentation process (40-48 hours) demonstrated a good growth of the yeast strain all almost culture media, especially when sunflower oil were use (3%).

It was found that maximum actities were obtained when the initial pH was adjusted to 6.0.Final pH of fermentation process for all variant of culture media is 5.5-6.5.

Table 2 Biosynthesis of lipase on different culture media with Yarrowia lipolytica

Culture Media	**pH**	**D.O. $_{570\ nm}$**	**Lipase activity (U/mL^{1})**
I.	5.5	1.83	**10,47**
II.	5.5	2.2125	**19,45**
III.	6.5	1.5975	**11,92**
IV.	5.5	2.080	**10,45**
V.	6.5	2.035	**13.44**
VI.	5.5	2.460	**16.44**
VII.	6.5	1.920	**13,44**
VIII.	5.5	2.475	**5,98**
IX.	5.5	2.500	**11,97**
X.	5.5	2.703	**20,93**

Note: initial pH 6.0

Effect of inducers source

In order to determine the influence of inducers on lipase production was used olive and sunflower oils in different concentrations. When were used these inducers at the same concentration (0.1%) and 1.5% glucose as carbon source, it was observed that is it not the difference in lipase activity, being approximate 13.44 U/mL (fig. 1).

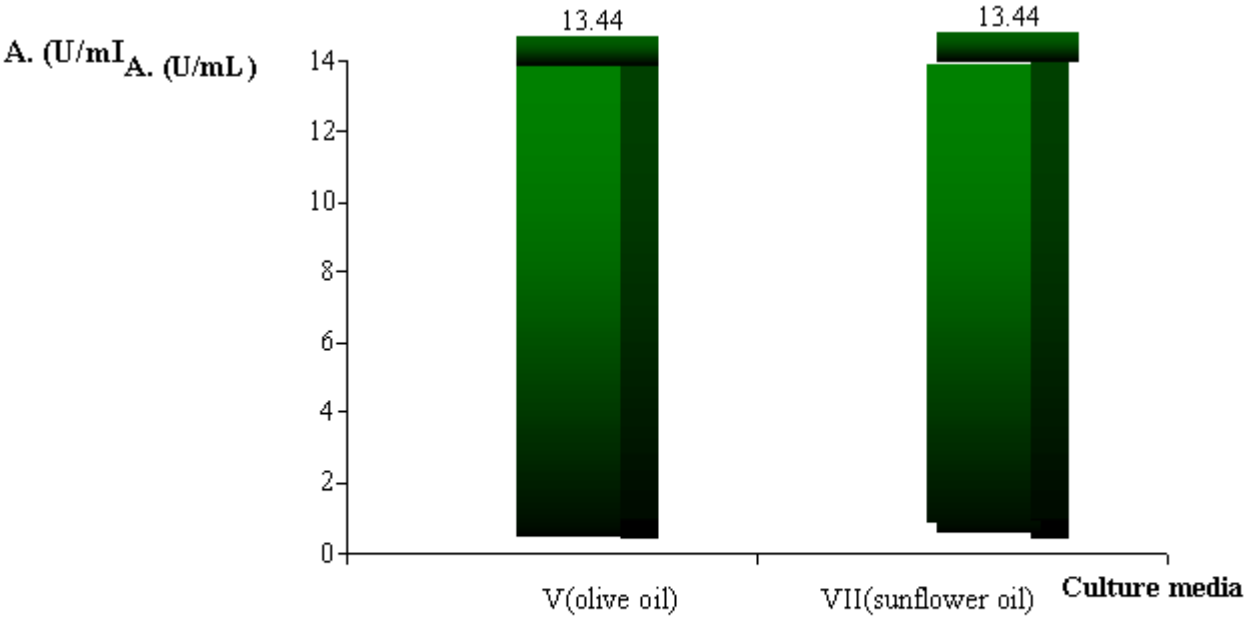

Figure 1 *Comparative influence of inductor (olive and sunflower oils) at 0.1% concentration on lipase biosynthesis*

We went to see if with the increased of inducer concentration and using as only carbon source enhance lipase activity. Lipase activity was approximate 3 fold higher when added 1,5% olive oil then when used sunflower oil in the same concentrations (fig. 2).

Sunflower oil was the carbon source employed in subsequent experiments. The conclusion that lipids were better carbon sources then carbohydrates for lipase production confirm the results reported for certain microbial lipase (3)

The increase sunflower concentration from 1.5% to 2.0 % leaded at doubling lipase activity, approximate 11,97 U/mL. The best result was obtained when it used 3% sunflower concentration, lipase activity increasing of approximate 4 fold higher (fig. 3).

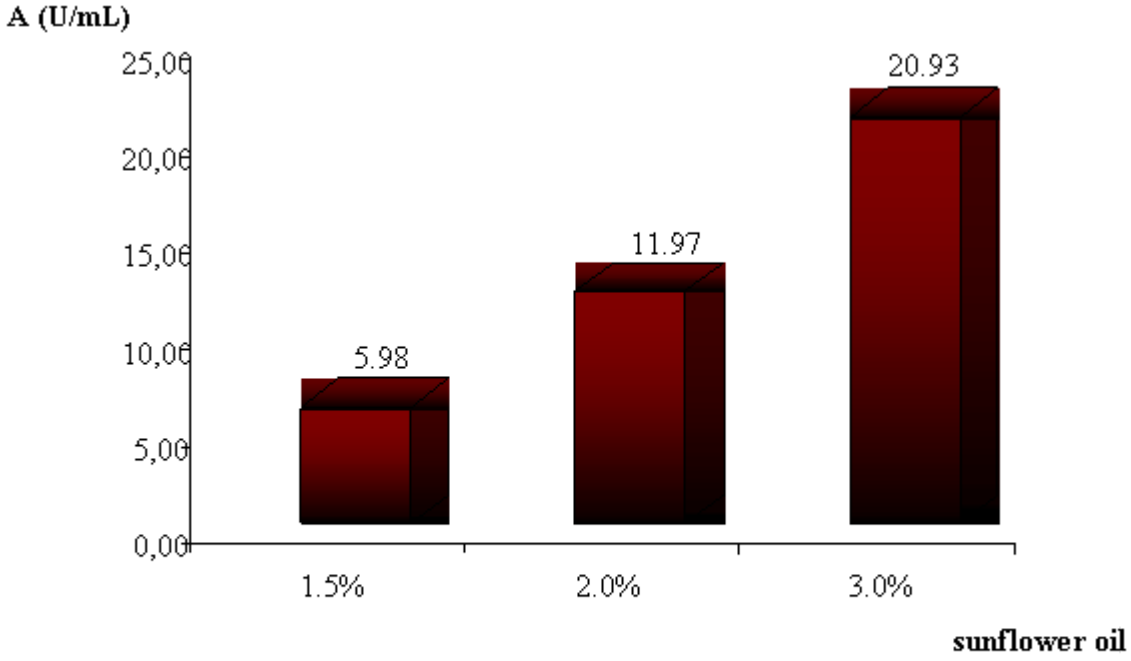

Figure 3. *Influence of concentration of sunflower oil (used as inductor) on lipase production*

Effect of nitrogen source

Nitrogen source mediated lipase activity was documented in submerged fermentation (2).Table 1 and 2 depicts the role of different sources on lipase activity by *Yarrowia lipolytica.*

Concerning the influence of nitrogen source on lipase biosynthesis was tested bactopepton and yeast extract at different concentrations (%) 0.5; 0.67; 1.0 and 2.0, 0.1; 0.5 and 1.0 respectively. The culture media was supplemented with 0.5% $(NH_4)_2HPO_4$ (inorganic nitrogen source).The lipase activity was stimulated in culture media containing proteic source supplemented with inorganic nitrogen source, the optimum rate of bactopepton: yeast extract: $(NH_4)_2HPO_4$, being 0.5:0.5:0.5. Ammonium phosphate was the best inorganic nitrogen and phosphorous source for increasing lipase biosynthesis.

Effect of mineral salts.

It has been analyzed the influence of phosphorous source on lipase biosynthesis by *Yarrowia lipolytica,* when added KH_2PO_4 and $(NH_4)_2HPO_4$ in culture medium. We are tested 3 concentrations of KH_2PO_4: 0.05%; 0.1%; 0.25%. The maximum yield, (20,93 U/mL) was obtained at 0,25% KH_2PO_4 and $(NH_4)_2HPO_4$, as shown in Table 2 (medium 10).

According literature date, magnesium ions were used in concentration of 0.1%.

The exclusion $NaNO_3$ from culture proved to have no negative influence on lipase activity. By exclusion of $(NH_4)_2HPO_4$ from culture media I, III and IV was observed a decreasing of lipase activity from 19.45 U/mL (culture medium II) to 11.92 U/mL (culture medium III) to 10.45 U/mL (culture media I, IV), the results obtained being in accordance with literature date [1].

Conclusions

The present study describes the influence of inducers, nitrogen source, and mineral salts on the production of extracellular lipase in submerged fermentation of selected strain *Yarrowia lipolytica*.

Good results were obtained when added 3% sunflower oil as carbon source and as inducers in culture medium, lipase activity being 20.93 U/mL.

It has been established the optimum rate between organic nitrogen source (bactopepton and yeast extract) and inorganic nitrogen source $(NH_4)_2 HPO_4$, being 0.5:0.5:0.5 respectively.

Finally, the composition of the culture medium, economically relevant, for lipase biosynthesis by *Yarrowia lipolytica* is (g/v): sunflower oil – 3.0 bactopepton - 0.5; yeast extract – 0.5; KH_2PO_4 – 0.25; $(NH_4)_2HPO_4$ -0.5; $MgSO_4$x 7 H_2O – 0.1, initial pH 6.0.

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