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Proceeding Paper

Investigating Culture Media for Obtaining Lipolytic Biocatalysts Based on *Rhizopus oryzae* Fungi †

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Abstract: *Rhizopus oryzae* is widely distributed in nature and can be isolated from different substrates such as decomposing vegetables, fruits and various soils. It is generally classified as GRAS filamentous fungi and commonly used in the production of oriental traditional food such as tempeh or peka. This microorganism has great industrial potential due to the capability to synthesize enzymes (glucoamylases, cellulases and lipases) and organic acids (lactic acid, fumaric acid). The most studied enzymes of the fungi are lipases (ROL). Therefore, the aim of the study was the selection of growth medium content and initial pH rate, which would provide high lipase synthesis yield in 5 days shaken cultures. Two fractions of lipases were investigated in order to obtain lipase biocatalysts: extracellular enzymes present in supernatant and cell-bound lipases in biomass. The used nutrient-rich media were: YPG (10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose), YPO (10 g/L yeast extract, 20 g/L peptone, 20 g/L olive oil), YMG (3 g/L yeast extract, 3 g/L malt extract, 5 g/L peptone, 20 g/L glucose), YMO (3 g/L yeast extract, 3 g/L malt extract, 5 g/L peptone, 20 g/L olive oil). The mineral media were: SMG (10 g/L peptone, 14 g/L KH_2PO_4, 2.4 g/L K_2HPO_4, 0.4 g/L $MgSO_4$, 20 g/L glucose) and SMO (10 g/L peptone, 14 g/L KH_2PO_4, 2.4 g/L K_2HPO_4, 0.4 g/L $MgSO_4$, 20 g/L olive oil). Fungi biomass and supernatant were separated and used to measure lipase activity by a spectrophotometric method based on the hydrolysis of *p*-nitrophenyl laurate. The results showed that the highest lipase activity after 5 days of cultivation was reached in YPO medium for biomass (from 7- to 60-fold higher results depending on the compared variant of culture media) and YMG for supernatant (from 3- to 6.5-fold higher results depending on the used variant of culture media). The addition of citric acid resulted in a two times increase of the activity of produced lipases after 5 days of cultivation.

Keywords: *Rhizopus oryzae*; lipases; culture medium

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1. Introduction

Rhizopus oryzae is widely distributed in nature, and mostly isolated from alcoholic beverages or oriental foods in China, Japan and Indonesia [1]. This microorganism is well known as a primary or secondary colonizer, due to its fast invasion of digestible substrates because of its rapid growth. *R. oryzae* can use different plant compounds and polysaccharides as an energy and carbon source. It is generally classified as a GRAS filamentous fungi and broadly employed in industry due to its capability to synthesize a great variety of products like enzymes (proteases, cellulases, lipases) and organic acids (lactic and fumaric acids) [2]. The most studied enzymes of this fungi are lipases, because they can carry out esterification and transesterification in organic media. They are classified as enzymes

that hydrolyze fats and oils with subsequent release of free fatty acids, diacyloglycerols, monoacylglycerols and glycerol. *R. oryzae* lipases (ROL) activity has a strong *sn*-1,3 regiospecificity which makes its activity attractive for several industrial processes such as fat and oil modification for structured lipids production [3,4]. Therefore, the aim of the study was the selection of growth medium content and initial pH rate, which would provide high lipase synthesis yield in shaken cultures of *R. oryzae*.

2. Materials and Methods

2.1. Materials

Microorganism *Rhizopus oryzae* DSM 2199 was purchased from the German Collection of Microorganisms and Cell Cultures (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, DSMZ, Braunschweig, Germany). Chemicals and medium ingredients were purchased from Sigma-Aldrich (Burlington, MA, USA) and Avantor Performance Materials (Gliwice, Poland).

2.2. Methods

2.2.1. Culture Media and Fungi Cultivation

Culture media used in the research are presented in Table 1. Inoculation was conducted by adding 1 mL of *R. oryzae* spore suspension to 200 mL sterile medium, and then cultured on a rotary shaker (150 rpm) for 3 and 5 days in 30 °C. To investigate the influence of medium acidification, media with 1 g/L NH_4NO_3, 1 g/L $(NH_4)_2SO_4$, 4 g/L K_2HPO_4, 2 g/L KH_2PO_4, 1 g/L NaCl, 10 g/L% olive oil and 1 g/L yeast extract were used. The media were acidified with 10% citric acid. At the end of experiment, biomass was separated from supernatant by filtration.

Table 1. Culture media composition.

Culture Media	Composition
YPG	10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose
YPO	10 g/L yeast extract, 20 g/L peptone, 20 g/L olive oil
YMG	3 g/L yeast extract, 3 g/L malt extract, 5 g/L peptone, 20 g/L glucose
YMO	(3 g/L yeast extract, 3 g/L malt extract, 5 g/L peptone, 20 g/L olive oil
SMG	10 g/L peptone, 14 g/L KH_2PO_4, 2.4 g/L K_2HPO_4, 0.4 g/L $MgSO_4$, 20 g/L glucose
SMO	10 g/L peptone, 14 g/L KH_2PO_4, 2.4 g/L K_2HPO_4, 0.4 g/L $MgSO_4$, 20 g/L olive oil

2.2.2. Hydrolytic Activity Measurements

Hydrolytic activity was evaluated by a spectrophotometric method of measuring the progress of the p-nitrophenyl laurate hydrolysis described by Kapturowska et al. [5]. Hydrolytic activity was measured in both fractions, i.e., extracellular enzymes present in supernatant and cell-bound lipases in biomass. The reaction was carried out in an Erlenmeyer flask. Firstly, the 0.3 mmol of p-nitrophenyl laurate was dissolved in 2 mL of heptane. Secondly, 1 g of biomass was dissolved in 15 mL of distilled water. In the reaction, 15 mL of supernatant was also used. Substrate and biocatalyst were stirred for 30 min at 37 °C. The absorbance was measured at 410 nm in a UV/Vis spectrophotometer. The unit of lipase enzymatic activity was 1 U, i.e., the amount of enzyme that released 1 µmol of p-nitrophenol per minute under the assay conditions.

2.2.3. Statistical Analysis

Statistical analysis was performed using Statistica 13.3 software (TIBCO Software Inc., Palo Alto, CA, USA). The Shapiro–Wilk test was used for the normality of data distribution, while the Brown–Forsythe test was used to assess the equality of variances. The results were analyzed using a one-way analysis of variance (ANOVA) and Tukey's post hoc test. The significance level was $\alpha = 0.05$.

3. Results

Two fractions of *R. oryzae* lipases were investigated in order to obtain biocatalysts: extracellular enzymes present in supernatant and cell-bound lipases in mold biomass. Different culture media were examined which contained glucose or olive oil as a carbon source. As can be seen in Figure 1, the highest lipolytic activity for cell-bound lipases was reached in 72 h culture in YPO medium (7.466 U/g), which was plentiful in nutrients and included olive oil abundant in oleic acid. In the rest of culture media, the lipolytic activity was significantly lower. For extracellular lipases the maximum lipase activity was obtained in YMG medium (0.571 U/mL) (Figure 2). The lipolytic activity was slightly higher than for other used media which contained glucose as carbon source (YPG, SMG) and considerably higher for media YPO, YMO, SMO.

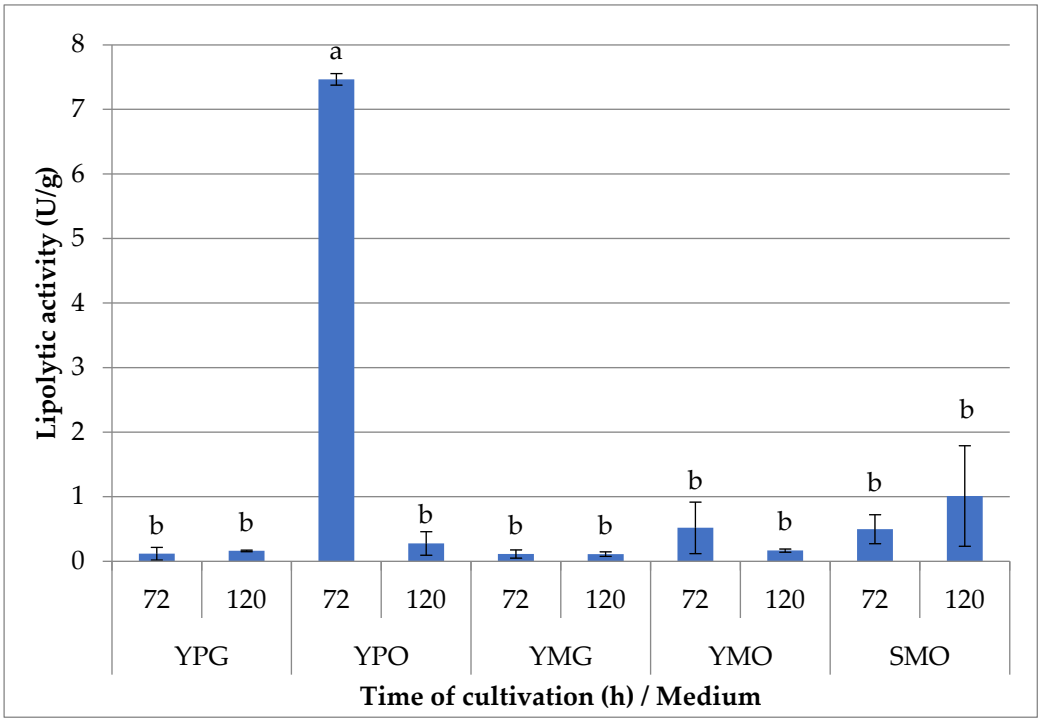

Figure 1. Lipolytic activity of cell-bound lipases in *R. oryzae* biomass in 72 h and 120 h shaken culture. Means with the same capital letter (a or b) did not differ significantly ($\alpha = 0.05$).

Similar culture conditions were used with a strain of *R. oryzae* WPG (ROL_W). Salah et. al. [6] observed that the maximal production of extracellular lipase was reached at pH 6, 30 °C and 72 h of growth in medium with glucose as a carbon source. The presence of triacylglycerols such as lipids in olive oil has not yet been examined for lipase synthesis by *R. oryzae* cells.

The second step of the research investigated the effect of citric acid addition to the medium on the production of lipases. In this experiment, different types of medium were used, and the results are presented in Table 2. The addition of citric acid to medium slightly increased the lipolytic activity in 120 h of culture growth but the difference was not relevant. According to other studies, it was shown that the lipase activity of *R. oryzae* is active at high pH but decreases in the presence of acetone, ethers, alkanes and chloroalkanes [1,3]. As can be seen, the lipolytic activity of lipases in supernatant was significantly lower comparing with previous experiments, and did not obtain satisfactory levels.

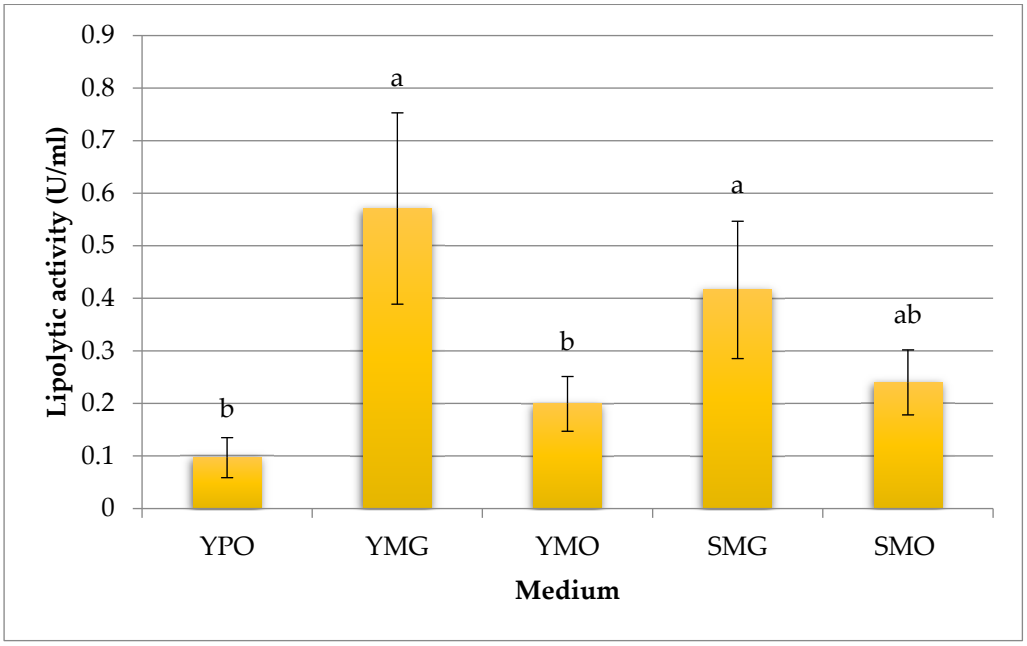

Figure 2. Lipolytic activity of *R. oryzae* extracellular enzymes in 72 h shaken culture. Means with the same capital letter (a or b) did not differ significantly ($\alpha = 0.05$).

Table 2. Lipolytic activity of extracellular enzymes produced by *R. oryzae*. Means with the same capital letter "a" in the case of 72-hour cultures and A or B in the case of 120-h cultures did not differ significantly ($\alpha = 0.05$).

Lipolytic Activity (U/mL)			
Medium with addition of citric acid Time cultivation:		Medium without addition of citric acid Time cultivation:	
72 h	120 h	72 h	120 h
0.018 ± 0.017 a	0.034 ± 0.010 A	0.008 ± 0.002 a	0.015 ± 0.004 B

4. Conclusions

In this study it was proven that obtaining two fractions of lipases, extracellular enzymes present in supernatant and cell-bound lipases in biomass from *Rhizopus oryzae*, is possible. More investigations for selecting conditions of microorganism growth are needed, because in the research only one medium—which consisted of olive oil (YPO)—achieved satisfying effects with high results for lipolytic activity. The secretion and lipolytic enzyme activity to culture media did not significantly change in pH rate 3 compared with pH rate 7.

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References

1. Ghosh, B.; Ray, R.R. Current Commercial Perspective of *Rhizopus oryzae*: A Review. *J. Appl. Sci.* **2011**, *11*, 2470–2486. [CrossRef]
2. Londono-Hernandez, L.; Ramirez-Toro, C.; Ruiz, H.A.; Ascacio-Valdes, J.A.; Aguilar-Gonazalez, M.A.; Rodriguez-Herrera, R.; Aquilar, C.N. *Rhizopus oryzae*—Ancient microbial resource with importance in modern food industry. *Int. J. Food Microbiol.* **2017**, *257*, 110–127. [CrossRef] [PubMed]
3. Lopez-Fernandez, J.; Benaiges, M.D.; Valero, F. *Rhizopus oryzae* Lipase, a Promising Industrial Enzyme: Biochemical Characteristics, Production and Biocatalytic Applications. *Catalyst* **2020**, *10*, 1277. [CrossRef]
4. Lopez-Fernandez, J.; Barrero, J.J.; Benaiges, M.D.; Valero, F. Truncated Prosequence of *Rhizopus oryzae* Lipase: Key Factor for Production Improvement and Biocatalyst Stability. *Catalyst* **2019**, *9*, 961. [CrossRef]
5. Kapturowska, A.; Stolarzewicz, I.; Krzyczkowska, J.; Białecka-Florjańczyk, E. Studies on lipolytic activity of sonicated enzymes from *Yarrowia lipolytica*. *Ultrason. Sonochem.* **2012**, *19*, 186–191. [CrossRef]
6. Salah, R.B.; Mosbah, H.; Fendri, A.; Gargouri, A.; Gargouri, Y.; Mejdoub, H. Biochemical and molecular characterization of a lipase produced by *Rhizopus oryzae*. *FEMS Microbiol. Lett.* **2006**, *260*, 241–248. [CrossRef] [PubMed]