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Optimization of lipase production by *Yarrowia divulgata*

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Introduction

Lipase is a highly valuable compound which has a wide range of usability, for example in the food-, pharmaceutical- and beauty industries. *Yarrowia lipolytica* is one of the most extensively studied yeast species, known from its remarkably high lipolytic and proteolytic activity. In the last few years some novel species belonging to the *Yarrowia* clade were described, justifying the interest to study their ability to produce lipase and other valuable compounds.
The main aims of this research were to evaluate the lipase producing ability of some novel *Yarrowia* strains, and to enhance the production by optimizing the operational parameters.

Materials and Methods

Thirty-five strains, belonging to the species *Yarrowia divulgata, Y. porcina* and *Y. bubula* isolated from raw, grounded pork or beef were screened by streaking them on the surface of Gorodkowa medium, supplemented with olive oil.
Inoculum (5%, v/v) was transferred into 500 mL flasks, containing 200 mL of fermentation medium (2% glucose, 0.64% peptone, 1% yeast extract) and supplemented with 1% olive oil and/or 0.05% Tween 80. Experiments were carried out for 72 hours at 28°C in a shaker (160 rpm).
Samples were centrifuged and supernatants were used for measurement of extracellular lipase activity; additionally, yeast cells were disrupted before the quantification of intracellular lipase. 25mM p-nitrophenyl-laurate was used as substrate to determine the lipase activity and the reaction was performed at 37°C in phosphate buffer (pH 7.2) for 10 min. Lipase activity was determined spectrophotometrically at 405 nm. One unit (U) of lipase activity was defined as the amount of enzyme that releases 1 µM of p-nitrophenol per minute (pH 7.2, 37°C).
Optical density (λ=600 nm) to quantify cellular growth and pH were also measured.

Results and Discussion

Almost all strain showed lipolytic activity, but *Y. divulgata* 5257 and 2062 were selected for further experiments. During submerged fermentation pH decreased, while OD kept growing. Exponential growth started after 8 h of cultivation for both strains in YEPD medium. In experiments with *Y. divulgata* strains, 4.03 U/ml and 8.11 U/ml of extracellular enzyme activity was attained after 8 h and 48 h, respectively. Olive oil and Tween 80 have been published to enhance lipase activity and in our experiments this positive effect has also been proved: maximal lipase activity rised to 6.59 U/ml and 25.17 U/ml at 48 hours in the prescence of olive oil, and to 139 U/ml and 141.76 U/ml when both additives were added.

Optimal temperature and pH for enzyme activity assay were also determined. Lipase activity was highest at 37 °C and at pH 6.5, and probably these parameters were also the most adequate for yeast growth and lipase production.

Y. divulgata 5257 was used to determine intracellular lipase activity, which was also significant (396 U/ml without Tween 80).

Conclusion

Yeasts of the *Yarrowia* genus are able to produce high amount of valuable compounds, such as the enzyme lipase. *Y. divulgata* strains showed the best performance and the addition of olive oil and Tween 80 led to the increase of lipase production. Thus besides *Y. lipolytica* members of other species may have great industrial potential and should be studied.

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