Enhancement of lipase production by *Yarrowia divulgata*

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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 known from its remarkably high lipolytic and proteolytic activity. In the last years some novel species belonging to the *Yarrowia* genus were described, and some of them also have the ability to produce lipase and other valuable compounds. The main aims of this research were to enhance the lipase production of some novel *Yarrowia* strains by using olive oil and Tween 80 supplements, and optimizing concentrations of olive oil.

Thirty-five strains of *Yarrowia divulgata, Y. porcina* and *Y. bubula* isolated from raw, grounded pork or beef were screened for lipase production 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%, 5%, 10%, 20% and 50% olive oil and 0.05% Tween 80. Experiments were carried out for 72 or 148 hours at 28°C in orbital 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 and the reaction was performed at 37°C in phosphate buffer (pH 7.2) for 10 min. Lipase activity was assayed 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).

Almost all examined *Yarrowia* strain showed lipolytic activity, but *Y. divulgata* 5257 and 2062 were selected for further experiments. In the cases of *Y. divulgata* strains, 4.03 U/l and 8.11 U/l of extracellular enzyme activities were attained after 8 h and 48 h, respectively. Olive oil and Tween 80 have been published (Corzo & Revah, 1999; Galvagno et al., 2011) to enhance lipase activity and in our experiments this positive effect has also been proved: maximal lipase activity rised to 6,59 U/l and 26.00 U/l at 48 and 64 hours in the prescence of 1% olive oil, and to 83.47 U/l and 203.61 U/l at 72 hours when both additives have been added. The effect of olive oil concentration was also dinvestigated. *Y. divulgata* 2062 showed the highest extra- and intracellular lipase activity in the prescence of 10% olive oil, although in case of *Y. divulgata* 5257, 5 and 50% olive oil supplementation was the most successful in case of both intra- and extracellular activity, which is controversial to previously published data (Pignède et al., 2000; Coca & Dustet, 2006). *Y. divulgata* 5257 showed hight intracellular lipase activity (924.44 U/l), and *Y. divulgata* 156.73 U/l without Tween 80.

Y. divulgata strains exhibited the best performance and the addition of proper concentration 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 are worth to study.

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