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Optimization of fermentation conditions for enhancement of lipase production by *Yarrowia* isolates

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ABSTRACT

Microbial lipases are widely used in biotechnology and industrial processes. *Yarrowia lipolytica* exhibits strong lipolytic activity and optimizing fermentation conditions is crucial for efficient enzyme production. This research investigated lipase production of some newly isolated *Yarrowia* strains, including *Yarrowia lipolytica, Yarrowia divulgata*, and *Yarrowia yakushimensis*. The *Y. lipolytica* 854/4 strain has reached the highest lipase activity after 3 days of fermentation, whereas other isolates require a longer fermentation time of 6–7 days. The lipase synthesis was not significantly affected by three tested cell count (10^6, 5^*10^6 and 10^7 CFU mL^{-1}) during optimization of the initial inoculum concentration. Different concentrations of Triton X-100 and Tween 80 supplementation enhanced the lipase production, although the extent of this effect varied from strain to strain. These findings highlight *Y. yakushimensis* as a promising species, in addition to *Y. lipolytica* for industrial application involving lipase production.

KEYWORDS

lipase, *Yarrowia yakushimensis*, *Yarrowia lipolytica*, inoculum concentration, Triton X-100, Tween 80

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INTRODUCTION

In recent decades, *Yarrowia* has received more and more attention in the fields of basic research and biotechnology (Bankar et al., 2009). The scientific name *Yarrowia* was first used by van der Walt and von Arx (1980) in recognition of a new genus identified by David Yarrow in 1972.

The genus *Yarrowia* belongs to the class Saccharomycetes and is considered the sister group of *Wickerhamiella* (Gouliamova et al., 2017; Kurtzman et al., 2011). The following species of the *Yarrowia* group are currently known: *Yarrowia alimentaria, Yarrowia brassicae, Yarrowia bubula, Yarrowia deformans, Yarrowia divulgata, Yarrowia galli, Yarrowia hollandica, Yarrowia keelungensis, Yarrowia lipolytica, Yarrowia oslonensis, Yarrowia parophoni, Yarrowia phangngensis, Yarrowia porcina, Yarrowia yakushimensis,* and *Candida hispaniensis* (Gouliamova et al., 2017; Liu et al., 2018). As the phenotypic diversity among members of *Yarrowia* is very limited, *Y. lipolytica* was considered the only species within this genus, until the 2010s. However, in recent years, molecular biological studies have shown that a significant part of the strains considered to be *Y. lipolytica* are actually different species (Nagy, 2015).

The most frequently studied species among its members is *Y. lipolytica*. This species is used in various industries due to its metabolic products. *Y. lipolytica* has a strong lipolytic activity (Nagy, 2015; Groenewald et al., 2014) and was first applied for single-cell protein fermentation in the 1960s using n-alkanes as substrate (Nicaud, 2012; Barth and Gaillardin, 1997). The microorganism produced lipase was proved to be useful in different fields of industrial processes. Lipases can be applied in the food industry to improve flavour, in detergents to hydrolyse oils and fats, in pharmaceuticals to synthesize chiral drugs, in paper processing to control pitch, in medicine to measure triglycerides, in cosmetics to exclude lipids, in wastewater treatment to decompose and remove oil, and in the leather industry to eliminate fat from animal skins (Sharma et al., 2017).

Y. divulgata was first described by Nagy and co-workers (Nagy et al., 2013). The strains of new species were isolated from different meat samples, one from a Danish bacon processing factory, two strains from chicken breast and liver in the USA and one originated from ground beef in Hungary. Based on the D1/D2 sequences and ITS regions, it showed that the isolated strains form a new species despite the phenotypic similarity to *Y. lipolytica* and *Y. deformans*.

Y. yakushimensis was first described in 2013 by Groenewald and Smith (Groenewald and Smith, 2013) together with another species belonging to the genus *Yarrowia* during the study of the yeast *Y. lipolytica*. The importance of this finding is that members of the closely related *Y. lipolytica* species, which have not yet been studied, or have been studied to a lesser extent, may also have outstanding potential for the industry in different metabolite production including lipase.

One of the key approaches to the efficient lipase production is to optimize fermentation technology, by adjusting environmental parameters including temperature, pH, media composition (nitrogen, carbon, and lipid), agitation, and inoculum concentrations. The optimization of inoculum concentration is crucial for fermentation processes, as increasing the size of the inoculum decreases the available nutrients. This phenomenon can negatively impact the lipase production. Similarly, low inoculum quantity may reduce enzyme secretion due to decreased cell numbers (Reddy et al., 2008). Certain inducers have a significant effect on increasing lipase production, including lipids, triglycerides, hydrolyzable esters, free fatty acids, and various surfactants, for example Tween 80 or Triton X-100 (Ghosh et al., 1996; Domínguez et al., 2003; Treichel et al., 2010). In biotechnological reactions, both the polyoxyethylene octylphenol

(Triton) and the nonionic polyethoxylated sorbitan (Tween) are widely known as carbon sources or inducers (Gutiérrez-Arnillas et al., 2017). This is because it changes the cell permeability and the way the surfactant affects the cell-bound lipase. However, surfactants do not always increase lipase production. It seems that their effects depend on the type of surfactant and the specific strain of bacteria being studied (de Almeida, 2013).

The main goal of this study is the investigation of the effects of inoculum volume and surfactant type and concentration on the lipase production of various *Yarrowia* isolates on olive oil substrate.

MATERIALS AND METHODS

Materials

All chemicals, substrate (p-nitrophenyl laurate) and reagents are analytical grades and either from Sigma-Aldrich Co. or Reanal Ltd. (Budapest, Hungary).

Strains, media and growth conditions

The National Collection of Agricultural and Industrial Microorganisms (NCAIM, Budapest) provides the *Yarrowia* strains and isolates for the research. They are originated from various meats and insects (Table 1).

YEPD agar plates were used for maintenance of the yeasts, containing 20 g L^{-1} glucose, 10 g L^{-1} peptone, 5 g L^{-1} yeast extract, and 25 g L^{-1} agar at 28 °C for 24 h. After growth the agar slants were kept in the refrigerator at 4 °C until use.

Methods

Fermentation

Yeast cells were preinoculated in 250 mL flasks containing 150 mL inoculum medium. This medium consists of 20 g L^{-1} glucose, 20 g L^{-1} peptone, 10 g L^{-1} yeast extract.

Enzyme fermentations were initiated with 24-h inoculum at 10^6, 5^*10^6 and 10^7 colony forming unit/mL (CFU mL^{-1}), using a shaker at 130 rpm in a volume of 125–200 mL at 28 °C for 168 h. The fermentation medium contained 20 g L^{-1} glucose, 6.4 g L^{-1} peptone, and 10 g L^{-1} yeast extract. Depending on the experimental design Tween 80 and/or Triton X-100 was added to the fermentation medium containing olive oil at different concentrations.

Table 1. Applied *Yarrowia* strains and their source of isolation

Strains	Source of isolation
Yarrowia divulgata NCAIM Y.02062	Chicken breast
Yarrowia divulgata 5257	Minced pork
Yarrowia yakushimensis NCAIM Y.02049	Insect (*Hodotermopsis sjoestedti*)
Yarrowia yakushimensis NCAIM Y.02050	Insect (*Hodotermopsis sjoestedti*)
Yarrowia yakushimensis NCAIM Y.02052	Insect (*Hodotermopsis sjoestedti*)
Yarrowia lipolytica 854/4	Meat

Lipase enzyme activity assay

Extracellular lipase activity was detected during the whole fermentation process. 5 mL of the ferment broth were taken at different time intervals. The samples were centrifuged at 10,000 rpm for 10 min at 4 °C and the supernatant was used to measure the extracellular lipase enzyme activity. The artificial substrate p-nitrophenyl laurate was applied to determine lipase activity spectrophotometrically at 405 nm using *Unicam Helios Alfa* equipment. The enzyme reaction was carried out at 37 °C and pH 7.2. The reaction mixture was prepared as described by Sipiczki et al. (2024).

Determination of cell number and optimization of inoculum

The cell count was determined using the Bürker counting chamber method with an Olympos microscope, 40× magnification. Twenty microliters of the tenfold diluted samples were pipetted into the Bürker chamber, and then the yeast cells were counted in 10 rectangles of the same size under a microscope. The results were averaged, and the cell number was given in cells/mL, taking the dilutions into account. In all cases, duplicates were performed.

For optimization of inoculum size, 10^6 CFU mL^{-1}, 5^*10^6 CFU mL^{-1} and 10^7 CFU mL^{-1} cell counts were applied to initiate the fermentation.

Optimization of fermentation conditions and statistical analysis

To optimize lipase enzyme fermentation Response Surface Methodology was used. Two independent variables as Triton X-100 and Tween 80 meanwhile the lipase enzyme activity (Y) as dependent variable were chosen.

The second order polynomial equation was used to obtain the response surfaces on the chosen model: $Y = b_0 + b_1X_1 + b_2X_2 + b_3X_1^2 + b_4X_2^2 + b_5X_1X_2$

Where Y represents the dependent variable, while X_1 and X_2 are the independent variables corresponding to Triton X-100 and Tween 80, respectively. The coefficients b_1–b_5 represent the independent variables, b_1 and b_2 correspond to the linear effects, b_3 and b_4 to the quadratic effects and b_5 represents the interaction term. The intercept is denoted by b_o. Experimental data were analyzed using Microsoft Excel and are presented as the mean ± Standard Error from independent replicates. A two-way analysis of variables (two-way ANOVA) was performed using STATISTICA software (version 9.0) to assess differences between the variances. Results with *P*-values below 0.05 were regarded as statistically significant.

RESULTS AND DISCUSSION

Optimizing the inoculum cell count

The effect of the initial inoculum size on lipase production was investigated in YEPD medium supplemented with olive oil and Tween 80 at a concentration of 1 % and 0.05 %, respectively, using 1 strain of *Y. lipolytica*, 3 strains of *Y. yakushimensis* and 2 strains of *Y. divulgata*. Three inoculum sizes were selected as follows: 10^6, 5^*10^6 and 10^7 CFU mL^{-1}. In addition to lipase activity, pH changes were also monitored throughout the fermentation process, and the results are shown in Table 2.

Table 2. Changes in pH values during lipase fermentation by different *Yarrowia* strains in YEPD medium containing olive oil

Strains	Cell concentration (CFU mL^{-1})	pH values					
		Day 0	Day 1	Day 2	Day 3	Day 6	Day 7
Y. lipolytica 854/4	10^6	6.39	6.06	5.34	5.17	5.32	6.06
	5^*10^6	6.41	5.91	5.48	5.16	5.53	6.15
	10^7	6.42	6.05	5.53	5.24	5.13	5.75
Y. yakushimensis NCAIM Y.02049	10^6	6.38	5.73	5.37	5.26	5.75	6.02
	5^*10^6	6.41	5.63	5.45	5.27	5.30	5.89
	10^7	6.46	5.74	5.52	5.41	5.26	5.20
Y. yakushimensis NCAIM Y.02050	10^6	6.19	5.57	5.75	5.61	5.67	6.62
	5^*10^6	6.14	5.45	5.66	5.29	5.25	5.60
	10^7	6.21	5.44	5.51	5.45	5.92	6.24
Y. yakushimensis NCAIM Y.02052	10^6	6.41	5.47	5.21	4.97	5.09	5.22
	5^*10^6	6.41	5.51	5.21	5.00	4.87	4.98
	10^7	6.43	5.47	5.24	5.01	4.90	4.98
Y. divulgata NCAIM Y.02062	10^6	6.23	5.81	5.32	5.02	4.94	5.04
	5^*10^6	6.20	5.72	5.38	5.18	7.01	7.88
	10^7	6.17	5.73	5.41	5.21	6.97	7.80
Y. divulgata 5257	10^6	6.16	5.68	4.86	4.59	7.29	6.64
	5^*10^6	6.11	5.48	4.78	4.54	5.50	5.63
	10^7	6.18	5.57	4.90	4.66	6.28	5.81

The initial pH values of the fermentation medium were around pH 6.0–6.5, and the pH started to decrease over time during fermentation until day 3 (Table 2). However, the pH values increased at the 7th day in almost all cases of concentrations and most strains, which may be due to cell lysis (Domínguez et al., 2003). The *Y. yakushimensis* NCAIM Y.02052 strain was the exception to this observation. The pH value decreased continuously or stagnated at pH 5.0–5.2 at all inoculum levels. A similar observation was obtained in the case of the *Y. yakushimensis* NCAIM Y.02049 strain at a concentration of 10^7 CFU mL^{-1}, as well as in the case of *Y. divulgata* NCAIM Y.02062 strain at a concentration of 10^6 CFU mL^{-1}. In most cases, the pH decreased to values between pH 5 and pH 5.5 after 3 days of fermentation, except for the *Y. divulgata* 5257 strain. At concentrations of 10^6 CFU mL^{-1} and 5^*10^6 CFU mL^{-1}, the pH reached pH 4.5 value. Domínguez et al. (2003) observed in their experiment that the pH decreased from an initial value of pH 6, which was likely due to the produced lipases. This finding is supported by the study of Válek and Pohanka (2021), who introduced a novel methodology for the determination of lipase activity. This method is based on monitoring pH changes using ISFET electrodes. The authors demonstrated that the presence of the lipase enzyme induced a decrease in pH in all tested samples. Furthermore, it has also been proven that slightly acidic pH values favour the production of lipase enzymes. Pereira et al. (2019) studied mango seeds and peels, – waste products of industrial fruit processing – as potential substrates for the lipase production by *Y. lipolytica*. They found that the optimal pH for lipase secretion was approximately pH 5. These results are consistent with the pH values and lipase activity observed in *Yarrowia* isolates (Fig. 1, Table 2).

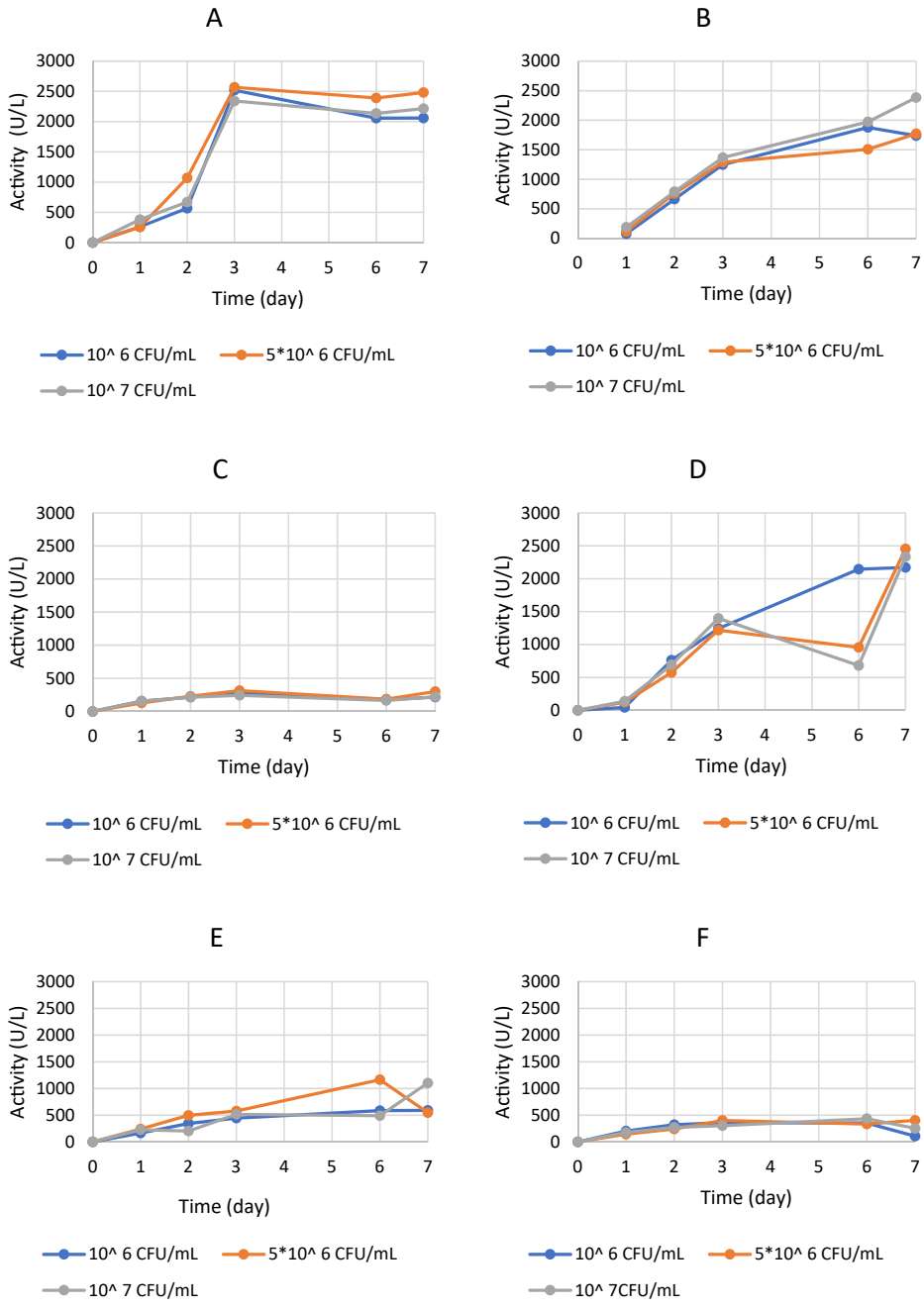

Fig. 1. Effect of inoculum concentration on the lipase enzyme production of *Y. lipolytica* 854/4 (A), *Y. yakushimensis* NCAIM Y.02049 (B), *Y. yakushimensis* NCAIM Y.02050 (C), *Y. yakushimensis* NCAIM Y.02052 (D), *Y. divulgata* NCAIM Y.02062 (E), and *Y. divulgata* 5257 (F) strains

As reported by Sipiczki et al. (2024), *Y. lipolytica* 854/4 exhibits good lipase production in YEPD medium with olive oil and Tween 80 supplementation. During fermentation, this strain demonstrated the highest lipase activity (2,567 U/L) among the tested *Yarrowia* strains in the fermentation medium inoculated with 5^*10^6 CFU mL^{-1} cells (Fig. 1A). Similar enzyme activities were observed at inoculum concentrations of 10^6 and 10^7 CFU mL^{-1}, reaching 2,518 U/L and 2,340 U/L, respectively. The most efficient lipase production was obtained on the 3rd day of fermentation at all inoculum levels, however, the initial cell count did not notably affect the enzyme activity.

Similar lipase activity can be achieved through prolonged fermentation in case of both *Y. yakushimensis* NCAIM Y.02049 strain (Fig. 1B) and *Y. yakushimensis* NCAIM Y.02052 strain (Fig. 1D). On the 7th day of fermentation at an inoculum cell count of 10^7 CFU mL^{-1} the enzyme activity achieved 2,382 U/L and 2,332 U/L, respectively. The maximum lipase activities remained below 1,900 U/L when lower initial cell concentrations (5^*10^6 CFU mL^{-1} or 10^6 CFU mL^{-1}) were used in the case of *Y. yakushimensis* NCAIM Y.02049 strain. The highest enzyme activity of *Y. yakushimensis* NCAIM Y.02052 strain (2,453 U/L) was measured in the fermentation medium with an inoculum concentration of 5^*10^6 CFU mL^{-1}. However, the inoculated cell concentration did not greatly influence the lipase production during the first three day of the fermentation. A decline in enzyme activity was noted from day 3 to day 6 at initial inoculum levels of 10^7 CFU mL^{-1} and 5^*10^6 CFU mL^{-1}, followed by a significant increase on the 7th day. The observed phenomenon could be explained by the presence of specific medium components, including organic nitrogen sources and long-chain fatty acids which stimulate lipase production. Otherwise, glucose inhibits it. Lipase production is under the catabolite repression by glucose, which may explain the observed trend of enzyme production increasing and then decreasing (Lock et al., 2007). The implication of glucose catabolic repression in the regulation of lipase synthesis is confirmed by several studies. Involvement of glucose catabolite repression was demonstrated with the analysis of the *Y. lipolytica* LgX64.81 mutant (Fickers et al., 2013). This strain exhibited a longer generation time and a decreased consumption rate of both glucose and fructose, which correlated with reduced hexokinase activity. Overexpression of *HXK1* in this strain resulted in increased kinase activity but was accompanied by a reduction in *LIP2* expression, as monitored using a β-galactosidase fusion reporter. A continuous increase in lipase activity of *Y. yakushimensis* NCAIM Y.02052 strain was observed during fermentation initiated with a concentration of 10^6 CFU mL^{-1} cells.

The lowest activity was shown by *Y. yakushimensis* NCAIM Y.02050 strain (Fig. 1C) among the investigated strains. Lipase production of this strain followed a pattern comparable to that observed in *Y. lipolytica* 854/4 strain; however, the maximum lipase activity was achieved just 314 U/L on the 3rd day of fermentation. Lipase activity did not differ significantly across the three different inoculum concentrations. The *Y. divulgata* 5257 strain exhibited only minimal enzyme activity (Fig. 1F). The maximal value (434 U/L) was achieved with an inoculum concentration of 10^7 CFU mL^{-1} on the 6th day. In contrast, the other strain of the *Y. divulgata* species, the NCAIM Y.02062 strain produces the enzyme much more efficiently compared to *Y. divulgata* 5257 strain. At an inoculum level of 5^*10^6 CFU mL^{-1}, the maximum lipase activity (1,164 U/L) was attained after 6 days.

These data confirm that the higher the initial inoculum concentration, the greater the lipase production. Meanwhile in the cases of *Y. lipolytica* 854/4 and *Y. yakushimensis* NCAIM Y.02052 strains, the inoculum concentration of 5^*10^6 CFU mL^{-1} proved to be the most effective for

lipase production, whereas in the case of *Y. yakushimensis* NCAIM Y.02049 strain, the inoculum size of 10^7 CFU mL^{-1} has resulted the highest activity. Colla et al. (2016) investigated the production of lipase by filamentous fungi *Aspergillus niger* and *A. flavus* during submerged fermentation. The results of lipolytic activity obtained from the Plackett–Burman design experiments were evaluated through analysis of variance showing no significant impact ($P > 0.10$) of either bran type or inoculum size — measured by fungal colony diameters of 1 or 2 cm — on lipase production in submerged fermentation. Godoy et al. (2009) during their solid-state fermentation (SSF) with the filamentous fungus, *Penicillium simplicissimum* also concluded that the concentration of the inoculum is not significant regarding lipase production.

When the pH of the medium increases or stagnates, then lipase enzyme production declines or remains constant. This phenomenon was clearly observed in the experiments, because for most strains the pH value either increased or stagnated on the final day of fermentation, which resulted in a stagnation of lipase activity. An exception to this trend was considered in *Y. yakushimensis* NCAIM Y.02049 strain at an inoculum concentration 5^*10^6 CFU mL^{-1}, and the *Y. divulgata* NCAIM Y.02062 strain at 10^7 CFU mL^{-1}, where lipase activity increased despite a simultaneous rise in pH compared to the previous day. The reason for this could be that the increase in pH favours the activity of the produced lipase. Experiments proved that *Y. lipolytica* lipases exhibit maximal hydrolytic activity under slightly acidic conditions, with the optimal pH ranging from 6 to 7.5 depending on the nature of the triglyceride substrate (tributyrin, trioctanoin, or triolein) and the specific assay conditions (Aloulou et al., 2007a, b; Yu et al., 2007). According to Carvalho et al. (2017), the lipase produced by *Y. lipolytica* IMUFRJ 50682 exhibited optimal activity within a pH range of 6–7. Consistently, other studies have also reported pH 7 as the optimum for *Y. lipolytica* lipase activity (Fickers et al. 2005, 2006). Optimal activity of the lipase enzyme was observed at pH 7.2 in Sorensen buffer, as reported by Sipiczki et al. (2024), while enzyme performance declined markedly at pH 5 and pH 8.

Effect of Triton X-100 and Tween 80 on lipase production

Response surface methodology coupled with a central composite design (CCD) was applied to evaluate the effect of different concentrations of Triton X-100 and Tween 80 on lipase production in a medium supplemented with 1% olive oil (Table 3). This model was chosen because the

Table 3. Experimental setup settings for lipase production by different *Yarrowia* strains in YEPD medium containing 1% olive oil, Triton X-100 and Tween 80

Setting	Triton X-100 level	Tween 80 level	Triton X-100 (%)	Tween 80 (%)
1	−1.41	0	0.0148	0.05
2	−1	1	0.025	0.075
3	0	1.41	0.05	0.0856
4	1	1	0.075	0.075
5	1.41	0	0.0856	0.05
6	1	−1	0.075	0.025
7	0	−1.41	0.05	0.0148
8	−1	−1	0.025	0.025
9	0	0	0.05	0.05
10	0	0	0.05	0.05

method was often used for optimization tasks, due to its efficiency, given the low number of samples (Nwabueze, 2010).

The one-week fermentation was initiated with the most optimal cell concentration for all strains, specifically, $5^{*}10^{6}$ CFU mL^{-1} culture was used for *Y. lipolytica* 854/4, *Y. yakushimensis* NCAIM Y.02050, *Y. yakushimensis* NCAIM Y.02052, and *Y. divulgata* NCAIM Y.02062, while 10^{7} CFU mL^{-1} was applied for *Y. yakushimensis* NCAIM Y.02049 and *Y. divulgata* 5257 to inoculate the fermentation medium.

During the fermentation of strains *Y. lipolytica* 854/4, *Y. yakushimensis* NCAIM Y.02049, *Y. yakushimensis* NCAIM Y.02052 and *Y. divulgata* 5257, the pH values rapidly decreased to pH 5–5.5 following a similar trend (Table 4). However, for the *Y. divulgata* NCAIM Y.02062 strain, the pH decreased more slowly. In contrast, for the *Y. divulgata* 5257 strain, the pH dropped to 4.5 under certain conditions, followed by a slight increase on the final day of fermentation.

Table 5 presents that the maximum activities were achieved on the 7th day of fermentation for almost all *Yarrowia* strains. An exception was *Y. divulgata* 5257 strain, which peak activity occurred on the 6th day. Among the five yeast strains tested, the highest lipase activity was measured for the *Y. lipolytica* 854/4 strain based on the experimental results.

Table 4. Values of the pH change in culture media supplemented with different concentrations of Triton X- and Tween 80. *Y. lipolytica* 854/4, *Y. yakushimensis* NCAIM Y.02049, *Y. yakushimensis* NCAIM Y.02052, *Y. divulgata* NCAIM Y.02062, and *Y. divulgata* 5257 strains

Day	Run	*Y. lipolytica* 854/4	*Y. yakushimensis* NCAIM Y.02049	*Y. yakushimensis* NCAIM Y.02052	*Y. divulgata* NCAIM Y.02062	*Y. divulgata* 5257
Day 0				6.5		
Day 2	1	5.35	5.6	5.24	6.25	4.79
	2	5.39	5.67	5.23	6.13	4.75
	3	5.49	5.69	5.32	6.51	4.82
	4	5.46	5.77	5.31	6.51	4.77
	5	5.49	5.85	5.36	6.54	4.78
	6	5.52	5.83	5.31	6.49	4.79
	7	5.37	5.8	5.32	6.52	4.8
	8	5.4	5.65	5.29	6.11	4.88
	9	5.46	5.75	5.33	6.48	4.8
	10	5.44	5.76	5.34	6.49	4.81
Day 7	1	5.1	5.35	5.05	5.48	4.65
	2	5.15	5.38	4.98	5.31	4.56
	3	5.43	5.36	4.98	5.38	4.77
	4	5.31	5.34	4.95	5.41	4.59
	5	5.4	5.61	5.00	5.37	4.67
	6	5.39	5.6	4.99	5.17	4.69
	7	5.36	5.53	5.01	5.33	4.45
	8	5.52	5.37	5.04	5.41	4.61
	9	5.06	5.4	5.05	5.11	4.58
	10	5.05	5.37	5.03	5.08	4.58

Table 5. Maximum lipase activities during 7-day fermentation of different *Yarrowia* strains in YEPD medium containing Tween 80, Triton X-100 and olive oil

Run	Lipase activity (U/L)				
	Y. lipolytica 854/4 (at day7)	*Y. yakishimensis* Y.02049 (at day7)	*Y. yakishimensis* Y.02052 (at day7)	*Y. divulgata* Y.02062 (at day7)	*Y. divulgata* 5257 (at day6)
1	2,297	1,688	1,475	1,238	1,037
2	2,415	2,101	1,543	1,444	1,113
3	2,550	2,077	1,395	1,202	987
4	1,753	1,927	1,398	1,227	1,147
5	1,952	1,950	1,115	1,203	1,036
6	1,615	1,745	1,187	1,205	1,008
7	2,494	1,798	1,290	1,262	942
8	2,290	1,142	1,336	1,275	1,436
9	2,261	1,220	1,112	1,239	1,484
10	2,249	1,217	1,274	1,281	1,445

Generally, higher levels of Triton X-100 did not support lipase production for the *Y. lipolytica* 854/4 strain. Optimal activity was detected at lower concentrations of Triton X-100 combined with higher concentrations of Tween 80, when both surfactants were used at equal concentrations. Applying lower but equal concentrations of the two surfactants resulted in the lowest lipase activity for *Y. yakushimensis* NCAIM Y.02049 strain, whereas the highest values for *Y. divulgata* 5257 strain. Similar activity values were measured for *Y. divulgata* NCAIM Y.02062 strain under almost all conditions regardless of the volume or type of supplementation.

According to the results of variance analysis (ANOVA) shown in Table 6, the most significant factor affecting enzyme production of the *Y. divulgata* NCAIM Y.02062 strain ($P = 0.01$) and the *Y. yakushimensis* NCAIM Y.02049 strain ($P = 0.01$) was the quadratic coefficient of Tween 80 (Tween 80 (Q)). In addition, the linear coefficient of Tween 80 also significantly influenced enzyme activity in both strains, with *P*-values ($P = 0.03$; $P = 0.05$) indicating significance at the 95% confidence level. Additionally, the quadratic coefficient of Tween 80 also had a significant impact on the enzyme activity of the *Y. divulgata* 5257 strain ($P = 0.04$) at the same confidence level. Triton X-100 significantly affected the enzyme production of the *Y. lipolytica* 854/4 strain and *Y. divulgata* NCAIM Y.02062 strain. However, the *Y. divulgata* 5257 strain was influenced by the quadratic Triton X-100 ($P = 0.07$; $P = 0.08$; $P = 0.07$). The enzyme activity of the *Y. yakushimensis* NCAIM Y.02049 strain was also significantly influenced by the quadratic coefficient of Triton X-100 ($P = 0.03$). For the *Y. yakushimensis* NCAIM Y.02052 strain, two linear variables had a statistically significant effect on enzyme production at the 90% confidence interval. The interaction between Tween 80 and Triton X-100 showed a significant effect on the lipase activity in *Y. yakushimensis* NCAIM Y.02049 ($P = 0.08$). Lipase synthesis was strongly enhanced by positive correlation of Tween 80 ($P = 0.08$) for the *Y. yakushimensis* NCAIM Y.02052 strain.

Table 6. Variance analysis for Triton X-100 and Tween 80 factors

Factor	*Y. lipolytica* 854/4		*Y. yakushimensis* NCAIM Y.02049		*Y. yakushimensis* NCAIM Y.02052		*Y. divulgata* Y.02062		*Y. divulgata* 5257	
	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*
Triton X-100 (%) (L)	5.97	**0.07**	2.7	0.17	9.97	**0.034**	2.27	0.21	0.86	0.4
Triton X-100 (%) (Q)	1.33	0.31	10.79	**0.03**	2.26	0.207	0.01	0.91	5.75	**0.07**
Tween 80 (%) (L)	0.21	0.67	10.17	**0.03**	4.97	**0.08**	0.23	0.65	0.08	0.79
Tween 80 (%) (Q)	0.2	0.67	16.24	**0.015**	4.27	**0.1**	0.002	0.96	8.47	**0.04**
Triton X-100 Tween 80	0.0007	0.98	5.2	**0.08**	0.0005	0.98	0.86	0.4	2.33	0.2

Values highlighted in bold indicate a significant effect at a confidence level of at least 90%.

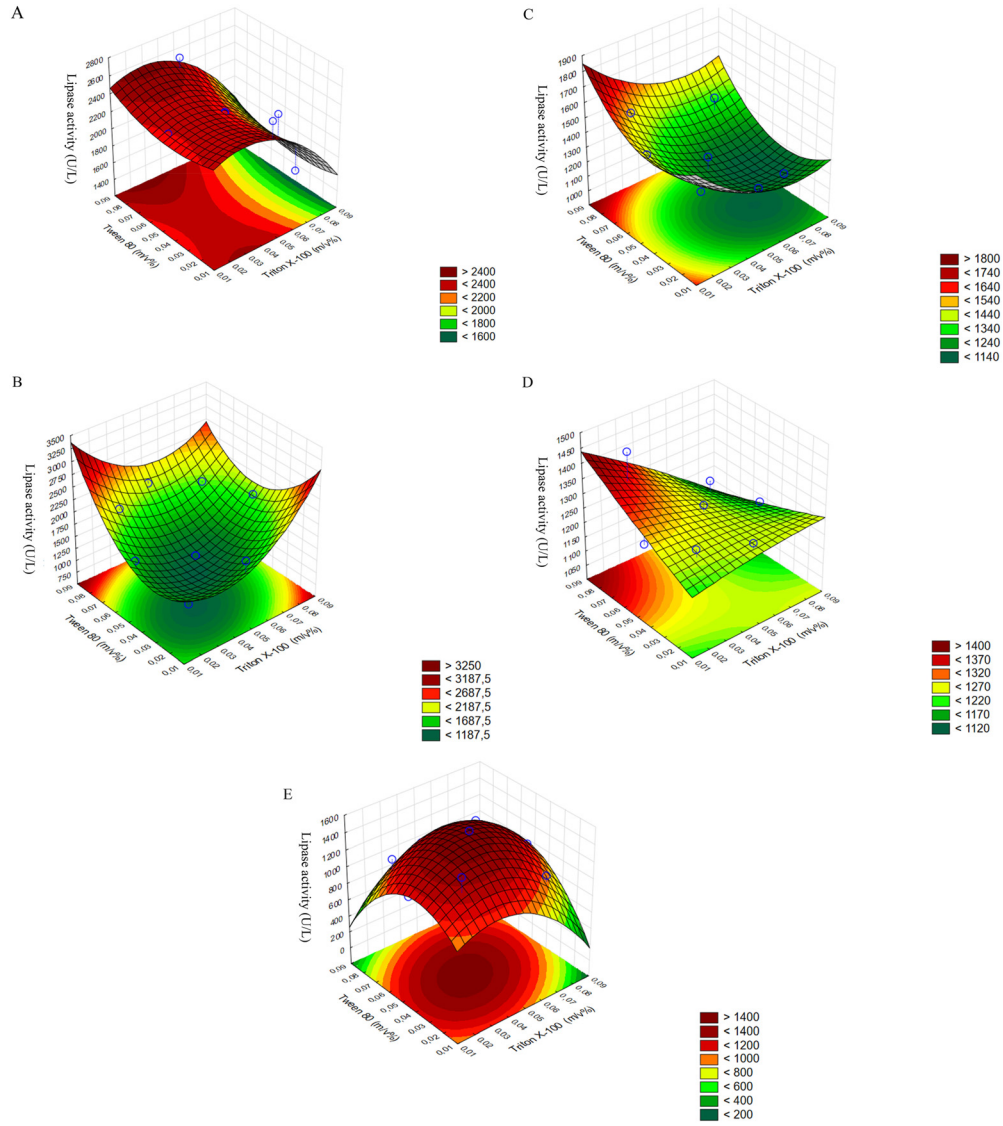

Fig. 2. Effect of different concentrations of Triton X-100 and Tween 80 on the lipase enzyme production of *Y. lipolytica* 854/4 (A), *Y. yakushimensis* NCAIM Y.02049 (B), *Y. yakushimensis* NCAIM Y.02052 (C), *Y. divulgata* NCAIM Y.02062 (D), and *Y. divulgata* 5257 (E) strains

Figure 2 presents the response surface plots illustrating lipase activity in relation to the concentration of Tween 80 and Triton X-100. According to the results, the optimal concentrations of the independent variables (Tween 80 and Triton X-100) within the tested range can be determined as follows:

- *Y. lipolytica* 854/4 strain: 0.09% Tween 80, 0.03% Triton X-100 (A)
- *Y. yakushimensis* NCAIM Y.02049 strain: 0.09% Tween 80 and 0.01% Triton X-100 (B)

- *Y. yakushimensis* NCAIM Y.02052 strain: 0.09% Tween 80 and 0.01% Triton X-100 (C)
- *Y. divulgata* NCAIM Y.02062 strain: 0.09% Tween 80 and 0.01% Triton X-100 (D)
- *Y. divulgata* 5257 strain: 0.05% Tween 80, 0.05% Triton X-100 (E)

Kanimozhi and Perinbam (2010) studied the enhancement of lipase production by *Pseudomonas* sp. Lp1 strain and evaluated the impact of various additives. Among the surfactants tested, Tween 80 proved to be more effective than Triton X-100 at a concentration of 0.2% (v/v). In this experiment, it was also observed that Triton X-100 induces enzyme production in yeasts; however, its application at higher concentrations did not stimulate lipase secretion. In *Candida rugosa* the addition of 0.45 g L^{-1} Triton X-100 increased enzyme production fivefold compared to samples without surfactants (Perna et al., 2017). Triton X-100, as a non-ionic surfactant, induces lipase activity by modulating enzyme-substrate interactions. At higher concentrations, Triton X-100 solubilizes membrane bound proteins and phospholipids, leading to lethal effects on yeast cells (Dinarvand et al., 2013). Based on the experimental results, it can be concluded that higher concentration (0.09%) of Tween 80 results in higher lipase activity for most of the tested strains. Furthermore, from the two surfactants Tween 80 has a greater effect on lipase activity than the other one. However, the results of *Y. divulgata* 5257 strain indicate that using both surfactants at equal concentrations is optimal for lipase production.

CONCLUSION

The experimental findings indicate that the most efficient lipase-producing strains in olive oil as a substate with Tween 80 supplementation were *Y. lipolytica* 854/4 strain, and two isolates of *Y. yakushimensis*, NCAIM Y.02049 strain and NCAIM Y.02052 strain. Lipase activities between 2,300 and 2,600 U/L were achieved by all three strains at the end of 1-week fermentation. However, *Y. lipolytica* 854/4 strain reached this value more rapidly, after 3 days of fermentation. This study confirmed a correlation between pH and lipase enzyme production. Over time, the presence of the lipase enzyme induced a decrease in pH in all tested samples and it has also been proven that slightly acidic pH values favour lipase production.

Optimization of initial inoculum concentration demonstrated that the inoculum size did not have a statistically significant effect on enzyme production among the tested strains. However, in the case of individual strains, different concentrations proved to be optimal.

Both Triton X-100 and Tween 80 stimulate lipase production, but the optimal concentration varies from strain to strain. In conclusion, these findings confirm that *Y. yakushimensis* strains NCAIM Y.02049 and NCAIM Y.02052 exhibit considerable lipase activity on olive oil supplemented with Tween 80 and Triton X-100. These isolates could serve as potential biocatalysts and alternatives to *Y. lipolytica* in industrial applications.

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REFERENCES

Aloulou, A., Puccinelli, D., De Caro, A., Leblond, Y., and Carrière, F. (2007a). A comparative study on two fungal lipases from *Thermomyces lanuginosus* and *Yarrowia lipolytica* shows the combined effects of detergents and pH on lipase adsorption and activity. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*, 1771(12): 1446–1456, https://doi.org/10.1016/j.bbalip.2007.10.006

Aloulou, A., Rodriguez, J.A., Puccinelli, D., Mouz, N., Leclaire, J., Leblond, Y., and Carrière, F. (2007b). Purification and biochemical characterization of the LIP2 lipase from *Yarrowia lipolytica*. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*, 1771(2): 228–237, https://doi.org/10.1016/j.bbalip.2006.12.006

Bankar A.V., Kumar A.R., Zinjarde S.S. (2009). Environmental and industrial applications of *Yarrowia lipolytica*. *Applied Microbiology and Biotechnology*, 84: 847–865, https://doi.org/10.1007/s00253-009-2156-8

Barth, G. and Gaillardin, C. (1997). Physiology and genetics of the dimorphic fungus *Yarrowia lipolytica*. *FEMS Microbiology Reviews*, 19(4): 219–237, https://doi.org/10.1111/j.1574-6976.1997.tb00299.x

Carvalho, T., Finotelli, P.V., Bonomo, R.C.F., Franco, M., and Amaral, P.F.F. (2017). Evaluating aqueous two-phase systems for *Yarrowia lipolytica* extracellular lipase purification. *Process Biochemistry*, 53: 259–266, ISSN 1359-5113, https://doi.org/10.1016/j.procbio.2016.11.019

Colla, L.M., Primaz, A.L., Benedetti, S., Loss, R.A., de Lima, M., Reinehr, C.O., Bertolin, T.E., and Costa, J.A.V. (2016). Surface response methodology for the optimization of lipase production under submerged fermentation by filamentous fungi. *Industrial Microbiology Brazilian Journal of Microbiology*, 47(2): Apr-Jun, https://doi.org/10.1016/j.bjm.2016.01.028

de Almeida, A.F., Tauk-Tornisielo, S.M., and Carmona, E.C. (2013). Acid lipase from *Candida viswanathii*: production, biochemical properties, and potential application. *Biomed Research International*, 1: 435818, https://doi.org/10.1155/2013/435818

Dinarvand, M., Rezaee, M., Masomian, M., Jazayeri, S.D., Zareian, M., Abbasi, S., and Ariff, A.B. (2013). Effect of C/N ratio and media optimization through response surface methodology on simultaneous productions of intra-and extracellular inulinase and invertase from *Aspergillus niger* ATCC 20611. *BioMed Research International*, 1: 508968, https://doi.org/10.1155/2013/508968

Domínguez, A., Deive, F.J., Sanroman, M.A., and Longo, M.A. (2003). Effect of lipids and surfactants on extracellular lipase production by *Yarrowia lipolytica*. *Journal of Chemical Technology and Biotechnology*, 78: 1166–1170, https://doi.org/10.1002/jctb.922

Fickers, P., Benetti, P.H., Waché, Y., Marty, A., Mauersberger, S., Smit, M.S., and Nicaud, J.M. (2005). Hydrophobic substrate utilisation by the yeast *Yarrowia lipolytica*, and its potential applications. *FEMS Yeast Research*, 527–543, https://doi.org/10.1016/j.femsyr.2004.09.004

Fickers, P., Ongena, M., Destain, J., Weekers, F., and Thonart, P. (2006). Production and down-stream processing of an extracellular lipase from the yeast *Yarrowia lipolytica*. *Enzyme and Microbial Technology*, 756–759, https://doi.org/10.1016/j.enzmictec.2005.08.005

Fickers, P., Sauveplane, V., and Nicaud, JM. (2013). The lipases from *Y. lipolytica*: genetics, production, regulation, and biochemical characterization. In: Barth, G. (Ed.), *Yarrowia lipolytica. Microbiology monographs*, Vol. 25. Springer, Berlin, Heidelberg, https://doi.org/10.1007/978-3-642-38583-4_3

Ghosh, P.K., Saxena, R.K., Gupta, R., Yadav, R.P., and Davidson, S. (1996). Microbial lipases: production and applications. *Science Progress*, 79(2): 119–157, http://www.jstor.org/stable/43421608.

Godoy, M.G., Gutarra, M.L.E., Maciel, F.M., Felix, S.P., Bevilaqua, J.V., Machado, O.L.T., and Freire, D.M.G. (2009). Use of a low-cost methodology for biodetoxification of castor bean waste and lipase production. *Enzyme and Microbial Technology*, 44(5): 317–322, 6 May, https://doi.org/10.1016/j.enzmictec.2009.01.002

Gouliamova, D.R.A., Guéorguiev, S.M.T., and Groenewald, M. (2017). Yarrowia parophonii. *Fungal Planet*, 628.

Groenewald M. and Smith M.T. (2013). The teleomorph state of *Candida deformans* Langeron & Guerra and description of *Yarrowia yakushimensis* comb. nov. *Antonie van Leeuwenhoek*, 103(5): 1023–1028, https://doi.org/10.1007/s10482-013-9882-8

Groenewald, M., Boekhout, T., Neuvéglise, C., Gaillardin, C., Van Dijck, P.W., and Wyss, M. (2014). *Yarrowia lipolytica*: safety assessment of an oleaginous yeast with a great industrial potential. *Critical Reviews in Microbiology*, 40(3): 187–206, https://doi.org/10.3109/1040841X.2013.770386

Gutiérrez-Arnillas, E., Arellano, M., Deive, F.J., Rodríguez, A., and Sanromán, M.A. (2017). Unravelling the suitability of biological induction for halophilic lipase production by *Halomonas* sp. LM1C cultures. *Bioresource Technology*, 239: 368–377, https://doi.org/10.1016/j.biortech.2017.04.128

Kanimozhi, S. and Perinbam, K. (2010). Optimization of media components and growth conditions to enhance lipase production by *Pseudomonas* sp. Lp1. *Biomedical and Pharmacology Journal*, 3(2), http://biomedpharmajournal.org/?p=1562.

Kurtzman, C.P., Fell, J.W., and Boekhout, T. (2011). *The yeasts, a taxonomic study*, Vol. 5. Elsevier, Amsterdam, p. 2080, ISBN: 9780444521491.

Liu, K.F., Li, X.H., and Hui, F.L. (2018). *Yarrowia brassicae* f.a., sp. nov., a new yeast species from traditional Chinese sauerkraut. *International Journal of Systematic and Evolutionary Microbiology*, 68(6): 2024–2027, https://doi.org/10.1099/ijsem.0.002783

Lock, L.L., Corbellini, V.A., and Valente, P. (2007). Lipases produced by yeasts: powerful biocatalysts for industrial purposes. In: *Tecnológica*, Vol. 11, Santa Cruz do Sul, pp. 18–25, 1 e 2, https://doi.org/10.17058/tecnolog.v11i1.153

Nagy, E.S. (2015). *Élelmiszerromlást okozó Yarrowia csoport biodiverzitása különböző élelmiszerekben*, Doktori disszertáció, Budapest, https://phd.lib.uni-corvinus.hu/855/2/Nagy_Edina_Szandra_thu.pdf.

Nagy, E., Niss, M., Dlauchy, D., Arneborg, N., Nielsen, D.S., and Péter, G. (2013). *Yarrowia divulgata* f.a., sp. nov., a yeast species from animal-related and marine sources. *International Journal of Systematic and Evolutional Microbiology*, 63: 4818–4823, https://doi.org/10.1099/ijs.0.057208-0

Nicaud, J.M. (2012). Yarrowia lipolytica. *Yeast (Chichester, England)*, 29(10): 409–418, https://doi.org/10.1002/yea.2921

Nwabueze, T.U. (2010). Basic steps in adapting response surface methodology as mathematical modelling for bioprocess optimisation in the food systems. *International Journal of Food Science and Technology*, 45: 1768–1776, https://doi.org/10.1111/j.1365-2621.2010.02256.x

Pereira, A.D.S., Fontes-Sant'Ana, G.C., and Amaral, P.F. (2019). Mango agro-industrial wastes for lipase production from *Yarrowia lipolytica* and the potential of the fermented solid as a biocatalyst. *Food and Bioproducts Processing*, 115: 68–77, https://doi.org/10.1016/j.fbp.2019.02.002

Perna, R.F., Tiosso, P.C., Sgobi, L.M., Vieira, A.M.S., Vieira, M.F., Tardioli, P.W., Soares, C.M.F., and Zanin, G.M. (2017). Effects of Triton X-100 and PEG on the catalytic properties and thermal stability of lipase from *Candida rugosa* free and immobilized on glyoxyl-agarose. *The Open Biochemistry Journal*, 11: 66–76, https://doi.org/10.2174/1874091X01711010066

Reddy, L.V.A., Wee, Y., Yun, J., and Ryu, H. (2008). Optimization of alkaline protease production by batch culture of *Bacillus* sp. RKY3 through Plackett-Burman and response surface methodological approaches. *Bioresource Technology*, 99: 2242–2249, https://doi.org/10.1016/j.biortech.2007.05.006

Sharma, P., Sharma, N., Pathania, S., and Handa, S. (2017). Purification and characterization of lipase by *Bacillus methylotrophicus* PS3 under submerged fermentation and its application in detergent industry, (2017). *Journal of Genetic Engineering and Biotechnology*, 15(2): 369–377, https://doi.org/10.1016/j.jgeb.2017.06.007

Sipiczki, G., Micevic, S.S., Kohari-Farkas, C., Nagy, E.S., Nguyen, Q.D., Gere, A., and Bujna, E. (2024). Effects of olive oil and Tween 80 on production of lipase by *Yarrowia* yeast strains. *Processes*, 12(6): 1206, https://doi.org/10.3390/pr12061206

Treichel, H., de Oliveira, D., Mazutti, M.A., Di Luccio, M., and Oliveira, J.V. (2010). A review on microbial lipases production. *Food and Bioprocess Technology*, 3: 182–196, https://doi.org/10.1007/s11947-009-0202-2

Válek, T. and Pohanka, M. (2021). The determination of lipase activity by measuring pH using ion-sensitive field-effect transistor. *International Journal of Electrochemical Science*, 16: 7, https://doi.org/10.20964/2021.07.59

van der Walt J.P. and von Arx J.A. (1980). The yeast genus *Yarrowia* gen. nov. *Antonie van Leeuwenhoek*, 46: 517–521, https://doi.org/10.1007/BF00394008

Yu, M, Qin, S, and Tan, T (2007). Purification and characterisation of the extracellular lipase Lip2 from *Yarrowia lipolytica*. *Process Biochemistry*, 42: 384–91, https://doi.org/10.1016/j.procbio.2006.09.019