Expression of native and mutant extracellular lipases from *Yarrowia lipolytica* in *Saccharomyces cerevisiae*

Farshad Darvishi*

Department of Microbiology, Faculty of Science, University of Maragheh, Maragheh 55181-83111, Iran.

Summary

*Saccharomyces cerevisiae* cannot produce extracellular lipase and utilize low-cost lipid substrates. This study aimed to express extracellular lipase from *Yarrowia lipolytica* in *S. cerevisiae*, construct recombinant oily substrate consumer strains, and compare the roles of native and mutant *Y. lipolytica* extracellular lipases in *S. cerevisiae*. The LIP2 gene of *Y. lipolytica* DSM3286 and its mutant *Y. lipolytica* U6 were isolated and cloned by expression vector in *S. cerevisiae*. New recombinant *S. cerevisiae* strains FDS100 containing the native LIP2 gene, and FDS101 containing the mutant LIP2 gene were produced 10 and 15 U ml\(^{-1}\) extracellular lipase respectively, on a production medium containing olive oil. New recombinant *S. cerevisiae* strains produce acceptable amount of extracellular lipase in comparison with *Y. lipolytica* wild-type strains. These strains can utilize olive oil and lipids as low-cost substrates to produce bioethanol, single cell protein and other biotechnologically valuable products. The recombinant *S. cerevisiae* strain with mutant LIP2 produced lipase with 1.5-fold higher activity. The LIP2 gene of *Y. lipolytica* was expressed in *S. cerevisiae* as a heterologous protein without any modifications. Strong components of the *Y. lipolytica* expression/secretion system could be used for high-level production of recombinant proteins in *S. cerevisiae*.

Introduction

For several centuries, *Saccharomyces cerevisiae* has been used in the production of food and ethanol, and today, this organism is also used as a platform for recombinant protein production (Nevoigt, 2008). *Saccharomyces cerevisiae* cannot acquire low-cost lipid substrates such as triacylglycerols from the growth media; therefore, this microorganism does not utilize these substrates for bioconversion to biotechnologically valuable products (Roermund et al., 2003; Ciafardini et al., 2006).

The yeast *Yarrowia lipolytica* utilizes hydrophobic substrates, including alkanes, fatty acids, fats and oils by activating several enzymes such as lipases/esterases (*LIP* genes), cytochromes P450 (ALK genes), and peroxisomal acyl-CoA oxidases (POX genes). Triglycerides are first hydrolysed by lipase into free fatty acids, which are then taken up by the cell (Hadeball, 1991; Iida et al., 1998; Sumita et al., 2002; Fickers et al., 2005a; Theveneau et al., 2007).

Depending on media composition and environmental conditions, *Y. lipolytica* is able to produce several extracellular, membrane-bound and intracellular lipases (Barth and Gaillardin, 1997; Pereira-Meirelles et al., 2000; Guieysse et al., 2004; Cancino et al., 2008). Extracellular lipase Lip2 (38.5 kDa) is encoded by the *LIP2* gene (GenBank Accession No. AJ012632). This gene is responsible for all the extracellular lipase activity of *Y. lipolytica*. It is synthesized as a 334-amino acid (aa) preproprotein containing a signal sequence, a stretch of four dipeptides X-Ala, X-Pro as cleavage sites of diaminopeptidase, followed by a 12-aa pro-region ending with a Lys-Arg dipeptide, which is the cleavage site for the endoprotease encoded by *XPR6* (Pignede et al., 2000). This extracellular lipase Lip2p was reported to hydrolyse long-chain triglycerides, with preference for oleyl residues. The enzyme is efficient for the hydrolysis of natural oils and fats, and stays active at low temperatures (4°C) and pH 3–4 (Barth and Gaillardin, 1997; Destain et al., 1997).

Extracellular lipase Lip2 of *Y. lipolytica* was expressed and characterized in *Pichia pastoris* (Yu et al., 2007). More recently, the *LIP2* gene of *Y. lipolytica* was expressed in *S. cerevisiae* with some modification containing the replacement of native Lip2p prepro sequence with the *S. cerevisiae* carboxypeptidase Y (CPY) signal sequence, as well as an additional serine residue for optimal recognition and cleavage by the *S. cerevisiae* Kex2 protease (*LIP2v* Ser) with an inserted serine codon at aa position 34 by site-directed mutagenesis in the N-terminal *LIP2* sequence. Furthermore, the synthetic gene *LIP2* was codon-optimized for expression in *S. cerevisiae* (Shockey et al., 2011). Fermentation of fatty low-value renewable carbon sources aiming at the production of various added-value metabolites presents a noticeable interest in the sectors of industrial microbiology and

Received 27 March, 2012; revised 16 May, 2012; accepted 22 May, 2012. *For correspondence. E-mail f.darvishi@ymail.com; Tel. (+98) 421 2276068; Fax (+98) 421 2276060.

© 2012 The Author

Microbial Biotechnology © 2012 Society for Applied Microbiology and Blackwell Publishing Ltd
biotechnology (Darvishi et al., 2009). The aims of the study were to express native and mutant extracellular lipases from Y. lipolytica in S. cerevisiae and to construct recombinant oily substrate consumer strains by different and easier strategies, and then compare these enzyme operations.

Results

Recombinant S. cerevisiae strains with Y. lipolytica extracellular lipase LIP2 gene

The yeast Y. lipolytica efficiently degrades hydrophobic substrates such as fatty acids, fats and oils. The LIP2 gene encodes the major extracellular lipase Lip2p, which is responsible for all the extracellular lipase activity of Y. lipolytica (Fickers et al., 2011).

More recently, Shockey et al. expressed codon-optimized LIP2 in S. cerevisiae under the control of the galactose-inducible GAL1 and fatty acid inducible PEX11 promoters with some modifications, including replacement of native Lip2p prepro sequence with the S. cerevisiae carboxypeptidase Y (CPY) signal sequence, and adding a serine residue for optimal recognition and cleavage by the S. cerevisiae Kex2 protease (LIP2-Set) with an inserted serine codon at the aa position 34 by site-directed mutagenesis in the N-terminal LIP2 sequence (Shockey et al., 2011).

In this study, the S. cerevisiae expression vector p426GPD with strong constitutive glycerol phosphate dehydrogenase (GPD) promoter was used for LIP2 gene expression (Fig. 1B). The native LIP2 gene from Y. lipolytica DSM3286 and the mutant LIP2 gene from the mutant Y. lipolytica U6 were cloned into the vector without any modifications. The resulting constructs designated pFDP100 and pFDP101 contained the native and mutant LIP2 gene respectively (Fig. 1C and D). The vectors were transformed to the S. cerevisiae strain CEN.PK 113-5D, and then, the new recombinant S. cerevisiae containing native and mutant extracellular lipase named S. cerevisiae FDS100 and FDS101 respectively.

Expression and secretion of Y. lipolytica extracellular lipase in S. cerevisiae

Extensive studies of the physiological behaviour of S. cerevisiae as well as the important ability of this yeast to express foreign genes in conjunction with its secretory apparatus make S. cerevisiae an attractive host for the production of certain heterologous proteins. Nevertheless, S. cerevisiae exhibits some disadvantages when used for the production of certain recombinant heterologous proteins (Ostergaard et al., 2000). To overcome these undesired modulations of the recombinant protein of interest, some modifications were proposed, including fusion of the prepro-leader sequence of S. cerevisiae to secrete the heterologous protein, codon optimization, etc. (Böer et al., 2007).

A different and easier strategy was used to investigate Y. lipolytica LIP2 gene expression elements in the S. cerevisiae expression system. For example, using a secretion signal peptide and pro-region cleavage site of the Y. lipolytica KEX2-like XPR6 endoprotease, and not using codon optimization to ensure similar S. cerevisiae codon usage.

Surprisingly, hydrolysis halo was detected during phenotypic analysis of the recombinant S. cerevisiae strains FDS100 and FDS101 on YNBT medium, indicating the production of extracellular lipase in these strains. The S. cerevisiae strain 5D had no lipase activity on the YNBT medium, which contained p426GPD vector without a Y. lipolytica extracellular lipase gene (Fig. 2). These results show that Y. lipolytica heterologous proteins, especially extracellular lipase LIP2, can be expressed in S. cerevisiae without any modifications. This is significant because strong components of the Y. lipolytica expression/secretion system could be used for high-level production of recombinant proteins in S. cerevisiae. These components include fatty acids, derivative-inducible POX promoters, peptone-inducible XPR2 promoter, fatty acids and derivatives, alkanes, ethanol- and acetate-inducible ICL1 promoter, LIP2 prepro and XPR2 prepro secretion signals, and LIP2t, XPR2t and PHO5t terminators (Madzak et al., 2004).

Analysis of Y. lipolytica native and mutant extracellular lipases expression in S. cerevisiae

Overexpression of the extracellular lipase in Y. lipolytica was initially achieved by two different approaches. In the Nicaud group, the LIP2 gene was cloned under the control of the strong, oleic acid-inducible POX2 promoter, by using multiple copies of the gene. The resulting strains are actually constructed by metabolic engineering, producing unstable amounts of lipase on expensive laboratory medium. In the Thonart’s group, overproducing mutants were isolated from the wild-type strain CBS6303 by successive rounds of chemical mutagenesis using N-methyl-N’-nitro-N-nitrosoguanidine. This led to the selection of the second-generation mutant LgX64.81, which produced stable amounts of lipase in cheap medium and is now used as an industrial strain (Fickers et al., 2011).

In the first step of our project on inverse metabolic engineering of Y. lipolytica extracellular lipase, an UV mutant strain was obtained that produced 10.5-fold lipase compared with the wild-type strain DSM3286, and could be used at the industrial scale (Darvishi et al., 2011). Sequence analysis showed strict identity between the
Fig. 1. Schematic representation of extracellular lipase gene (LIP2), p426GPD, pFDP100 and pFDP101 vectors. A. Schematic representation of extracellular lipase gene (LIP2) and situation of forward primer (Ylip2START) and reverse primer (Ylip2STOP). Shown are the putative 13-aa signal sequence (SS), followed by a stretch of four dipeptides (DP); a short 12-aa pro region (PRO), including the Lys-Arg (KR) cleavage site of the KEX2-like XPR6 endoprotease; and the mature 301-aa lipase (MATURE). The diamonds indicate the positions of the potential signals for asparagine-linked glycosylation (Asn-X-Thr/Ser). The promoter (P) and terminator (T) regions, consisting of 1.06 and 0.97 kb fragments situated upstream and downstream from the yLIP2 ORF respectively. B. Schematic representation structure of the p426GPD Saccharomyces cerevisiae expression vector. GPD promoter, glycerol phosphate dehydrogenase promoter; Lac promoter, lac promoter; T3, T3 phage promoter; T7 promoter; T7 phage promoter; AmpR, ampicillin resistance gene; URA3, URA3 marker; 2-micron ORI, yeast 2 μ expression replication origin; pMB1 ORI, origin of replication of E. coli; F1 ORI, origin of replication. C. The structure of pFDP100 vector containing extracellular lipase ORF of native Y. lipolytica strain DSM3286 (Y. DSM3286 Lip2). D. The structure of pFDP101 vector containing extracellular lipase ORF of the mutant Y. lipolytica strain U6 (Y. U6 Lip2).
LIP2 sequences of *Y. lipolytica* DSM3286 and its mutant strain U6. However, only two silent substitutions at the positions 362 and 385 were observed in the open reading frame (ORF) region of *LIP2* gene. Two single substitutions and two duplications of the T nucleotide were also detected in its promoter region (Darvishi et al., 2011).

The effects of these genetic changes on *LIP2* expression must be analysed stepwise in a suitable host. To address this problem, the ORF regions in the mutant and native *LIP2* genes were selected for expression in *S. cerevisiae*. Multiple sequence alignment obtained with ClustalW program of the extracellular lipase *LIP2* from *Y. lipolytica* strains CLIB122, DSM3286 and U6 show that threonine at the positions 121 and 129 is replaced with isoleucine and serine in the mutant lipase of *Y. lipolytica* U6 mutant strain compared with the native lipase of wild-type strains (Fig. 3).

The phenotype of the recombinant lipase strains were further investigated by following the growth and lipase activity of strains in liquid medium containing olive oil as the sole carbon source. The *S. cerevisiae* FDS101 containing mutant *LIP2* produced 1.5-fold higher levels of lipase than *S. cerevisiae* FDS100 with the wild-type gene (Fig. 4). Lipase production increased only 1.5-fold by using substitutions in the ORF region. Figure 5 shows the time course of biomass production of *S. cerevisiae* strains 5D, FDS100 and FDS101 during culture in olive oil medium. The growth curves were similar for *S. cerevisiae* strains FDS100 and FDS101. Crude supernatant preparations of enzyme analysed by SDS-PAGE show one main band corresponding to 38 kDa, similar to *Y. lipolytica* extracellular lipase enzyme (data not shown).

**Discussion**

Current agricultural and industrial practices have led to the generation of large amounts of various low-value or negative-cost crude fatty materials. On the other hand, the fermentation of these renewable materials as carbon sources for the production of various added-value metabolites such as enzymes and recombinant protein is of great interest in the industrial microbiology and biotechnology sectors (Darvishi et al., 2009; 2011; Darvishi, 2012).

Free fatty acids are readily taken up by *S. cerevisiae*. Most oils exist as triacylglycerols, a form of lipid that cannot be directly taken up by *S. cerevisiae*. The first step of their catabolism involves hydrolysis into free fatty acids and glycerol by lipolytic enzymes. *S. cerevisiae* cannot produce extracellular lipases and utilize low-cost lipid substrates (Roermund et al., 2003; Fickers et al., 2005a). The *Y. lipolytica* extracellular lipase Lip2p was reported to hydrolyse long-chain triglycerides, with a preference for oleyl residues, and it is the best candidate gene to equip *S. cerevisiae* for utilizing low-cost lipid substrates. In the previous studies, researchers have reported that codon usage in *Y. lipolytica* appears to be rather different compared with that in *S. cerevisiae*, but similar to that in *Aspergillus* genus (Gaillardin and Heslot, 1988; Spencer et al., 2002). However, the heterologous genes expressed in *Y. lipolytica* were not modified to fit this peculiar codon usage (Madzak et al., 2004). The native *LIP2* gene from *Y. lipolytica* DSM3286 and mutant *LIP2* gene from its mutant *Y. lipolytica* U6 were cloned into the vector without any modifications, unlike the work done by Shockey et al., where superabundant modifications for the cloning and expression of *LIP2* gene in *S. cerevisiae* were used (Shockey et al., 2011).

Surprisingly, recombinant *S. cerevisiae* strains expressed and secreted *Y. lipolytica* extracellular lipase, especially on olive oil medium. These results show that heterologous proteins of *Y. lipolytica* could be expressed in *S. cerevisiae* without any modifications. It is important because strong components of the *Y. lipolytica* expression/secretion system could be used for high-level production of recombinant proteins in *S. cerevisiae*. Engineered *S. cerevisiae* strains by Shockey et al. produced lipase on semisynthetic mineral media containing appro-
appropriate amino acid supplements, and either free linoleic acid or trilinolein as well as on laboratory and expensive media (Shockey et al., 2011). *Yarrowia lipolytica* wild-type strains generally have lipase activities ranging from 1.8 U ml\(^{-1}\) to 45.5 U ml\(^{-1}\) (Papanikolaou et al., 2007). Hence, new recombinant *S. cerevisiae* strains produce acceptable amount of extracellular lipase in comparison with *Y. lipolytica* wild-type strains. Furthermore, the recombinant *S. cerevisiae* strains constructed in this study can utilize olive oil and 

![Fig. 3](image-url) Multiple sequence alignment obtained with the ClustalW program of the extracellular lipase LIP2 from *Y. lipolytica* strains CLIB122 (GenBank Accession No. XP000282), DSM3286 (GenBank Accession No. ADL57141), and U6 (GenBank Accession No. ADL57145). Asterisks with grey boxes indicate that threonine at the positions 121 and 129 is replaced with isoleucine and serine for the mutant lipase of *Y. lipolytica* U6 mutant strain in comparison with the native lipase of wild-type strains.

![Fig. 4](image-url) Time course of lipase activity of *Saccharomyces cerevisiae* strain 5D, *S. cerevisiae* strain FDS100, and *S. cerevisiae* strain FDS101 during culture in olive oil medium (as a basal medium for lipase production). Error bars in graph represent the mean standard error from three independent experiments.

![Fig. 5](image-url) Time course of biomass production of *Saccharomyces cerevisiae* strain 5D, *S. cerevisiae* strain FDS100, and *S. cerevisiae* strain FDS101 during culture in olive oil medium (as a basal medium for lipase production). Error bars in graph represent the mean standard error from three independent experiments.
lipids as low-cost substrates to produce bioethanol and other biotechnologically valuable products.

In the literature, it is suggested that lower biomass yield on substrate (Yf) values around 0.5–0.7 g g⁻¹ obtained from high-lipase producing wild Y. lipolytica strains are very satisfactory for single-cell protein fermentation from fatty substrates (Darvishi et al., 2009). The recombinant S. cerevisiae strains have lower Yf values around 0.7 g g⁻¹ and can be used for single-cell protein production.

Inverse metabolic engineering by elucidation of a metabolic engineering strategy involves the following steps: first, identifying, constructing or calculating a desired phenotype; second, determining the genetic or the particular environmental factors conferring that phenotype; and third, endowing that phenotype on another strain or organism by directed genetic or environmental manipulation (Bailey et al., 2002). Now, inverse metabolic engineering is a good strategy in microbial enzyme biotechnology. To successfully attain extracellular lipase inverse metabolic engineering in Y. lipolytica, we constructed the high-level lipase producer strain U6 by UV mutagenesis (Darvishi et al., 2011). The effects of genetic changes in LIP2 expression must be analysed stepwise in a suitable host. To address this problem, the ORF regions of mutant and native LIP2 were selected for expression in S. cerevisiae.

The recombinant S. cerevisiae with mutations in the LIP2 ORF region produced lipase with 1.5-fold higher activity compared with the wild-type ORF region. Therefore, the ORF region is not a good target for inverse metabolic engineering and site-directed mutagenesis of extracellular lipase in Y. lipolytica. Fickers et al. detected a single silent substitution of T for C at the LIP2 coding region as well as six single substitutions and duplication of the ACAGATCAT sequence in the promoter region of the industrial mutant strain LgX64.81 (Fickers et al., 2003). In conclusion, instead of the ORF region, it will be more useful if the studies on mutant LIP2 promoters from the industrial Y. lipolytica strains propose to construct stable and high-level extracellular lipase producing yeast strains.

**Experimental procedures**

**Strains and culture conditions**

*Yarrowia lipolytica* strain DSM3286 was obtained from the culture collection of the DSMZ, Germany. The *Y. lipolytica* U6 mutant strain was obtained by physical mutagenesis (with ultraviolet radiation) from the wild strain DSM3286 (Darvishi et al., 2011). *Saccharomyces cerevisiae* strain CEN.PK 113-5D (MATA ura3-52 suc2 met2-8) strains. Yeasts were grown on YPD (glucose, 1.5%; yeast extract, 1%; casein peptone, 1%) or YNB (yeast nitrogen base without amino acids and ammonium sulfate) supplemented according to auxotrophic requirements. For selection of Ura⁺ clones, transformants were plated on YNBura (YNB with 0.01% uracil). YNBt (YNB with 1% of tributyrin) was used for the qualitative evaluation of lipolytic activity of the yeast strains.

*Escherichia coli* strain DH5α ( Gibco-BRL, Rockville, MD, USA), used for transformation and amplification of recombinant plasmid DNA, were grown at 37°C in Luria–Bertani medium supplemented with ampicillin (100 µg ml⁻¹), when required (Fickers et al., 2005b).

The composition of the basal medium for lipase production was as follows: olive oil, 10 ml; yeast extract, 2 g; KH₂PO₄, 0.5 g; K₂HPO₄, 0.5 g; MgSO₄·7H₂O, 0.5 g; CaCl₂, 0.1 g; and NaCl, 0.1 g l⁻¹. Initial pH of the medium was adjusted to 6. At cultivation, the yeast strains were grown in 50 ml of YPD or production medium in 250 ml flasks at 30°C and 200 r.p.m. (Darvishi et al., 2009).

**General genetic techniques**

Standard molecular genetic techniques were used (Sambrook and Russell, 2001). Genomic DNA from *Y. lipolytica* strains were extracted and used as a template for polymerase chain reaction (PCR). Primers were designed and checked by OLIgo software (version 5.0, w. Rychlik) and blasted using BLAST facilities available on the NCBI web site against the LIP2 nucleotide sequence from *Y. lipolytica* strain DSM3286 (GenBank Accession No. HM488699). Primers were as follows: Ylip2START (5'-ACGGGATCCATGAAAGCTTTCACCACATCCCC-3') with BamHI restriction site as the forward primer and Ylip2STOP (5'-TCGGAATTCCGCTTATGACACAGACACCCTC-3') with the EcoRI restriction site as reverse primer (Fig. 1A).

The PCR amplification was performed in a thermal cycler (Eppendorf, Germany) with a final volume of 50 µl containing 5 µl of 10× buffer (100 mM Tris-HCl, pH 9, 500 mM KCl and 15 mM MgCl₂), 200 mM dNTP (200 mM each dATP, dCTP, dGTP and dTTP), 30–60 ng of template, 50 pmol appropriate primers and 0.5 U each of Taq or Pfu (Fermentas, Canada) DNA polymerases. Twenty-five PCR cycles were carried out with the following programme: denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min kbp⁻¹. DNA fragments were recovered from agarose gels by using a Bioneer gel extraction kit (Bioneer, Korea) (Darvishi et al., 2011).

**Construction of LIP2 expression vector and transformation of yeast cells**

The purified PCR product and *S. cerevisiae* expression vector p426GPD (Yeast 2 µ expression vector with GPD promoter and URA3 marker; GenBank Accession No. D0019861) were digested with BamHI and EcoRI restriction enzymes, and then ligated with T4 ligase. The resulting vector was transformed into the *S. cerevisiae* strain CEN.PK 113-5D by using the lithium acetate method (Mumberg et al., 1995; Fickers et al., 2005b).

**Analytical methods**

Qualitative evaluation of yeasts was performed on H/C diameter of YNBT medium, where the H/C ratio represents the
ratio of the diameter of the hydrolysis halo (H) to the diameter of the cell colony (C). An H/C ratio of 1 means no tributyrin hydrolysis, and thus no lipase production, whereas a higher H/C ratio indicates lipase secretion. The H/C ratios of the mutant and wild-type strains were compared. Microbial growth was monitored by optical density measurements at 600 nm (Fickers et al., 2005c). The extracellular lipase activity was determined using a titrimetric assay performed in olive oil emulsion as the enzyme substrate. One unit (U) of lipase activity is defined as the amount of enzyme that produces 1 µmol of fatty acid per minute at 37°C and pH 7, as described previously (Kar et al., 2008). The molecular weight of the enzyme was determined by analysing the supernatant by using SDS-PAGE (Destain et al., 1997).

Acknowledgements

Author would like to thank Professor Nahvi, Dr Asadollahi and Mr Ziaee for their kindly helps.

References

Bailey, J.E., Sburlati, A., Hatzimanikatis, V., Lee, K., Renner, W.A., and Tsai, P.S. (2002) Inverse metabolic engineering: a strategy for directed genetic engineering of useful phenotypes. Biotechnol Bioeng 79: 568–579.

Barth, G., and Gaillardin, C. (1997) Physiology and genetics of the dimorphic fungus Yarrowia lipolytica. FEMS Yeast Res 19: 219–237.

Boer, E., Steinborn, G., Kunze, G., and Gellissen, G. (2007) Yeast expression platforms. Appl Microbiol Biotechnol 77: 513–523.

Cancino, M., Bauchart, P., Sandoval, G., Nicaud, J.-M., Andre, I., Dossat, V., and Marty, A. (2008) A variant of Yarrowia lipolytica lipase with improved activity and enantioselectivity for resolution of 2-bromo-arylacetic acid esters. Tetrahedron, Asymmetry 19: 1608–1612.

Ciafardini, G., Zullo, B.A., and Iride, A. (2006) Lipase production by yeasts from extra virgin olive oil. Food Microbiol 23: 60–67.

Darvishi, F. (2012) Microbial biotechnology in olive oil industry. In Olive Oil – Attributes, Quality, Health Properties and Bioconversions. Dimitrios, B. (ed.). Rijeka, Croatia: InTech publisher, pp. 309–330.

Darvishi, F., Nahvi, I., Zarkesh-Esfahani, H., and Momenbeik, F. (2009) Effect of plant oils upon lipase and citric acid production in Yarrowia lipolytica yeast. J Biomed Biotechnol 2009: 1–7.

Darvishi, F., Destain, J., Nahvi, I., Thonart, P., and Zarkesh-Esfahani, H. (2011) High-level production of extracellular lipase by Yarrowia lipolytica mutants from methyl oleate. N Biotechnol 28: 756–760.

Destain, J., Roblain, D., and Thonart, P. (1997) Improvement of lipase production from Yarrowia lipolytica. Biotechnol Lett 19: 105–107.

Fickers, P., Nicaud, J.M., Destain, J., and Thonart, P. (2003) Overproduction of lipase by Yarrowia lipolytica mutants. Appl Microbiol Biotechnol 63: 136–142.

Fickers, P., Benneti, P.H., Wache, Y., Marty, A., Maurseeberger, S., Smit, M.S., and Nicaud, J.M. (2005a) Hydrophobic substrate utilisation by the yeast Yarrowia lipolytica, and its potential applications. FEMS Yeast Res 5: 527–543.

Fickers, P., Fudalej, F., Dall, M.T.L., Casaregola, S., Gaillardin, C., Thonart, P., and Nicaud, J.M. (2005b) Identification and characterisation of LIP7 and LIP8 genes encoding two extracellular triacylglycerol lipases in the yeast Yarrowia lipolytica. Fungal Genet Biol 42: 264–274.

Fickers, P., Fudalej, F., Nicaud, J.-M., Destain, J., and Thonart, P. (2005c) Selection of new over-producing derivatives for the improvement of extracellular lipase production by the non-conventional yeast Yarrowia lipolytica. J Biotechnol 115: 379–386.

Fickers, P., Marty, A., and Nicaud, J.M. (2011) The lipases from Yarrowia lipolytica: Genetics, production, regulation, biochemical characterization and biotechnological applications. Biotechnol Adv 29: 632–644.

Gaillardin, C., and Heslot, H. (1988) Genetic engineering in Yarrowia lipolytica. J Basic Microbiol 28: 161–174.

Guieysse, D., Sandoval, G., Faure, L., Nicaud, J.M., Monsan, P., and Marty, A. (2004) New efficient lipase from Yarrowia lipolytica for the resolution of 2-bromo-arylacetic acid esters. Tetrahedron, Asymmetry 15: 3539–3543.

Hadeball, W. (1991) Production of lipase by Yarrowia lipolytica. Acta Biotechnol 11: 159–167.

Iida, T., Ohta, A., and Takagi, M. (1998) Cloning and characterisation of an n-alkane-inducible cytochrome P450 gene essential for n-decane assimilation by Yarrowia lipolytica. Yeast 14: 1387–1397.

Kar, T., Delvigne, F., Masson, M., Destain, J., and Thonart, P. (2008) Investigation of the effect of different extracellular factors on the lipase production by Yarrowia lipolytica on the basis of a scale-down approach. J Ind Microbiol Biotechnol 35: 1053–1059.

Madjak, Z., Gaillardin, C., and Beckerich, J.-M. (2004) Heterologous protein expression and secretion in the non-conventional yeast Yarrowia lipolytica: a review. J Biotechnol 109: 63–81.

Mumberg, D., Muller, R., and Funk, M. (1995) Yeast vectors for the controlled expression of heterologous proteins in different genetic backgrounds. Gene 156: 119–122.

Nevoigt, E. (2008) Progress in metabolic engineering of Saccharomyces cerevisiae. Microbiol Mol Biol Rev 64: 34–50.

Ostergaard, S., Olsson, L., and Nielsen, J. (2000) Metabolic engineering of Saccharomyces cerevisiae. Microbiol Mol Biol Rev 64: 34–50.

Papanikolaou, S., Chevalot, I., Galiotiou-Panayotou, M., Komaitis, M., Marc, I., and Aggelis, G. (2007) Industrial derivate of tallow: a promising renewable substrate for microbial lipid, single-cell protein and lipase production by Yarrowia lipolytica. Electron. J Biotechnol 10: 425–435.

Pereira-Meirelles, F.V., Rocha-Leao, M.H.M., and Sant’ Anna, G.L. (2000) Lipase location in Yarrowia lipolytica cells. Biotechnol Lett 22: 71–75.

Pigneda, G., Wang, H.J., Fudalej, F., Gaillardin, C., Seman, M., and Nicaud, J.M. (2000) Characterization of an extracellular lipase encoded by LIP2 in Yarrowia lipolytica. J Bacteriol 182: 2802–2810.

Roermund, C.W.T., Waterham, H.R., Ijlst, L., and Wanders, R.J.A. (2003) Fatty acid metabolism in Saccharomyces cerevisiae. Cell Mol Life Sci 60: 1838–1851.
Sambrook, J., and Russell, D.W. (2001) Molecular Cloning: A Laboratory Manual. New York, NY, USA: Cold Spring Harbor Laboratory Press.

Shockey, J., Chapital, D., Gidda, S., Mason, C., Davis, G., Klasson, K., et al. (2011) Expression of a lipid-inducible, self-regulating form of Yarrowia lipolytica lipase LIP2 in Saccharomyces cerevisiae. Appl Microbiol Biotechnol 92: 1207–1217.

Spencer, J.F.T., de Spencer, A.L.R., and Laluce, C. (2002) Non-conventional yeasts. Appl Microbiol Biotechnol 58: 147–156.

Sumita, T., Iida, T., Hirata, A., Horiuchi, H., Takagi, M., and Ohta, A. (2002) Peroxisome deficiency represses the expression of n-alkane-inducible YIALK1 encoding cytochrome P450ALK1 in Yarrowia lipolytica. FEMS Microbiol Lett 214: 31–38.

Thevenieu, F., Le Dall, M.T., Nthangeni, B., Mauersberger, S., Marchal, R., and Nicaud, J.M. (2007) Characterization of Yarrowia lipolytica mutants affected in hydrophobic substrate utilization. Fungal Genet Biol 44: 531–542.

Yu, M.R., Lange, S., Richter, S., Tan, T.W., and Schmid, R.D. (2007) High-level expression of extracellular lipase Lip2 from Yarrowia lipolytica in Pichia pastoris and its purification and characterization. Protein Expr Purif 53: 255–263.