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SHORT COMMUNICATION

Catalytic activity of baker's yeast in ester hydrolysis

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Abstract
The hydrolysis of phenyl esters of alkane carboxylic acids in the presence of lyophilized *Saccharomyces cerevisiae* has been studied. In the case of phenyl acetate the hydrolysis obeyed Michaelis–Menten kinetics, behavior typical of esterase-catalyzed reactions. For phenyl laurate our experiments provided evidence for the growth-associated production of lipase by baker's yeast.

Keywords: *Baker's yeast, hydrolysis, lipase*

Introduction

Baker's yeast has significant potential as a catalyst in organic chemistry (Servi 1990; Csuk & Glanzer 1991; D'Arrigo et al. 1994) owing to its ease of handling and broad substrate acceptability. It is non-pathogenic, inexpensive, simple to grow in the laboratory at any scale and accessible in stable dried (lyophilized) form. Moreover, baker's yeast possesses GRAS (Generally Recognized As Safe) status, which is an advantage in the production of compounds that are intended for human consumption. It is well established that baker's yeast has a high catalytic capacity for stereoselective reductions (Servi 1990; Csuk & Glanzer 1991; D'Arrigo et al. 1994). The use of a whole-cell biocatalyst offers the possibility of internal cofactor regeneration, achieved by adding co-substrates such as glucose.

The reduction of prochiral carbonyl groups by baker's yeast is well understood and β-ketoesters are unquestionably the compounds of reference; the reduction of ethyl-3-oxobutanoate to ethyl-(*S*)-3-hydroxybutanoate is the most thoroughly investigated reaction (Sybesma et al. 1998). Stereocontrol in baker's yeast reduction leads to the synthesis of natural products or pharmaceuticals. Ethyl-(*S*)-3-hydroxybutanoate can be used as a precursor of carbapenem antibiotics, insect pheromones or natural products (Sato & Fujisawa 1990; Kanno & Kawamoto 2000). Although the enantiomeric excess exceeds 99%, the overall yield of the reaction only achieves 50–70% on account of the presence of a vast variety of other enzymes in the cell – including several types of oxidoreductases (Rodriguez et al. 2001) and hydrolases (Chin-Joe et al. 2000).

The hydrolytic abilities of baker's yeast, initially discovered as an undesired side reaction (Csuk & Glanzer 1991), have found interesting applications in the chemoselective hydrolysis of ester bonds (Białecka-Florjańczyk & Majewska 2010) or the resolution of enantiomers of amino acids by hydrolysis of the *N*-acetyl derivatives of ethyl esters (Glanzer et al. 1987).

Several hydrolytic enzymes, such as sterol ester hydrolase (Taketani et al. 1981), carboxylic ester hydrolase (Degrassi et al. 1999) and lipases (Schousboe 1976), have been isolated from *Saccharomyces cerevisiae*, but systematic investigations of the hydrolytic activity of this yeast have not been performed so far.

Lipases are defined as carboxylesterases, catalyzing the hydrolysis and synthesis of long-chain triacylglycerols. Enzymes that are active towards water-soluble short-chain glycerol esters are classified as esterases. The essential difference between lipases and esterases is the fact that the former act

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at the water–lipid interface; that is, require micelle formation by a water-insoluble substrate (Gill & Parish 1997). In the case of triacylglycerols, chain lengths over ten carbon atoms can be regarded as a lipase substrate. Lipases can catalyze a wide range of chemical reactions – hydrolysis, esterification, alcoholysis, acidolysis and transesterification (Reetz 2000). Ester hydrolysis and esterification are reversible reactions and the positions of the equilibria depend on the water activities in the reaction mixtures; hence low water content generally favors lipase-catalyzed esterification. For this reason the hydrolytic activities of different lipases always give a good indication of the potential synthetic activity.

Materials and methods

Materials

S. cerevisiae (lyophilized) was obtained from Lesaffre (Wołczyn , Poland); enzymes were supplied by Sigma (Steinheim, Germany) (lipase from *Candida antarctica*) and Fluka (Steinheim, Germany) (esterase from *S. cerevisiae*); sucrose was purchased from BTL (Łodź, Poland); chloroform and Celite were purchased from POCH (Gliwice, Poland); phenyl esters were synthesized in our laboratory.

Hydrolysis

Phenyl ester hydrolysis was carried out in Erlenmayer flasks (500 mL). The esters – acetate (0.075–3 mmol), propanoate (1.5 mmol), hexanoate (1.5 mmol), heptanoate (1.5 mmol), octanoate (1.5 mmol) or laurate (1.5 mmol) – were added with vigorous stirring to a suspension of dry yeast (3 g) and sucrose (3 g) in distilled water (60 mL). The reaction was allowed to proceed at room temperature with shaking at 200 rpm at room temperature. The conversion (%) of the hydrolysis reaction was calculated from the production of phenol after a given time. Samples of reaction mixtures were extracted with chloroform, which was then vacuum-filtered through Celite. Reactant and product contents were determined by GC with flame ionization detector using a BPX 70 column. Nitrogen was used as the carrier gas. After injection of samples, the temperature of the column oven was kept constant for 1 min (120 C), increased linearly at 30 C min^{-1} to 180 C and 5 C min^{-1} to 230 C (for phenyl laurate) or at 10 C min^{-1} to 210 C (for the other esters), and kept at 230 C or 210 C for the remaining time of analysis.

Reactions with the addition of lipase from *C. antarctica* or esterase from *S. cerevisiae* were carried out and analyzed similarly.

Results and discussion

The aim of this work was to investigate the hydrolytic abilities of baker's yeast. We started with the typical conditions applied for baker's yeast-catalyzed reactions, i.e. lyophilized baker's yeast and sucrose as nutrient (Roberts 1999). Studies focused on the hydrolysis of phenyl esters of alkane carboxylic acids in the presence of lyophilized *S. cerevisiae* (Scheme 1).

By changing *n* from 0 to 10 in the phenyl esters (see Scheme 1) we could investigate the predisposition of baker's yeast to hydrolyze substrates of different lipophilicity. This should potentially provide evidence for the production of different types of hydrolytic enzymes.

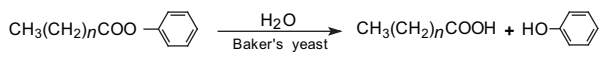
Initially, the kinetics of hydrolysis of phenyl acetate was studied, varying the substrate concentration from 10^{-3} to 5×10^{-2} mol L^{-1} in the reaction mixture and keeping all other parameters constant (Table I).

In the lower concentration range the reaction obeyed Michaelis–Menten kinetics for a single substrate, but for the highest concentrations we observed pseudo first-order kinetics of hydrolysis. This behavior is typical of esterases, which obey classical Michaelis–Menten kinetics in solution. With an ester concentration of 2.45×10^{-2} mol L^{-1} the reaction was completed after about 4 h.

Taking into consideration that one can find reports of lipolytic abilities of *S. cerevisiae* in the literature (Schousboe 1976; Shirazi et al. 1998; Vakhlu & Kour 2006), we compared the reactivity towards other phenyl esters of alkane carboxylic acids under the previous conditions (ester concentration 2.45×10^{-2} mol L^{-1}) and the resulting reaction constants are collected in Table II.

The rate of hydrolysis diminished with the growing number of carbon atoms in the alkyl chain of the ester molecule. In the case of phenyl laurate, an example of a fatty acid ester, we did not observe measurable quantities of phenol after a comparable period of time. This suggests that the hydrolysis only involved esterases and no lipolytic activity was evident during 4 h of hydrolysis. But if we continued the reaction for a longer period of time the hydrolysis of laurate started after 6 h, and after 72 h the reaction was nearly complete (Figure 1).

To confirm that lipases are the only catalyst active in the hydrolysis of phenyl laurate we performed the *S. cerevisiae* esterase-assisted reaction,



Scheme 1. Hydrolysis of phenyl esters of alkane carboxylic acids in the presence of lyophilized *S. cerevisiae*.

Table I. Kinetic parameters of the hydrolysis of phenyl acetate.

	Ester concentration (mol L^{-1})	Reaction rate, v (mol L^{-1} s^{-1})	Order of reaction
1	1.22×10^{-3}	1.02×10^{-6}	Pseudo zero
2	6.12×10^{-3}	1.63×10^{-6}	Pseudo zero
3	2.45×10^{-2}	1.59×10^{-6}	Pseudo zero
4	4.90×10^{-2}	9.00×10^{-7} [a]	Pseudo first-order; $k = 1.67$ s^{-1}

[a]Initial rate.

and compared it with the hydrolysis catalyzed by *C. antarctica* lipase. In contrast to lipase we observed no hydrolysis in the case of the esterase-assisted reaction (Table III).

Thus, we can conclude that baker's yeast is able to produce lipolytic enzymes, but that their activity is observed only after a long period of time. This result may be interpreted in two different ways: one possibility is that diffusion limits the lipase activity, because some of it is located in lipid particles (Athenstaedt & Daum 2005; Ham et al. 2010) or membrane-anchored (Koffel et al. 2005); on the other hand, taking into account that microbial lipases are usually produced extracellulary by fungi (Vakhlu & Kour 2006), the secretion of the enzyme could be growth-associated.

Triacylglycerol lipases of *S. cerevisiae* are poorly characterized. Schousboe (1976) described a triacylglycerol lipase activity in the yeast mitochondrial fraction, but the polypeptide catalyzing this reaction was not identified. During investigation of intracellular lipid particles in *S. cerevisiae* (lipid particles serve as energy sources and a source of building blocks) proteins Tgl1 and Tgl2 of unknown function were detected. Tgl1 and Tgl2 have been proposed to be triacylglycerol-specific lipases on the basis of their homology to lipases from humans and rats, and the enzymatic activity of Tgl1 against triacylglycerol and steryl esters has been demonstrated (Jandrositz et al. 2005). Athenstaedt & Daum (2005) identified some other proteins of yeast lipid particles as enzymes catalyzing degradation of triacylglycerols (TGl3p) and steryl esters (TGl4p, TGl5p). These three yeast enzymes

Table II. The rate of hydrolysis of phenyl esters by *S. cerevisiae*.

	Phenyl ester	Reaction rate, v (mol L^{-1} s^{-1})
1	Acetate	1.59×10^{-6}
2	Propanoate	1.50×10^{-6}
3	Hexanoate	0.90×10^{-6}
4	Heptanoate	0.75×10^{-6}
5	Octanoate	0.48×10^{-6}
6	Dodecanoate (laurate)	No reaction after 4 h

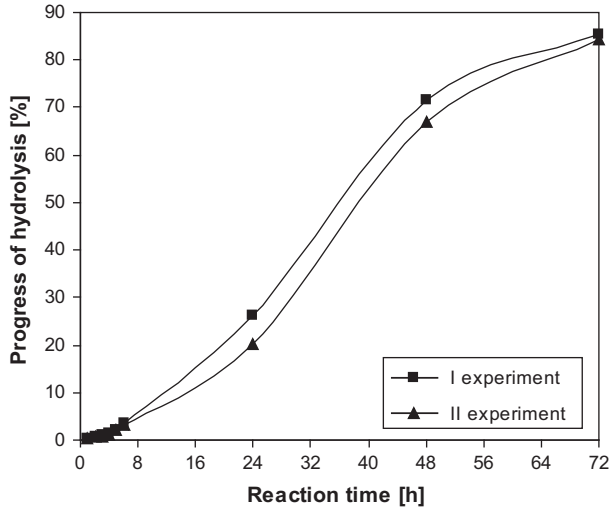


Figure 1. Time course of hydrolysis of phenyl laurate by *S. cerevisiae*.

constitute a novel class of lipases that differ in topology and subcellular localization.

Regarding extracellular lipases, Shirazi et al. (1998) found the highest lipase activity for *S. cerevisiae* strain DSM 1848 cultivated on lipid substrates, but when the strain was grown in MYGP medium (malt extract 3 g; yeast extract 3 g; glucose 10 g; peptone 5 g; deionized water to 1000 mL), in which glucose was the main carbon source, no lipase activity was detected. Under our conditions, glucose would have been present in reaction media as a product of sucrose hydrolysis, exerting an inhibitory effect on lipase production. Depletion of glucose during fermentative growth is connected with a change of cellular metabolism called diauxic shift (Stahl et al. 2004). In our case the production of lipases probably begins after the diauxic shift during a second respiratory growth phase, in contrast to esterase activity in *S.* cerevisiae which, according to Wheeler & Rose (1973), is high in the early exponential phase of growth and declines thereafter.

For other important lipase-producing yeasts e.g. *Candida rugosa*, a strong but ambiguous effect of the

Table III. Conversion (%) of phenyl laurate to phenol in the presence of *C. antarctica* lipase and *S. cerevisiae* esterase.

	Conversion [%]	
Reaction time (h)	*C. antarctica* lipase	*S. cerevisiae* esterase
1	0.7	0
2	9.1	0
3	36.3	0
4	43.3	0
5	60.4	0
24	74.9	0.1

culture medium composition has been observed (Dalmau et al. 2000). Valero et al. (1991) reported that lipase production was sensitive to glucose repression, but Chang et al. (1994) showed that lipase was produced in the presence of glucose.

Conclusions

Our experiments have provided further evidence for the possibility of lipase production by baker's yeast. It appears that it is induced during growth of baker's yeast and probably depends on culture medium composition, as has been observed for other yeast strains. This may also be true of other yeasts considered to be non-lipolytic strains.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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