Ultrasonics Sonochemistry 19 (2012) 186–191

Contents lists available at ScienceDirect

Ultrasonics Sonochemistry

journal homepage: www.elsevier.com/locate/ultsonch

Studies on the lipolytic activity of sonicated enzymes from *Yarrowia lipolytica*

Agata Urszula Kapturowska *, Izabela Agnieszka Stolarzewicz, Jolanta Krzyczkowska, Ewa Białecka-Florjańczyk

Department of Chemistry, Faculty of Food Sciences, Warsaw University of Life Sciences, 02-787, Poland

ARTICLE INFO

Article history:
Received 15 April 2011
Received in revised form 23 June 2011
Accepted 24 June 2011
Available online 2 July 2011

Keywords:
Lipase
Protein release
Sonication
Yarrowia lipolytica

ABSTRACT

The aim of this study was to evaluate the efficiency of sonication in releasing protein from a widespread lipase-producing yeast, *Yarrowia lipolytica* KKP 379, and to examine the impact of ultrasound waves generated in a horn-type sonicator on the lipolytic activity of *Y. lipolytica* in the hydrolysis of *p*-nitrophenyl laurate. In this paper, we focused on a few parameters of ultrasound cell disruption, such as the time of sonication, acoustic power, storage time of the frozen yeast biomass used in sonication and the solvent used to suspend the yeast cells which were considered as the most important part in the process of obtaining a biocatalyst from *Y. lipolytica* for organic synthesis. The most effective additive in protein release proved to be 2% Tween 80; other ideal parameters of the process were ultrasonic power at 150 W for 15 min and 9 weeks of frozen biomass storage time. The sonication parameters, which were the best for protein release, did not seem to be the most effective for obtaining high lipolytic activity due to denaturation as an effect of cavitation.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

Cell disruption is an important step in isolating intracellular products such as proteins, enzymes and polysaccharides and there is a wide range of physical, chemical and biological methods of disrupting the cell wall and cell membrane structures [1,2]. The sonication method takes advantage of high intensity acoustic waves (20–100 kHz). The ultrasound effect on microorganisms is caused by cavitation (implosion of gas bubbles) and shock waves generated as an effect of sound energy passing through the medium [3,4]. Observations made by Oyane et al. support the suggestion that OH radicals and hydrogen peroxide formed during the sonolysis of water damage yeast cells [5].

Ultrasonics is a rapidly growing field of research for biotechnology, green chemistry and food technology and there are many reports which include ultrasound as a tool for extracting plant-origin metabolites and bioactive compounds from plant or animal material. Still, ultrasound is not widely recognized as a tool for protein extraction [6]. High intensity ultrasound has become a tool in the permeabilization of cell membranes and disrupting cell walls in addition to its use as a laboratory preparative technique [3,7] and method for intensifying the performance of biocatalysts; the general effect depends on the sonicated organism and process parameters [8]. Sonication could be also a technique for basic research on yeast cells including localization of enzymatic proteins, for example lipases.

Commercially useful lipases are commonly obtained from microorganisms. Microbial lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) have many applications, including organic synthesis, bioconversions, in the food, detergent, paper and oleochemical industries, in cosmetics, medicine and in waste treatment [9,10]. The majority of commercially significant lipases are produced by *Candida rugosa, Candida antarctica, Aspergillus niger, Rhizomucor miehei* and *Rhizopus arrhizus* [11–13]. Lipases are expressed intracellularly or extracellularly, although there is a wide range of cell wall-bound enzymes [14].

Yarrowia lipolytica is commonly known as a widespread lipase-producing yeast species, but there is little data about the impact of ultrasound on the lipolytic activity of *Yarrowia* strains. The aim of this study was to evaluate the efficiency of sonication in terms of protein release from yeast cells and to examine the impact of ultrasound waves generated in a horn-type sonicator on the lipolytic activity of *Y. lipolytica* in the hydrolysis of *p*-nitrophenyl laurate. In this paper, we focused on a few parameters of ultrasound cell disruption, such as the time of sonication, acoustic power, storage time of the frozen yeast biomass used in sonication and the solvent used to suspend the yeast cells which were considered as the most important in a process of preparing biocatalysts from *Y. lipolytica* for organic synthesis.

* Corresponding author. Address: Department of Chemistry, Faculty of Food Sciences, Warsaw University of Life Sciences, Nowoursynowska Street 159, 02-787, Poland. Tel.: +48 22 59 37 621.
E-mail address: agata_kapturowska@sggw.pl (A.U. Kapturowska).

1350-4177/$ - see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.ultsonch.2011.06.015

2. Experimental

2.1. Chemicals and culture media

Modified YPG medium (2% glucose, 1% yeast extract, 2% peptone, 2% olive oil, pH 5.0) was used for the cultivation of yeast. Sabourand agar medium with chloramphenicol was used for enumeration of yeast cells. The media were prepared with distilled water. All medium ingredients were purchased from BTL (Łódź, Poland). Tween 80 was purchased from Acros Organics (Geel, Belgium). TRIS buffer, all salts, sodium hydroxide and Folin–Ciocalteu reagent were purchased from POCH (Gliwice, Poland). Phenyl esters were synthesized in our laboratory. Bovine serum albumin was purchased from Sigma Aldrich (Poznań, Poland).

2.2. Microorganism

Yeast *Y. lipolytica* KKP 379 were purchased from the Collection of Industrial Microorganisms at the Institute of Agricultural and Food Biotechnology in Warsaw. Yeast were cultivated for 32 h at 28 °C, with a rotation of 400 rpm in a BioFlo 3000 bioreactor (New Brunswick Scientific, Germany). Yeast biomass was characterized by cell dry mass measured by the thermogravimetric method at 105 °C and determined as cfu/g.

2.3. Methods

2.3.1. Sonication

A 20 kHz horn-type sonicator (OmniRuptor Ultrasonic Homogenizer, Kennesaw, USA) with a maximum acoustic power output of 300 W was used for ultrasonic disruption. The sonication of cells was performed using an 80% duty cycle in 40 ml of selected solution with different parameters for time and the ultrasound power. The variability introduced by culture conditions was avoided by using aliquots from the same cultivation process. The following solutions were used: TRIS buffer at pH 7.0, 2% Tween 80 and a salt solution at pH 6.3 (0.025 M $MgCl_2$; 0.05 M KH_2PO_4) with and without tributyrine. The yeast biomass was cooled during sonication in an ice water bath. After centrifugation, the sonicated biomass and supernatants of sonicated cells were stored at −25 °C for up to 4 months. Cells were also disrupted mechanically at 10,000 rpm, 10 min, 20 °C to compare to the activity from sonicated yeast biomass.

The amount of protein released from yeast cells was measured by a modified spectrophotometric Lowry's method at 750 nm [15]. Bovine serum albumin was used as the protein standard.

2.3.2. Measurement of lipase activity

Measures of enzymatic activity were carried out using a modified spectrophotometric method described previously [16] which is based on the hydrolysis of *p*-nitrophenyl laurate. Reactions were carried out in Erlenmeyer flasks (100 ml) for 30 min. 0.3 Mmol of substrate was suspended in 2 ml of heptane. *p*-Nitrophenyl laurate was added to 1 g of yeast biomass suspended in 15 ml of distilled water or to 15 ml of supernatant with vigorous stirring. Activity of the sonicated *Y. lipolytica* preparations (both yeast biomass and supernatant) was measured using a standard curve evaluated with *p*-nitrophenol standards at a concentration from 4.4×10^6 M/ml to 3.7×10^7 M/ml. One unit of enzyme activity was defined as the enzyme quantity that liberated 1 μmol of *p*-nitrophenol per minute under the assay conditions at 37 °C.

2.3.3. Scanning electron microscopy (SEM)

SEM was used for visualizing cell disruption. Cell slurries sonicated for 15 min, at an acoustic power of 150 W and a 90% duty cycle were fixed with a glutaraldehyde solution and then with osmium tetroxide. The slurries were filtered through carbonate filters and carefully dehydrated in an increasing graded ethanol series and then in acetone. The filters with slurries were critically point dried with Polaron CPD 7501. Samples were coated with gold in a Joel Fine Coater JFC – 1300. Biological samples were examined in Quanta 200 FEJ Elektron Optics scanning electron microscope.

2.4. Statistical analysis

Statistical analyses of the results were performed by repeated measurements with multiple-way ANOVA in STATGRAPHICS Plus for Windows 4.1©Statistical Graphics Corp., followed by Tukey's multiple comparison test. *P*-values of $p \leqslant 0.05$ were considered to be statistically significant.

3. Results

3.1. Disruption of Yarrowia cells using ultrasound waves

Y. lipolytica KKP 379 cells were sonicated in a 20 kHz horn-type sonicator with different process parameters. Scanning electron microscopy investigations of the effects of ultrasound on yeast cells are shown in Fig. 1. It can be seen that sonication at 150 W, at an 80% duty cycle for 15 min is not enough to disrupt all of the cells. The most sensitive were *Yarrowia* hyphae (Fig. 1b), while oval cells were more resistant to acoustic waves (Fig. 1c).

Shear stress associated with microstreaming surrounding bubbles may be large enough to generate pores in cell wall structures. A cell may be able to survive with previously "opened" membrane (reparable sonoporation), or it can be lethally damaged (lethal sonoporation) [17]. An effect of both phenomena could cause a protein leak. It should be noted that the degree of destruction in the photographs does not reflect the amount of enzymatic proteins released from the cells or the activity of biomass or supernatant from sonicated *Y. lipolytica* KKP 379, because ultrasound is known to generate both positive and negative impacts on the ability of enzymes to catalyze certain reactions. A high level of protein release does not necessarily correspond with higher activity of the enzyme. This is why these two occurrences were investigated simultaneously.

3.2. Protein leak from sonicated Y. lipolytica cells

Our results show that during this process *c.a.* 100–200 mg proteins/g d.m could be released. In this paper, we discuss a significant impact of time, ultrasound power and the type of solution used for cell dilution on protein leak.

It can be assumed that the higher ultrasonic power, the more intracellular proteins are released (Fig. 2a). As the acoustic power increased from 30 to 150 W, the amount of protein released increased from 59.4 to 241.0 mg/g d.m.

A similar effect of time was observed as the effect of acoustic power (Fig. 2c). A 3-fold shorter sonication time resulted in nearly 100 mg/g d.m. less protein in the supernatant compared to the amount of protein after 15 min of exposure to ultrasound. A sonication time of 15 min was maintained in further experiments on the influence of solution used for cell dilution on protein release.

To study the effect of different solutions, in which yeast biomass was blended, 4 g of *Y. lipolytica* was suspended in 40 ml of one of the following solutions: TRIS buffer (pH 7.0), 2% Tween 80 and salt solution (pH 6.3; 0.025 M $MgCl_2$; 0.05 M KH_2PO_4), prepared with and without tributyrine (Fig. 2b). The salt solution was prepared according to earlier experiments on *Saccharomyces cerevisiae* (unpublished results). Mg^{2+} ions as well as TRIS buffer

Download English Version:

https://daneshyari.com/en/article/1270177

Download Persian Version:

https://daneshyari.com/article/1270177

Daneshyari.com