AMYLOLYTIC ACTIVITY OF KLUYVER-POSITIVE DEBARYOMYCES OCCIDENTALIS CELLS IMMOBILIZED IN FOAMED ALGINATE GEL

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ABSTRACT

This study investigates the amylolytic activity of Kluyver-positive yeast strain Debaryomyces occidentalis immobilized in foamed alginate gels. Encapsulation was performed through the traditional process of droplet formation from a foamed alginate solution. The beads were coated with a layer of 3% alginate. Amylolytic enzyme activities were determined in the presence of different carbon sources – glucose, maltose, starch or dextrin – in both complex and minimal culture media. The yeast was capable of producing inducible alpha-amylase (EC 3.2.1.1) and glucoamylase (EC 3.2.1.3). The highest level of cell proliferation was observed in the complex medium with glucose. Immobilized cells showed the highest amylolytic activities in culture media with maltose. Both alpha-amylase and glucoamylase activities were higher in minimal media than in complex media.

Keywords: Debaryomyces occidentalis; encapsulation; foamed alginate; Kluyver effect; alpha-amylase; glucoamylase; ethanol

INTRODUCTION

Microbial amylases began to be produced on an industrial scale during the last century. They are the most important group of enzymes, in terms of biotechnological applications. In particular, α-amylase (1,4-α-D-glucan glucanohydrolase, EC 3.2.1.1) and glucoamylase (exo-1,4-α-D-glucan glucoamylase, EC 3.2.1.3) have found a wide variety of uses in a number of industrial processes, including fermentation, food production and textile and paper manufacturing (Monteiro de Souza and de Oliveira e Magalhães, 2010). Other amylolytic enzymes can bypass the α-1,6 branch points in the starch molecule (debranching activity) (Hui et al., 2012). Amylases are produced by a wide range of organisms, including yeasts (Yalçın and Çorbacı, 2013). The main industrial use of amylases is in the starch saccharification process (Hostínová and Gašperík, 2010).

The unconventional yeast Debaryomyces occidentalis (syn. Schwanniomyces occidentalis) was identified as early as the 1980s as a ‘super yeast’ because of its ability to hydrolyze whole starch to glucose completely without prior hydrolysis (Wang et al., 1999). The ability of Debaryomyces spp. to tolerate extreme stress could be an additionally advantage in low-cost fermentation processes (Johnson and Echavarri-Erasun, 2011). However, D. occidentalis exhibits the Kluyver effect for maltose and starch (Weusthuis et al., 1994). Fermentation of maltose and starch is blocked under anaerobic conditions. This effect may be caused by the rapid down-regulation of sugar carrier capacity, which occurs under oxygen-limited conditions, since certain yeasts require oxygen to transport sugars into their cells and to produce extracellular amylases (Barnett and Entian, 2005).

Kluyver effect positive yeasts play an important role in the study of immobilized cells, as they provide a specific indicator for the availability of oxygen within alginate beads. This study is the continuation of previous research on yeast immobilization, in which Debaryomyces spp. cells were grown inside foamed alginate beads without undergoing irreversible structural changes to their Ca-alginate networks (Kregiel et al., 2013). These results suggested a further avenue of inquiry, into amylolytic activity and ethanol formation in Kluyver-positive yeast cells encapsulated in foamed alginate. This manuscript describes the continuation of previous research, with a special focus on two enzymes: alpha-amylase and glucoamylase. The formation of ethanol, as the end product of yeast fermentation, is also discussed.
0.1% Triton X-100 (MERCK-MILLIPORE). Triton X-100 was used as the foaming agent in a novel procedure. The toxicity of this compound was verified at the beginning of this research on foamed alginate (unpublished data). This compound at a concentration of 0.01% did not show any toxicity towards yeasts. Only chemicals of the highest purity were used. The solutions and materials were sterilized before use at 121 °C, except for the sodium alginate solution which was sterilized using tyndalization. The mixture, which contained around 1×10⁷ cells per 1 mL of gel, was dropped through a 1.8 mm needle into 0.15 M CaCl₂ solution. The beads (φ ~3 mm) were hardened in 0.15 M CaCl₂ solution for 60 minutes and then washed in distilled water (5 min). To study the internal morphology beads were frozen (-20°C) and cut with a sharp razor blade. Slices of alginites were observed under a light microscope CX41 (OLYMPUS). The procedure for creating the foamed gels was described in Polish Patent No. 210458 (Ambroziak et al., 2012). The resultant cores of foamed alginate consisted of micro-spheres approximately 50-200 microns in diameter and had a specific internal bubble structure (Fig. 1). The foamed beads were vented inside a sterile syringe under a mild vacuum at 10 °C for 24 hours in a liquid complex medium with glucose, maltose, starch or dextrin, in order to introduce nutrients into the bead spheres. To increase mechanical stability, the foamed beads were given an additional alginate coating by placing them in a solution of 3% alginate (FLUKA) for 15 minutes. They were then washed in sterile water (Kregiel et al., 2013).

A quantitative assessment of the immobilization was performed by dissolving the alginate beads in 0.2 M Na₂HPO₄ (in a ratio of 1 bead per 1 mL). The number of immobilized cells was determined using the microscopic method. The number of cells per single tested bead was calculated and presented as a logarithmic function.

Alpha-amylose and fermentation activities

Glucoamylase activity was estimated using the 3,5-dinitrosalicylic acid method on the basis of the amount of reducing sugars released during starch hydrolysis (Damian-Almazo et al., 2008). The reaction was carried out in 1 mL of 10 mg/mL soluble starch from corn (73% amylopectin and 27% amylose) (SIGMA) dissolved in 50 mM KH₂PO₄-NaOH buffer (pH=6.0) at 45 °C for 10 minutes. One unit of enzyme activity was defined as the amount of maltose equivalents (μmol) released per minute and per mL of supernatant (Moranelli et al., 1987; Ryu and Sung, 1993).

Glucoamylase activity was determined from the reaction of 1 mL supernatant (after cultivation in 1 mL of 10 mg/mL soluble starch from corn (SIGMA) in 50 mM citrate buffer (pH=5) at 50 °C for 20 min. The glucose released was measured using 3,5-dinitrosalicylic acid reagent with glucose as a standard. One glucoamylase activity unit (U) was defined as the amount of enzyme that releases one μmol of glucose equivalent per minute per mL (Clementi and Rossi 1986; Ryu and Sung, 1993).

The fermentation performance of the immobilized cells was evaluated based on the ethanol content in the precipitate, determined using an AGILENT 6890 gas chromatograph equipped with headspace autosampler, capillary INNOVAX column (60 m × 0.32 mm) and flame ionization detector (FID) (Kregiel et al., 2012).

RESULTS AND DISCUSSION

Yeast cell growth inside gel beads

After a 5-day incubation period in complex medium with glucose YPD, the number of immobilized yeast cells increased from an initial 2×10⁶ to 1.2×10⁷ per bead, indicating significant colonization of the alginate cores (Fig. 2). Multiplication inside beads incubated in complex media with other carbon sources was slightly lower at 5-6 ×10⁶ cells/bead.

Oxygen may influence the production of glycolytic enzymes (Fukuhara, 2003). Violle et al. (1992) have shown that amylase and glucosidase are not synthesized in the presence of maltose under anaerobic conditions. However, maltose permease is synthesized in anaerobiosis without its functioning being inhibit. In our studies, foamed alginate cores with special interior spaces provided favorable conditions for the synthesis of amylolytic enzymes.

The alpha-amylase activity in rich culture media gradually increased, reaching the highest value (2.65 U/mL) in YPM medium with maltose as the carbon source (Fig. 3A). No such gradual increase in alpha-amylase activity was observed during the cultivation of immobilized yeast cells in minimal medium Mo, although the highest value of alpha-amylase activity was again in the medium with maltose as the carbon source (3.9 U/mL) (Fig. 3B).

The maximal activities of glucoamylase were also noted in culture media with maltose, at 0.08 U/mL and 0.11 U/mL for rich and minimal medium, respectively (Fig. 4 AB).


