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Evaluation and comparison of microbial cells disruption methods for extraction of pyruvate decarboxylase

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Abstract

An enzymatic biotransformation process based on extracted pyruvate decarboxylase (PDC) overcomes the problem of by-product benzyl alcohol production. Seven methods of cells disruption which included application of glass beads, freezing/thawing sequence, silver nanoparticles, ultrasonication, freezing/thawing sequence with glass beads, freezing/thawing sequence with silver nanoparticles, and freezing/thawing sequence with ultrasonication were investigated to choose the best method for partial isolation of PDC from *Candida tropicalis* TISTR 5350. Ultrasonication was an effective method for cells disruption with the corresponding specific PDC activity of 0.36 ± 0.01 U/mg of protein. Ultrasonication method should thus be selected as the method of choice to preserve enzyme activity. The PDC from *C. tropicalis* TISTR 5350 released from ultrasonic cells disruption method was prepared for partial purification in comparison with 40-60% (w/v) ammonium sulphate and 50% (v/v) acetone precipitation techniques. Specific PDC activity, purification and percentage recovery (yield) of precipitated PDC enzyme based on 50% (v/v) acetone at 4°C were higher (0.53 ± 0.02 U/mg protein, 1.24 ± 0.10, and 94.41 ± 2.12 %, respectively) than the precipitation obtained using the 40 to 60% (w/v) ammonium sulphate saturation (0.49 ± 0.01 U/mg protein, 1.13 ± 0.07, and 92.87 ± 2.50%, respectively). The result indicated that the precipitation of PDC using the 50% (v/v) acetone was the most effective strategy for partially purifying PDC and suitable for application in a commercial process which had a relatively low cost and simple process.

Introduction

Microorganisms consisted of a semipermeable, tough, rigid, outer cells wall surrounding the cytoplasmic membrane, and cytoplasm. The cytoplasm was made up of nucleic acids, proteins, carbohydrates, lipids, enzymes, inorganic ions, vitamins, pigments, inclusion bodies, and about 80% water. In order to isolate and extract these substances from inside the cells, it was necessary to break the cells wall and protoplasmic membrane (APV, 2009). Cells could be disrupted in various ways, which included; (1) mechanical methods such as homogenization (shear force), cells rupture by pressure, sonication, grinding, osmotic shock, as well as freezing and thawing sequence; (2) enzymatic method such as lysozyme, zymolase, cellulase, glycanase, protease, and mannase; (3) chemical methods such as treatment with detergents, silver nanoparticles, and solvents (Stephanova and Topouzova, 2001).

An enzymatic process with free pyruvate decarboxylase (PDC) successfully eliminated benzyl alcohol formation since there were no electron regeneration donors (e.g. NADH) (Shin and Rogers, 1996; Rosche *et al.*, 2002). PDC extracted from the yeast has been intensively studied in the development of enzymatic PAC production processes which was a precursor for the commercial production of ephedrine and pseudoephedrine. These substances were used primarily as bronchial dilators and nasal decongestants (Suresh *et al.*, 2009). Moreover, PAC production should preferably exhibit high levels of PDC (Chen, 2006).

Candida tropicalis could utilize wide variety of carbon sources including many saccharides, phenols, alkanes, alkane derivatives, and fatty acids (Kawachi *et al.*, 1997). This yeast was considered strong candidate for thermotolerance and ethanol tolerance required to produce ethanol from lignocellulosic biomass (Jamai *et al.*, 2001). As previous studied from Tangtua *et al.* (2013), *C. tropicalis* TISTR 5350 was the most interesting candidate microbial strain which observed the highest PDC activities (0.39 ± 0.06 U/ml) and PAC production (19.83 ± 3.36 mM). The cytoplasm of yeast cells was a rich source of bio-products (proteins, cytoplasmic enzymes, polysaccharides, etc.). *C. tropicalis* was a simple eukaryotic cells with a relatively rigid cells wall in addition to cell membrane. Hence, efficient breakage of the cells walls was a necessary step to recover

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