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Short communication

Extraction penicillin G acylase from *Alcaligenes faecalis* in recombinant *Escherichia coli* with cetyl-trimethylammoniumbromide

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Abstract

Cells were permeabilized with cetyl-trimethylammoniumbromide (CTAB) to extract penicillin G acylase (sp. *Alcaligenes faecalis*) in recombinant *Escherichia coli*. The optimum conditions were 0.5% (w/v) CTAB, 0.5 M ionic strength, pH 8.0 and at 4 °C for 28 h. The maximum relative enzyme activity obtained was 93.4%, and the specific activity increased about 1.6 times than that by ultrasonic fragmentation. Furthermore, CTAB-extracted enzyme solution had fine stability, and almost no activity decreased as the enzyme was preserved at 4 °C for 30 days. From these results, the method can be used as a high-performance system about the extraction of penicillin G acylase from *A. faecalis* that resides mainly in the outer periplasmic space of *E. coli*.

Keywords: *Alcaligenes faecalis*; Cell disruption; CTAB; Enzyme activity; Enzyme extraction; Penicillin G acylase

1. Introduction

As one of the most important industrial enzymes, penicillin acylase (PA, E.C 3.5.1.11) catalyzes the formation of 6-aminopenicillianic acid (6-APA) and 7-aminodesacetoxycephalosporanic acid (7-ADCA), which are further processed in the production of various important semi-synthetic antibiotics [1,2]. It is also used in peptide synthesis [3], resolution of amino acids [4], amine [5] and amino alcohol [6], etc.

Therefore, much effect has been directed to isolate new penicillin G acylase (PGA) from different microorganisms, including bacterial, yeast and filamentous fungi, and then to extend its industrial applications [7]. The relatively unknown penicillin G acylase from *Alcaligenes faecalis* (*A. faecalis* PGA) shares only 49% of protein sequence and 53% DNA sequence homology to *E. coli* PGA [8], which has several unique outstanding characteristics including a broad optimum pH range, significantly higher thermal stability, higher enantioselectivity [5,9,10]. So there are more and more attentions to its new applications.

Recently we have obtained high activity of *A. faecalis* PGA in recombinant *E. coli* by optimizing the conditions of the enzyme production, which was about 45,000 U/L with batch cultivation in shaken flasks, and the specific enzyme activity was above 10 U per mg protein. But biochemically active PGA in *E. coli* resides mainly in the outer periplasmic space [11]. In order to make the enzyme be released, the traditional extraction methods, for example high-pressure homogenization and sonication, have been broadly used. However, those methods can contaminate the target enzyme because of other proteins released synchronously in cells with similar physicochemical properties [12]. So chemical treatment has been used for selective enzyme extraction [11–13], which is a potential substitute for mechanical disruption [14].

There are several literatures about chemical methods in which the aim proteins were extracted from whole microorganism cells with anionic or nonionic detergents [15,16], but little was reported using cationic detergents, especially CTAB. CTAB has been widely used for the analysis about some metabolic processes such as DNA replication [17] and RNA isolation [18], etc. Moreover, CTAB-treated whole cells have been studied as a source of intracellular enzymes instead of expensive enzymes isolated. Nagalakshmi and Pai treated cells with CTAB and the penicillin acylase activity increased about two-fold [19]. We previously also used CTAB to enhance the activity of whole cell *A. faecalis* PGA, which indicated that the enzyme activity increased about 7.5-fold as compared to that of untreated cells,

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but only 16% of total active enzyme was released [20]. In this paper, *A. faecalis* PGA expressed in *E. coli* was extracted with CTAB.

2. Materials and methods

2.1. Chemicals and microorganism

Potassium penicillin G and 6-aminopenicillanic acid (6-APA) were kind gifts from Shijiazhuang Pharmaceutical (China). Cetyl-trimethylammoniumbromide (CTAB) was purchased from Amresco. *p*-Dimethylaminobenzaldehyde (PDAB) was supplied by Sssreagent Company (China). All other reagents and chemicals used were of analytical grade.

E. coli strain DH5α was used as the host for the production of *A. faecalis* PGA. The recombinant plasmid, pSMLFPGA, is a medium copy-number plasmid, which carries the *A. faecalis* pga gene, rrnB transcript terminator, medium-copy replicon (p15A) and a tetracycline resistant gene. The expression of pga gene is controlled by the *trc* promoter, which needs not be induced by isopropyl-β-thio-galactopyranoside (IPTG) because of no $lacI^q$ gene.

2.2. Treatment of cells with CTAB

Cells were harvested during stationary phase then separated by centrifugation at 12,000 × *g* and 4 °C for 5 min. The packed cells were resuspended in different pH buffers (1.0 g wet cell/10 ml), CTAB was added to final concentration (0.05–0.65%, w/v), and then stirred gently on a rotary shaker (150 rpm) at different temperature and ionic strength for different time.

2.3. Treatment of cells by sonication

The centrifuged cells were resuspended in 50 mM pH 8.0 phosphate buffer (1.0 g wet cell/10 ml), and then diluted sixfold. Cell wall was disrupted with the ultrasonic cell disruptor (Ningbo Scientz Biotechnology, JY88-II, China) in cold ice-water bath, which was at 400 W for 99 times (working 5 s and intervals 5 s as one cycle).

The treated sample was observed with microscope (10 × 100, XSP-10C, Shanghai Optical Instrument, China) after it was diluted some times and stained with Crystal violet, which was found be disrupted entirely. If it was disrupted again with the same conditions, the enzyme activity did not increase. So the active enzyme in *E. coli* cells was considered be released completely.

2.4. Determination of PGA activity and total protein content

The treated samples were centrifuged at 4 °C and 12,000 × *g* for 10 min, and the supernatants were carried out for the measurements of PGA activity and total protein content. Total protein content was determined by Lowry method using Folin phenol reagent [21]. The enzyme activity was measured with potassium penicillin G (PGK) solution. The hydrolysis of PGK by PGA yields 6-APA which is followed by a spectrophotometric assay with *p*-dimethylaminobenzadehyde as a colorimetric substrate [22]. One enzyme activity unit (U) is defined as the amount of enzyme required to produce 1 μmol of 6-APA per min in 4% (w/v) PGK solution at pH 8.0 and 37 °C. The relative activity and specific activity of *A. faecalis* PGA were calculated as follows:

$$\gamma\,(\%) = \frac{\alpha}{\beta} \times 100 \tag{1}$$

$$\omega = \frac{\theta}{\lambda} \tag{2}$$

where γ is the relative activity (%), α the CTAB-treated enzyme activity (U/l), β the enzyme activity of ultrasonic sample (U/l), ω the specific activity (U/mg protein), θ the *A. faecalis* activity (U/l), and λ is the total protein content (mg/l).

3. Results and discussion

3.1. Effect of extraction time

The extraction time was established at the optimum conditions where cells were permeabilized with CTAB for cell immobilization [20]. 35.9% of total enzyme activity for the best result was obtained at 28 h (Fig. 1), and the relative enzyme activity slowly declined after the time. The reason was probably that the enzyme was deactivated as time prolonged at 25 °C.

3.2. The concentration of CTAB

The effect of this parameter on the enzyme extraction process was similarly investigated (Fig. 2), where the detergent concentration ranged from 0.05% to 0.65% (w/v). The maximum percent of released *A. faecalis* PGA was 45.3% at 0.5% (w/v) CTAB concentration. When the detergent concentration was above 0.6% (w/v), the relative activity was sharply reduced due to deactivation of the enzyme by higher CTAB concentration [11].

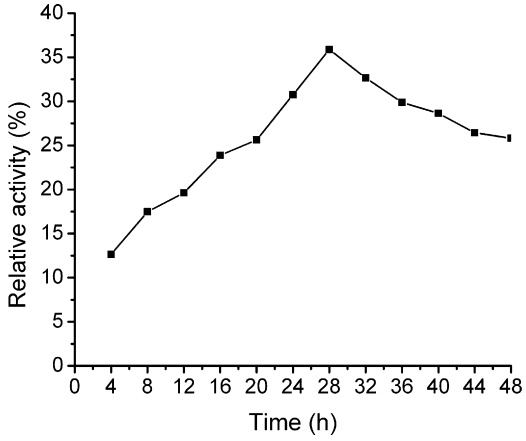

Fig. 1. Time effect on the extraction yield of *A. faecalis* PGA. Conditions: CTAB concentration, 0.3% (w/v); pH, 8.0; phosphate buffer, 50 mM; temperature, 25 °C.