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ScienceDirect
Bioresource Technology 99 (2008) 699–702
BIORESOURCE TECHNOLOGY

Production of a thermostable uricase by a novel *Bacillus thermocatenulatus* strain

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Received 31 December 2006; received in revised form 27 January 2007; accepted 30 January 2007
Available online 28 March 2007

Abstract

A novel uricase-producing bacterium was identified based on its 16S rRNA sequence as *Bacillus thermocatenulatus*. The kinetic constants for this uricase, determined with uric acid as the substrate, were a V_{max} of 0.99 U/ml of enzyme and a K_m of 0.25 mM. After heat treatment at 75 °C for 45 min, the uricase retained about 100% of its initial activity. The uric acid showed to be an inducer for uricase production. The effects of different factors on the enzyme production were studied. Pretreated cane molasses and corn steep liquor were the most promising carbon and nitrogen sources, respectively. When the strain was cultured at 30 °C at pH 7.0 for 30–36 h, the uricase activity peaked at 1.25 U/ml.

Keywords: *Bacillus thermocatenulatus*; Uricase; Cane molasses; Corn steep liquor

1. Introduction

Uricase (urate oxidase, EC 1.7.3.3) catalyzes the oxidative opening of the purine ring of urate to yield allantoin, carbon dioxide, and hydrogen peroxide. It has vast and beneficial uses both in vitro and in vivo. Determining the urate concentration in blood and urine is required for the diagnosis of gout as urate accumulation is a causative factor of gout in humans. Uricase is useful for enzymatic determination of urate in clinical analysis by coupling with 4-aminoantipyrine-peroxidase system (Gochman and Schmitz, 1971). It can be also used as protein drug for treatment of hyperuricemia, as Rasburicase (Colloc'h et al., 1997).

Many organisms including higher plants and microorganisms are able to produce uricase. *Pseudomonas aeruginosa* (Frank and Hahn, 1955) and *P. acidovorans* (Sin, 1975) are capable of degrading uric acid by unstable membrane-bound uricase. *Arthrobacter globiformis* (Nobutoshi et al., 2000), *Bacillus subtilis* (Hunag and Wu, 2004), *Nocardia farcinica* (Ishikawa et al., 2004) and *Microbacterium* sp. (Zhou et al., 2005) have been used to produce uricase.

The aim of the present work is to investigate the ability of *Bacillus thermocatenulatus* as a novel uricase producer and to optimize the enzyme productivity.

2. Methods

2.1. Strain

The organism used throughout this study is a new Egyptian soil *B. thermocatenulatus* isolate. A single isolated colony was selected and the bacterium was identified by 16S rRNA gene sequencing (El-Helow, 2001) and blasting the results in the GenBank (accession number DQ887577). Biology WorkBench, showed 99.998% identity to the homologous fragments of *B. thermocatenulatus*.

2.2. Enzyme production conditions

Cultures were allowed to grow at 30 °C with shaking at 200 rpm, in 250 ml conical flasks containing 50 ml aliquots

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doi:10.1016/j.biortech.2007.01.048

pre-culture basal medium of the following composition (g/l): peptone, 20; glucose, 30; KH_2PO_4, 1; $MgSO_4 \cdot 7H_2O$, 0.5; and uric acid, 0.3. Then 0.5 ml of the overnight culture was centrifuged, washed with sterile saline and consequently used as inoculum for the basal production medium of the following composition (g/l): K_3PO_4, 10; $MgCl_2$, 1; and uric acid, 3. Bacterial growth was monitored turbidimetrically at 550 nm. At the indicated time, 2 ml of the growing cultures was taken and centrifuged at 8000 rpm for 2 min. The cell free supernatant was used as crude enzyme preparation for further determinations.

2.3. Enzyme assay

The principle of enzyme measurement was as follow: uricase could catalyze the oxidation of uric acid into allantoin and H_2O_2, which was then measured by using a reaction system containing 4-aminoantipyrine, phenol and peroxidase as chromogen. In practical analysis, 0.1 ml enzyme solution was incubated with a mixture of 0.6 ml sodium borate buffer (pH 8.5, 0.1 M) containing 2 mM uric acid, 0.15 ml 4-aminoantipyrine (30 mM), 0.1 ml phenol (1.5%), 0.05 ml peroxidase (15 U/ml) at 37 °C for 20 min (Masaru, 1981). The reaction was stopped by addition of 1.0 ml ethanol, and the absorbance at 540 nm was read against the blank by a spectrophotometer. One unit of enzyme was defined as the amount of enzyme that produces 1.0 mmol of H_2O_2 per minute under the standard assay conditions.

2.4. Determination of the kinetic constants K_m and V_{max}

The kinetic constants K_m and V_{max} were estimated by the method of Lineweaver and Burk (1934).

2.5. Effect of temperature on uricase activity

Crude enzyme solution was incubated in water bath at different temperatures ranged from 25 to 80 °C for 45 min. The remaining activities were determined after their cooling to 37 °C.

2.6. Biomass determination

The biomass of cells was determined by measuring the absorbance at 550 nm.

2.7. Effect of carbon and nitrogen source

Different carbon sources (citric acid, sucrose, glucose, lactose, starch and cane molasses pretreated with 15 g/l calcium phosphate) and nitrogen sources (potassium nitrate, sodium glutamate, ammonium sulfate, peptone, yeast extract, maize milk, beef extract, soybean flour and corn steep liquor) were tested in order to determine their influences in the uricase production. Uric acid (0.3% (w/v)) was used in all these experiments as an inducer.

2.8. Effect of aeration and initial pH

The effect of aeration on uricase production was examined using 100 ml conical flasks containing 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60 ml of medium and incubated as described above. To determine the optimal pH for uricase production, the bacterium was cultured in media, of which the pH was adjusted to 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5 and 10.0.

3. Results

The time course of uricase production is shown in Fig. 1. The maximum enzyme activity (0.45 U/ml) was observed at 30 h cultivation.

The Lineweaver–Burke plot of *B. thermocatenulatus* crude uricase (Fig. 2) showed V_{max} and K_m of 0.99 U/ml and 0.25 mM, respectively. Fig. 3 illustrates the effect of different temperatures on uricase. The enzyme retained 100% of its initial activity after been treated at 75 °C for 45 min.

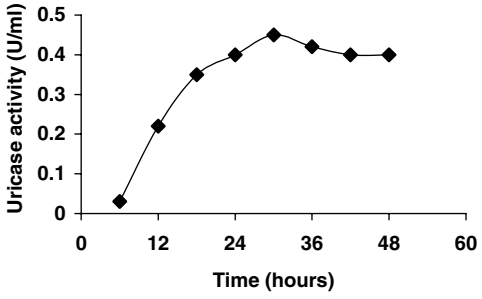

Fig. 1. Time course of uricase production.

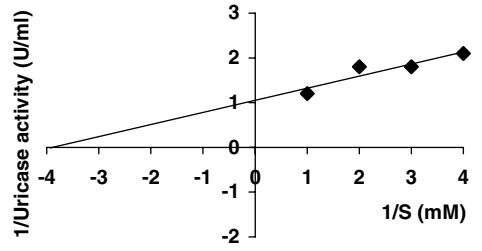

Fig. 2. The K_m and V_{max} values of uricase produced by *B. thermocatenulatus*.

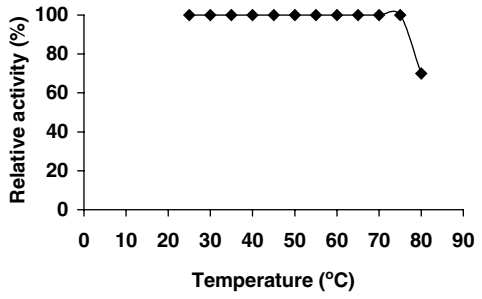

Fig. 3. Thermal stability of crude uricase.