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Isolation of a thermostable uricase-producing bacterium and study on its enzyme production conditions

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Abstract

A uricase-producing bacterium was isolated from soil with a medium containing uric acid as the only carbon source. Based on its morphological and physiological characteristics, as well as 16S rDNA sequence and phylogenetic tree analysis, this new isolate belong to the genus *Microbacterium*. After heat treatment at 70 °C for 30 min, the uricase retained about 100% of the initial activity. The enzyme activity remained largely unchanged when it was stored in borate buffer at pH 8.5 at 37 °C for 40 days. The effects of different factors on the enzyme production were studied. Maize milk was the best C and N resources, and the uric acid showed to be an inducer for uricase production. When the strain was cultured at 30 °C at pH 7.5 for 30–36 h, the uricase activity peaked at 1.0 U/ml.

Keywords: Uricase; Thermostable enzyme; *Microbacterium* sp

1. Introduction

Uricase (urate oxidase EC 1.7.3.3, UC) catalyses the oxidation of uric acid, a final product of purine catabolism, to allantoin, which is more soluble and more easily to be excreted than the starting compound [1]. This enzyme is widely present in most vertebrates but is absent in humans [2]. When the level of uric acid increases in blood over than the normal value it can cause renal failure and may contribute to a group of diseases such as gout, leukemia, toxemia of pregnancy, severe renal impairment and idiopathic calcium urate nephrolithiasis [3]. Consequently, uric acid determination is one of the parameters monitored in urine and blood. At present, different methods of uric acid analysis such as chemistry [4], colorimetry [5], mass fragmentography [6], radiochemical-HPLC method [7] and enzyme electrode [8] are available. However, among all these methods, the colorimetric one employing uricase and peroxidase is a relative simple, sensitive and specific method. Furthermore, it does not require expensive apparatus and have been proved to be more suitable for routine test. This method is so popular that its commercial kits are already available [9]. Being as a commercial kit, the enzyme to be used in it should has some special quality such as high stability and activity.

Some microorganisms such as *Arthrobacter globiformis* [10], *Bacillus subtilis* [11] and *Nocardia farcinica* [12] have been used to produce uricase. However, the uricase produced by these bacteria are not thermal stable and at the temperatures higher than 60 °C will lost their activities short periods [2].

The purpose of present study was to investigate the uricase production ability of the strain, and some physicochemical parameters were tested to optimize uricase productivity.

2. Materials and methods

2.1. Organisms

All strains investigated in this study were isolated from the soil samples collected from Hangzhou, Zhejiang Province, China. The cultures were maintained on Luria-Bertani (LB) agar and stored at 4 °C.

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2.2. Media

The composition of enrichment medium was 0.8% Uric acid, 0.2% K_2HPO_4, 0.05% KH_2PO_4, 0.01% $MgSO_4{\cdot}7H_2O$, 0.01% NaCl and 0.01% $CaCl_2$. The pH was adjusted to 7.5. The basal medium consisting of 0.3% uric acid, 0.2% K_2HPO_4, 0.05% KH_2PO_4, 0.01% $MgSO_4{\cdot}7H2O$, 0.01% NaCl and 0.01% $CaCl_2$ and 1.0% (v/v) trace elements solution containing of 5.2% $ZnSO_4$, 5.0% $FeSO_4{\cdot}7H_2O$, 5.0% $CuSO_4{\cdot}7H_2O$ and 0.05% $MnSO_4{\cdot}H_2O$.

2.3. Culture condition for bacteria

The strain from a slant was transferred to a 100 ml Erlenmeyer flask containing 10 ml of sterilized medium. It was then incubated in an orbital shaker operating at 120 rpm at 28 °C for 24 h. At the end of the incubation, 300 μl of the culture was transferred to another 100 ml Erlenmeyer flasks containing 30 ml medium, and was used for the study of the conditions of the fermentation. The culture condition experiments were performed in triplicate. During the fermentation, a 4 ml sample of the culture was withdrawn at 6 h intervals and the cells were collected by centrifugation at 5000 × *g* for 10 min. The cells were suspended again in 4 ml of borate buffer (pH 8.5) and treated by an ultrasonic device to lease the enzyme. It was then centrifuged at 5000 × *g* for 20 min, the supernatant was used for analysis enzyme activity.

2.4. 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, 0.10 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 [13]. The reaction was stopped by addition of 1.0 ml ethanol, and the absorbance at 540 nm was read against the blank in a spectrophotometer. One unit of enzyme was defined as the amount enzyme that produces 1.0 μmol of H_2O_2 per minute under the standard assay conditions.

2.5. Biomass determination

The biomass of cells was determined by measuring the absorbance at 600 nm.

2.6. Effect of carbon and nitrogen source

Different carbon sources (citric acid, sodium acetate, saccharose, glucose, lactose, starch and fructose) and nitrogen sources (sodium glutamate, ammonium sulfate, peptone, yeast extract, maize milk, beef extract and tyrosine glycine) were tested in order to determine their influences in the uricase production. Uric acid (0.2% (w/v)) was used in all these experiments as an inducer.

2.7. Effect of aeration and initial pH

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

2.8. Identification of strain ZZJ4-1

Physiological characteristics were determined by the procedures outlined in Manual of Methods for General Bacteriology [14]. Cell morphology was observed using a transmission electronic microscope after staining cells negatively with 1% (w/v) phosphotungstic acid. The 16S rDNA sequences and phylogenetic tree analysis were determined as described by Rainey et al. [15].

3. Results

3.1. Isolation of the strain

Forty strains of bacteria were able to grown in the medium containing uric acid. Five cultures showed with high uricase production ability were selected and intensely studied. The analysis of their uricase thermal stability (Fig. 1) and Km values (Fig. 2) was carried out, and the strain ZZJ4-1, which had the highest thermal stability and the lowest Km value, was selected for further study.

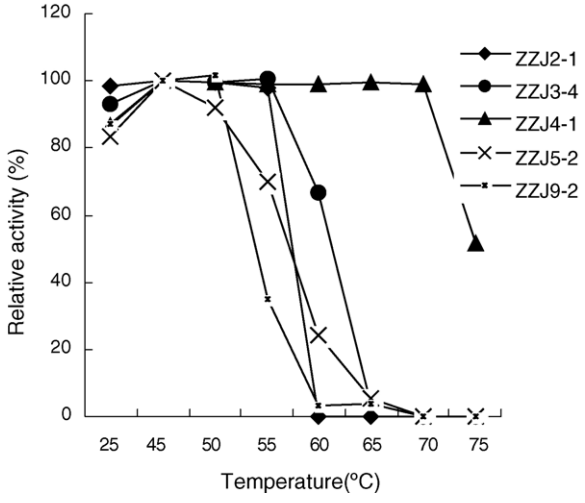

Fig. 1. Effect of temperature on different uricase activities. Crude enzyme solution was incubated in water bath at different temperatures for 30 min respectively. The remaining activities were determined after their cooling to 37 °C.