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Production and Partial Characterization of Uric Acid Degrading Enzyme from New Source *Saccharopolyspora* sp. PNR11

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Abstract: The strain PNR11 was isolated from gut of termite during the screening for uric acid degrading actinomyces. This strain was able to produce an intracellular uricase when cultured in fermentation medium containing uric acid as nitrogen source. Base on its morphological characters and 16S rDNA sequence analysis, this strain belong to the genus *Saccharopolyspora*. This is the first report of uricase produced from the genus *Saccharopolyspora*. The aim of this study was to investigate the effects of different factors on uricase production by new source of *Saccharopolyspora*. *Saccharopolyspora* sp. PNR11 was cultured in production medium in order to determine the best cultivation period. The result showed that the time period required for maximum enzyme production was 24 h on a rotary shaker operating at 180 rpm. Optimized composition of the production medium consisted of 1% yeast extract, 1% maltose, 0.1% K_2HPO_4, 0.05% $MgSO_4$ $7H_2O$, 0.05% NaCl and 1% uric acid. The optimum pH and temperature for uricase production in the optimized medium were pH 7.0 and 30°C, respectively. When the strain was cultured at optimized condition, the uricase activity reached to 216 mU mL^{-1} in confidential level of 95%. The crude enzyme had an optimum temperature of uricase was 37°C and it was stable up to 30°C at pH 8.5. The optimum pH of uricase was 8.5 and was stable in range of pH 7.0-10.0 at 4°C. This strain might be considered as a candidate source for uricase production in the further studies. Present finding could be fulfill the information of uricase produce from actinomycetes.

Key words: Optimization, uricase, *Saccharopolyspora*, actinomycetes

INTRODUCTION

Uricase (Urate oxidase, EC 1.7.3.3) catalyzes the oxidative opening of the purine ring of urate to yield alantoin, carbondioxide and hydrogen peroxide. It has vast and beneficial uses both *in vitro* and *in vivo*. Urate is a final oxidation product of purine catabolism (Bertrand *et al*., 2008). Determining the urate concentration in blood and urine is required for the diagnostic of gout as urate accumulation. It 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 hyperucicemia, as Rasburicase (Bomalaski and Clark, 2004; Haidari *et al*., 2008).

Many organisms including plant and microorganisms are able to produced uricase. To date, pure cultures of bacteria capable of producing uricase that have been documented are only *Pseudomonas aeruginosa* (Frank and Hahn, 1955), *P. acidovorans* (Sin, 1975), *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; Kai *et al*., 2008). But very limited research has been directed towards uricase production from termite actinomycetes. The genus *Saccharopolyspora* contains twenty described species. *Saccharopolyspora* is a well know producer of macrolide antibiotic such as erythromycin and spinosad. However, the production of uricase has not been reported.

The aim of the present study was to investigate the ability of *Saccharopolyspora* sp. PNR11 as a novel uricase producer. In addition, fermentation medium composition and relevant conditions were tested to optimized uricase enzyme productivity.

MATERIALS AND METHODS

This research project was conducted from January 2010 to December 2010 at Faculty of Science and Technology, Phranakhon Rajabhat University, Thailand.

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Microorganism: *Saccharopolyspora* sp. PNR11 in this study was isolated from gut of termites in genus of *Termes* collected from Sakaerat Environmental Research Station in Nakhon Ratchasima province, Thailand. The culture was maintained on Yeast extract-Malt extract (ISP2) agar. Based on its partial 16S rDNA sequence and morphological characters, strain PNR11 was classified to the member of genus *Saccharopolyspora*.

Medium: The composition of pre-culture medium was 1% peptone, 1% glucose, 0.1% K_2HPO_4, 0.05% $MgSO_4 \bullet 7H_2O$ and 0.05% NaCl. The pH was adjusted to 7.0. For production medium, 1% uric acid was added into pre-culture medium and 1% maltose was used as a carbon source.

Culture condition for bacteria: The strain from a slant was transferred to a 500 mL Erlenmeyer flask containing 50 mL of sterilized pre-culture medium. It was then incubated in a rotary shaker operating at 180 rpm at 30°C for 48 h. At the end of incubation, 1 g of wet weight was transferred to another 500 mL Erlenmeyer flask containing 50 mL of production medium and used for the study of the fermentation condition. The uricase production was evaluated each 6 up to 36 h. Uricase activity and intracellular protein were performed. The data of experiments were analyzed by GNU PSPP Statistical Analysis Sofware Release 0.6.2.

Effect of carbon and nitrogen source: For the effect of carbon sources on uricase production, the 500 mL Erlenmeyer flasks were prepared containing 50 mL of the basal medium (1% peptone, 0.1% K_2HPO_4, 0.05% $MgSO_4 \bullet 7H_2O$, 1% uric acid and 0.05% NaCl) supplemented with 1% of different carbon sources (maltose, glucose, galactose, fructose, lactose, sucrose, soluble starch and glycerol). The absent of carbon source was set for a control experiment. Uricase assay was performed after 24 h cultivation.

To detect the effect of nitrogen sources on uricase production, the 500 mL Erlenmeyer flasks were prepared containing 50 mL of the basal medium containing 1% of maltose as a carbon source and supplemented with 1% of different nitrogen sources (peptone, tryptone, corn steep liquor, yeast extract, ammonium acetate, sodium nitrate and potassium nitrate) in order to determine their influences in the uricase production. Uricase assay was performed after 24 h cultivation.

Determination of temperature and pH on uricase production: Effect of temperature on the enzyme production was studied in the suitable production medium from above experiment at different temperature ranging from 20 to 37°C. The effect of pH was also studied by adjusting the pH of the production medium to different level ranging from pH 5.0 to 9.0.

Preparation of crude intracellular uricase: The cells were collected by centrifugation at 13,000 rpm for 5 min and washed twice time with 0.85% NaCl. The cells were suspended in 10 volumes of wet basis of 50 mM borate buffer (pH 7.0) and treated by an ultrasonic device to lease the enzyme. It was then centrifuged at 13,000 rpm for 5 min; the supernatant was used for analysis of uricase activity.

Enzyme assay: Uricase activity was measured from cell-free extract. The assay mixture contained 0.5 mL of enzyme solution in 50 mM borate buffer (pH 8.5) and 0.01% uric acid in a final volume of 3.0 mL. Incubation was carried out at 37°C for 5 min. The reaction was terminated by the addition of 200 μL of 20% KOH. The absorbance was measured at 293 nm using spectrophotometer. As a control, the solution of KOH was added to substrate before the addition of the enzyme solution. One unit of enzyme was defined as the amount of enzyme necessary to transform 1 μmol of uric acid into allantoin in 1 min at 37°C

Protein determination: Protein was estimated by determination of intracellular protein. Cell mass was separated from the medium, disrupted and released protein was determined by the method of Lowry *et al.* (1951) using Bovine Serum Albumin (BSA) as a standard for calibration.

Determination of optimum pH and temperature of uricase activity: Investigation to find out the optimum pH for uricase activity was carried out in 50 mM buffers with various pH values ranging from pH 4.0 to 10.0. Acetate buffer was used in the pH range of 4.0 and 5.0. Phosphate buffer was used for pH 6.0 and 7.0. Borate buffer was used for pH 7.0, 7.5, 8.0, 8.5 and 9.0. Glycine buffer was used for pH 10.0 and then incubated at 37°C for 5 min. Amount of uricase activity was determined. To determine the pH stability of the enzyme, the enzyme was incubated in different pH as mentioned above at 4°C for 10 min. The remaining uricase activity was measured under standard assay procedure.

The optimum temperature for uricase activity was determined by incubating the enzyme in 50 mM borate buffer (pH 8.5) at various temperatures (20-55°C) for 5 min. Amount of uricase activity was determined. For stability,

those reactions were pre-incubated at various temperatures for 10 min. The remaining uricase activity was measured under standard assay procedure.

RESULTS AND DISCUSSION

Uricase production: The uricase activity was measured in both the cultural supernatant as well as the intracellular fluid. No uricase activity was found in the supernatant while uricase was detectable in biomass. The uricase production level obtained from *Saccharopolyspora* sp. PNR11 was determined when it was culture in the medium containing 1% peptone, 1% maltose, 0.1% K_2HPO_4, 0.05% $MgSO_4$ $7H_2O$, 0.05% NaCl and 1% uric acid. The uricase production rapidly increased during the first 18 h of cultivation, yielding 150 mU mL^{-1} and then slowly increased until it reached the maximum activity of 168 mU mL^{-1} after 24 h cultivation. The enzyme activity was decreased to 130 mU mL^{-1} after 30 h cultivation (Fig. 1). The intracellular protein of cell mass was increased which was related to the enzyme production. The cultivation time of *Saccharopolyspora* sp. PNR11to reach the maximum enzyme production was shorter than that of *Microbacterium* ZZJ4-1 for 12 h (Zhou *et al*., 2005). Therefore, *Saccharopolyspora* sp. PNR11 was considered as an intracellular uricase producer. Most of the microbial uricase from *Microbacterium* ZZJ4-1 and *Streptomyces cyanogenus* origin (Zhou *et al*., 2005; Kai *et al*., 2008; Yokoyama *et al*., 1988) are intracellular and cell disruption is necessary in order to obtain the enzyme. However, in some microbial resource such as *Bacillus fastidiosus* (Bongaerts *et al*., 1978), *Mucor hiemalis* (Yazdi *et al*., 2006) and *Pseudomonas aeruginosa* (Saeed *et al*., 2004) extracellular uricase with no need of cell disruption have been reported.

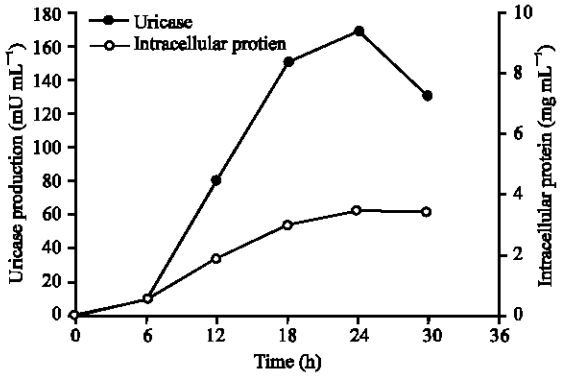

Fig. 1: Time course of uricase and intracellular protein produced by the *Saccharopolyspora* sp. PNR11 cultured at 30°C in a medium containing maltose as the carbon source

Effect of carbon and nitrogen source: The influence of different carbon sources was studied and the results presented in Fig. 2a. The effect of carbon sources showed that the uricase production was most affected by the addition of maltose with activity of 168 mU mL^{-1} where it activity was approximately 148 and 151 mU mL^{-1} higher than that of control (production medium without carbon source) and glucose as a carbon source, respectively. This indicated that glucose could not stimulate uricase productivity. However, low level of uricase activity was detected in intracellular fluid due to the present of uric acid as an inducer. Moreover, the result also showed that uricase obtained from *Saccharopolyspora* sp. PNR11 was inducible enzyme. Addition of maltose also affected on uricase production of *Mucor hiemalis* with the activity of 67% higher than control (Yazdi *et al*., 2006).

The production medium having maltose as carbon source was used for nitrogen source optimization. The effect of nitrogen source, several inorganic and organic nitrogen sources were evaluated (Fig. 2b). Result showed that simple nitrogen source had less positive effect on the enzyme production. Among the various complex nitrogen source, yeast extract partially increased enzyme activities up to 216 mU mL^{-1} that was 76 and 48 mU mL^{-1} higher than that of control and peptone, respectively. However, the enzyme activity was detected in control experiment containing uric acid alone without any other nitrogen source. This result showed that uric acid not only inducer but also served as a nitrogen source for uricase production. It has been reported that the highest uricase induction levels for *Candida utilis* (Jianguo *et al*., 1994) and *Mucor hiemalis* (Yazdi *et al*., 2006) were obtained in the medium mainly containing uric acid. Addition of corn steep liquor, sodium nitrate, potassium nitrate and ammonium acetate did not enhance the enzyme production comparing to control. Most complex nitrogen sources significantly increased enzyme production. In general, uricase production was far more enhanced by using the organic nitrogen than the inorganic nitrogen. It was possible that organic nitrogen may contains most kinds of amino acids for the growth of bacterium that could be metabolized directly by cells, consequently promoting the uricase production (Lotfy, 2008). Therefore, uric acid itself was sufficient to induce the enzyme production.

Optimum pH and temperature for uricase production: The effect of initial pH on uricase production was performed on a fermentation medium containing maltose and yeast extract as a suitable C and N source,

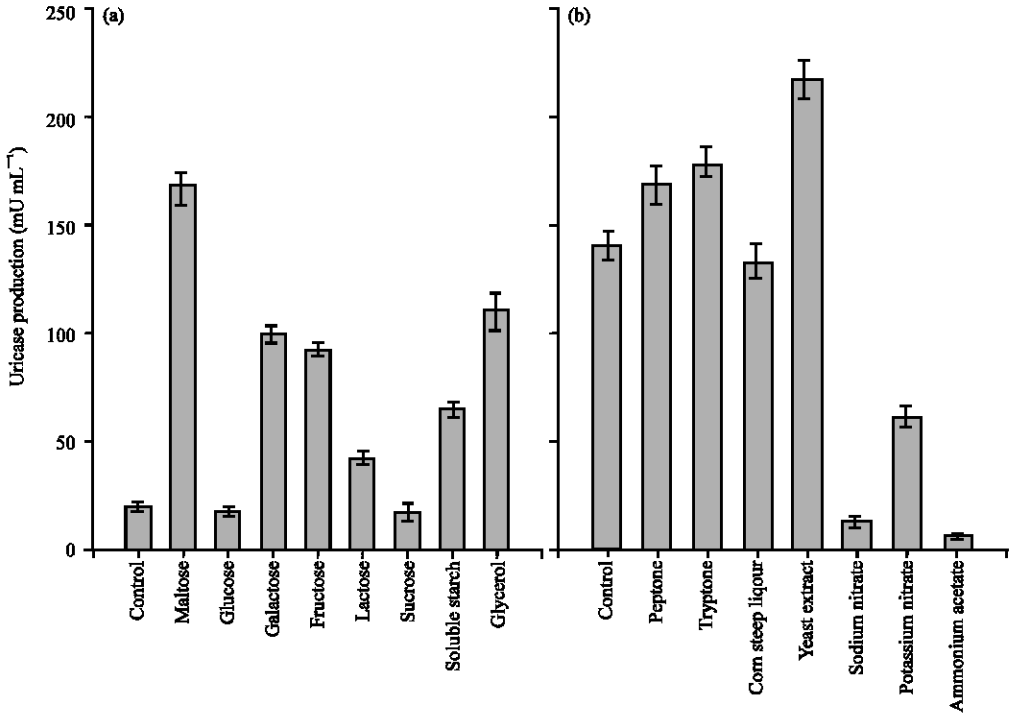

Fig. 2: Effects of carbon source (a) and nitrogen source (b) on uricase production by *Saccharopolyspora* sp. PNR11

respectively. The enzyme production was observed at initial pH of 5.0 to 9.0. Uricase activity was rapidly increased during pH 5.0 to 7.0 of cultivation and achieved to maximum level at pH 7.0 with the activity of 216 mU mL^{-1} (Fig. 3a). The productivities were slowly decreased at alkali condition ranging of pH 8.0 to 9.0 in which was 16 and 81 mU mL^{-1} lower than that of the maximum activity, respectively. The optimum pH for uricase production of this strain was higher than that of uricase produced by *Mucor hiemalis* (Yazdi *et al*., 2006) but lower than that of the enzyme produced by *Microbacterium* sp. ZZJ4-1 (Zhou *et al*., 2005) and *Gliomastix gueg* (Atalla *et al*., 2009).

The study on influence of temperature on enzyme production was carried out at various temperatures in the production medium (pH 7.0) containing maltose and yeast extract. Optimum temperature for uricase production of *Saccharopolyspora* sp. PNR11 was 30°C with the activity of 216 mU mL^{-1} (Fig. 3b). Moreover, it was found that the production of enzyme severely decreased to 180 mU mL^{-1} when the cultures were grown at 37°C. This result was similar to uricase production by *Gliomastix gueg* (Atalla *et al*., 2009). This indicated that less efficient on the enzyme production was due to high temperature that is inheritance behavior of this strain. In addition, the incubation at high temperature affected to reduce the enzyme production might cause of less thermostability of the enzyme.

Optimization of pH and temperature on uricase activity and stability: Optimum pH for the uricase activity of *Saccharopolyspora* sp. PNR11was pH 8.5 and the activities gradually decreased when the pH raised (Fig. 4a). Notice that the relative activity at pH 8.0 and 9.0, activities of the enzymes still exhibited 84 and 60%, respectively. For storage, these enzymes had full activity when kept at 4°C for 10 min in a range of pH 7.0 to 10.0 as shown in Fig. 4a. Thus, this enzyme could storage without lose its activity at high pH that would protect them from bacteria.

Optimum temperature to exhibit its activities of uricase of *Saccharopolyspora* sp. PNR11 was 37°C incubating for 5 min (Fig. 4b). However, the enzymes exhibited low activities at over temperature of 45°C and 20% of its optimum activity was remained when incubated at 55°C. This enzyme was stable up to 30°C for 10 min (Fig. 4b). When the enzyme was kept at 37°C, this enzyme was denatured rapidly. At 55°C, its activities remained only 9% of original activity. The optimum pH and temperature of this enzyme was closed to other uricase produced by *Streptomyces* sp. THPN 58 that were in range of temperatures of 35°C and pH 8.5 (Khucharoenphaisan and Sinma, 2010). It could be observed that the enzymatic activity was notably dropped at high temperature of 45°C. *Norcardia farcinica* also has been reported to produce non-thermal stable uricase and rapidly lost most of it activity at 60°C

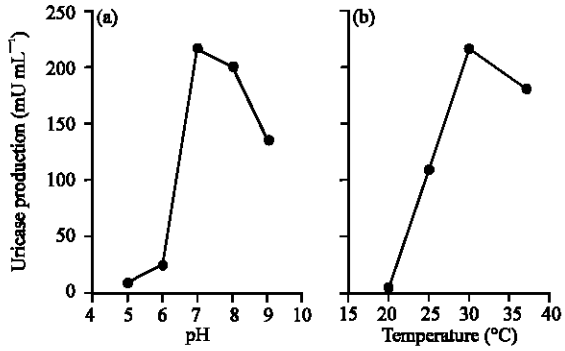

Fig. 3: Effect of initial pH (a) and temperature (b) on uricase production in 24 h by *Saccharopolyspora* sp. PNR11

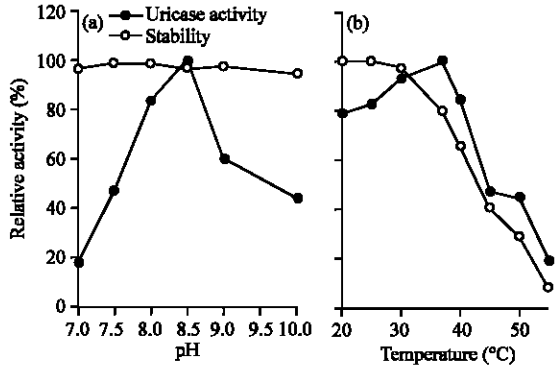

Fig. 4: Effect of pH (a) and temperature (b) on crude uricase activity and stability from *Saccharopolyspora* sp. PNR11. For the effect of pH, the reaction mixture was incubated at 37°C with various buffers and for the temperature analysis the sample were incubate at each temperature for 5 min in 50 mM borate buffer pH 8.5.

(Schiavon *et al*., 2000). In contrast, *Microbacterium* ZZJ4-1 was an excellent thermostable uricase producer; its enzyme was stable at 65°C for 30 min (Kai *et al*., 2008).

CONCLUSION

The actinomycetes, *Saccharopolyspora* sp. PNR11 produced an intracellular uricase. By optimizing cultural condition, the highest level of uricase activity attained at 216 mU mL^{-1}. The optimum pH and temperature of this enzyme was pH 8.5 and 37°C, respectively. This is the first report on the production of uricase from *Saccharopolyspora*. This strain might be considered as a candidate source for uricase production in the further studies.

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