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ผลขององค์ประกอบในอาหารต่อการผลิตเอนไซม์ย่อยโปรตีนในสภาวะด่างโดย *Bacillus* sp.PS121

Effect of Medium Composition on Alkaline Protease Production by *Bacillus* sp.PS121

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บทคัดย่อ

ผลขององค์ประกอบในอาหารต่อการเจริญและการผลิตเอนไซม์ย่อยโปรตีนในสภาวะด่างโดย *Bacillus* sp.PS121 เมื่อทำการเลี้ยงในอาหารที่ประกอบด้วย 0.5% glucose, 0.75% peptone, 0.5% $MgSO_4$, 0.5% KH_2PO_4 และ 0.01% $FeSO_4$ ที่ pH 9.0 อุณหภูมิ 40°C และเมื่อทำการเปลี่ยนแปลงแหล่งคาร์บอนและแหล่งไนโตรเจนทั้งในรูปสารอินทรีย์และสารอนินทรีย์ พบว่าแหล่งคาร์บอนและแหล่งไนโตรเจนที่เป็นสารอินทรีย์ที่ดีที่สุดสำหรับแบคทีเรียชนิดนี้คือกลูโคส และเปปโตน ตามลำดับ ในขณะที่แหล่งไนโตรเจนที่เป็นสารอนินทรีย์ที่ดีที่สุดคือแอมโมเนียมคาร์บอเนต ส่วนไกลซีนเป็นกรดอะมิโนที่ดีที่สุดสำหรับการผลิตเอนไซม์ชนิดนี้ และการเติม Mg^{2+} ในอาหารเลี้ยงเชื้อส่งผลให้การผลิตเอนไซม์ดีขึ้น

คำสำคัญ : อัลคาไลด์โปรตีเอส บาซิลลัส องค์ประกอบในอาหาร

Abstract

Study on effect of medium components for maximum growth rate and alkaline protease production by *Bacillus* sp.PS121. The growth rate and proteolytic activity was observed when the bacterial was growth in medium containing 0.5% glucose, 0.75% peptone, 0.5% $MgSO_4$, 0.5% KH_2PO_4 and 0.01% $FeSO_4$ at pH 9.0 and temperature of 40°C. Different carbon and nitrogen sources in the form of fine powder of organic and inorganic were studied for alkaline protease production. The best of carbon and organic nitrogen sources for this bacterium were glucose and peptone, respectively. While the most effective inorganic nitrogen sources was ammonium carbonate. The study was also shown that glycine was the best amino acid for protease production. Supplementation of the culture medium with Mg^{2+} improved the protease production substantially.

Keywords : Alkaline protease, *Bacillus* sp., Medium composition

INTRODUCTION

Alkaline proteases, an important group of industrial enzymes are produced by a wide range of microorganisms including fungi, animal and bacteria. Bacteria which produce proteases include *Bacillus* sp., *Alcaligenes faecalis*, *Pseudomonas fluorescens* and *Aeromonas hydrophilia.* (Gupta and Lorenz, 2002, pp.15-32). *Bacillus* sp. are specific producers of extracellular protease. (Priest, 1977, pp.711-735). These enzymes have wide industrial application, including pharmaceutical industry, leather industry, manufacture of protein hydrolizates, food industry and waste processing industry. (Pastor, *et al.*, 2001, pp.1-8). Studies on other strains of *Bacillus* showed that nutritional, chemical and physical factors can influence protease production. Nutritional factors include the sources of carbon, nitrogen and metal ions. In addition, availability of metal ions in growth media was also shown to affect protease production. (Adinarayana, *et al.*, 2003, pp.1-9). Fe^{3+} and Ca^{2+} were reported to increase enzymatic activity while Mg^{2+}, Na^{+}, Zn^{2+} and Cu^{2+} interfered with protease production by *Bacillus* sp.SSR1. (Singh, *et al.*, 2001, pp.781-785). Besides nutritional factors, physical factors such as temperature, pH and incubation time (Rahman *et al.*, 2005, pp.429-436) also significantly affect protease production.

In this paper *Bacillus* sp.PS121 was investigated for screening of medium components and culture condition for protease production.

METERIALS AND METHODS

Organism

The bacterial strain used in this study was *Bacillus* sp.PS121 isolated from soil sample collected from shrimp pond in Pattani province, Thailand.

Enzyme production

The culture medium used in this work for protease production contained 0.5% glucose, 0.75% peptone, 0.5% $MgSO_4$, 0.5% KH_2PO_4 and 0.01% $FeSO_4$. The pH was adjusted to 9.0 with Tris-HCl buffer and this basal medium was sterilized by autoclaving at 121°C for 15 min. The medium (50 mL in 250 mL Erlenmeyer flasks) was inoculated with 5 mL of overnight culture and incubated at 37°C in a rotary shaker operated at 150 rpm for 24, 48 and 72 h. Before assay, the cells were separated by centrifugation at 4,000 rpm for 15 min and supernatant was used as crude enzyme preparation.

Enzyme assay

Protease activity was determined by using 2% (W/V) casein in 50 mM Tris-HCl buffer (pH 9.0) as a substrate. Casein solution with an equal volume of suitable diluted enzyme solution was incubation at 37°C. After 10 minutes the reaction was terminated by addition of 1 mL of 10% trichloroacetic acid. The mixture was centrifuged and supernatant (2 mL) was taken, to this 4 mL of NaOH (0.1 M) and 0.5 mL of Folin-ciocalteau reagent : distilled water (1:1 V/V). After 30 minutes the color developed was read at 670 nm against reagent blank prepared in same manner. Tyrosine served as the reference standard. The optical density of these solutions was measured. One unit (U) of enzyme activity was defined as the amount of enzyme that released 1 *mg* of tyrosine per minute, under assay conditions.

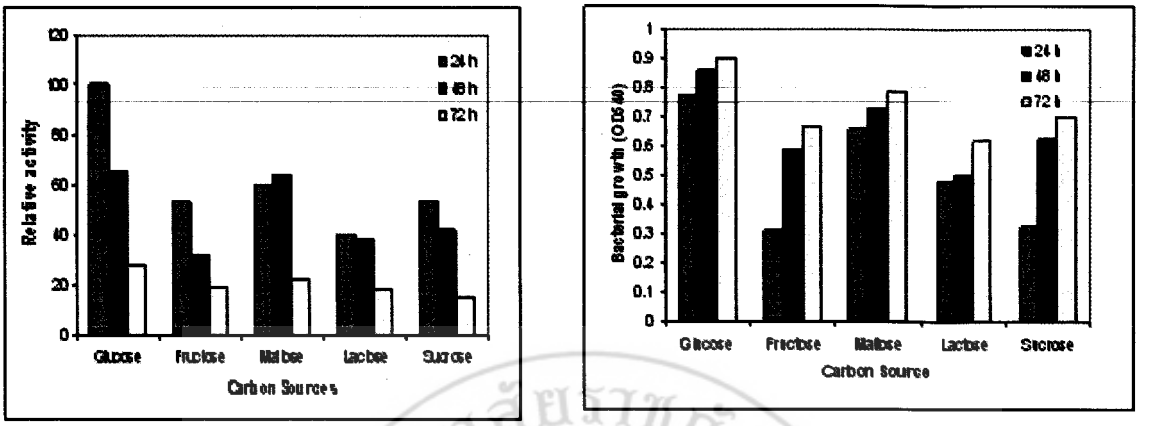
Effect of culture condition on enzyme production

Carbon sources chosen for the study were glucose, fructose, maltose, sucrose and lactose. These carbon sources were used to replace the carbon source available in media. In this study, three sources of nitrogen were used. They were organic nitrogen (peptone, tryptone, yeast extract and nutrient broth), inorganic nitrogen (ammonium nitrate, ammonium carbonate and urea) and amino acid (alanine, glutamic acid and glycine). Metal cations tested to replace metal ion source in the media were Mg^{2+}, Ca^{2+}, Cu^{2+} and Mn^{2+}.

RESULTS AND DISCUSSION

Effect of carbon sources on protease production

Results obtained showed that glucose instigated highest protease production and bacterial growth compared to other carbon sources at 24, 48 and 72 h of incubation (Fig 1a,b). Fructose, maltose, lactose and sucrose also showed high protease expression at 24 h but reduced by 48 and 72 h of incubation. This observation agrees with previous report which suggested that sources of carbon affected production of enzyme by bacteria. (Gupta, *et al.*, 2002, pp.381-398). At 72 h incubation, low protease production was detected in all the samples. The prolonged incubation time perhaps led to autodigestion of protease and proteolytic attacked by other proteases. (Priest, 1977, pp.711-753). This results is similar to previous report which showed that glucose caused high level of enzyme expression in *Bacillus subtilis* PE-11. (Adinarayana, *et al.*, 2003, pp.1-9).



(a) (b)

Fig 1 Effect of carbon sources on protease production and growth of *Bacillus* sp. PS121. Five different sources of carbon were tested in this experiment. Culture were incubated at 37°C, 150 rpm for 24, 48 and 72 h (a) : Quantitative analysis of alkaline protease activity was performed on samples harvested after the incubation periods. (b) : Bacterial growth in response to each carbon sources was also determined.

Effect of organic nitrogen sources on protease production

Production of extracellular protease has been shown to be sensitive to repression by different carbohydrate and nitrogen source.(Haulon, *et al.*, 1982, pp.845-851). In the present investigation, results obtained showed that peptone resulted in the highest level of protease activity and bacterial growth compared to other sources of organic nitrogen (Fig 2a,b). This result is in agreement with Adinarayana, *et al.*, (2003) which reported an increased production of protease by *Bacillus subtilis* PE-11 in the presence of peptone. Previous studies also reported that protease production by *Bacillus licheniformis* (Fereshteh, *et al.*, 2003, pp.183-185), *Bacillus stearothermophilus* F1 (Rahman, *et al.*, 2003, 199-210) and *Bacillus* sp. SMIA2 (Wellingta and Meire, 2004, pp.91-96) was best in presence of organic nitrogen sources. In some organism, however organic nitrogen sources were found to be better nitrogen sources both for growth and also protease production. (Phadatare, *et al.*, 1993, pp.72-76).

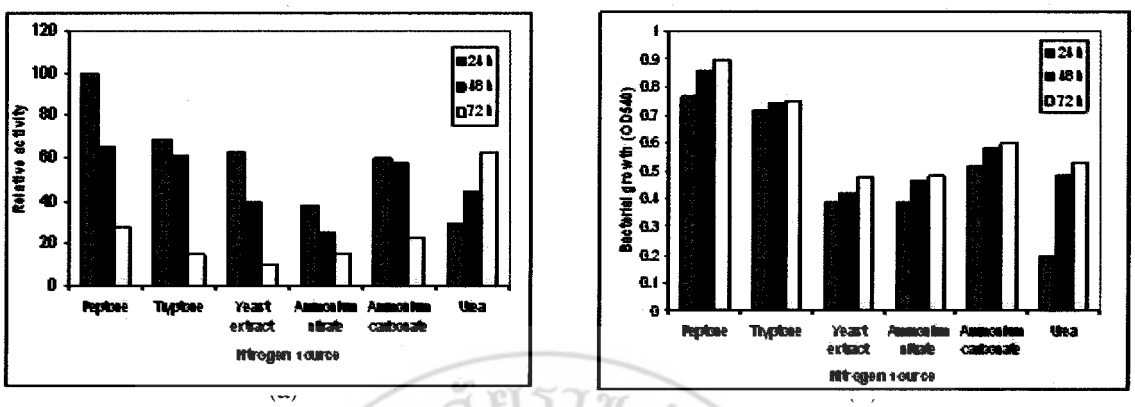


Fig 2 Effect of nitrogen sources on protease production and growth of *Bacillus* sp. PS121. Selected organic nitrogen sources were added to culture media and incubated at 37°C, 150 rpm for 24, 48 and 72 h. (a) : Quantitative analysis of alkaline protease activity was performed on samples harvested after the incubation periods. (b) : Bacterial growth in response to each nitrogen sources was also determined.

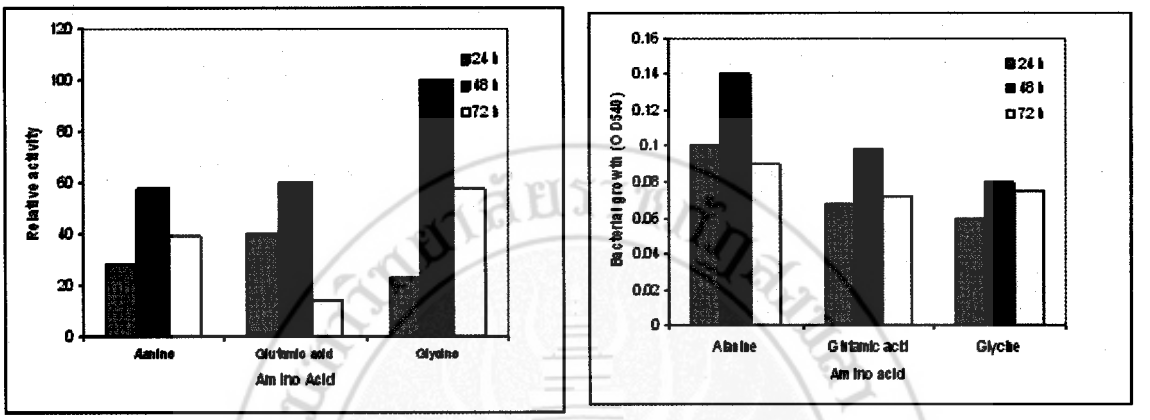
Effect of inorganic nitrogen sources on protease production

Inorganic nitrogen sources were also tested on the growth and protease production of *Bacillus* sp. PS121. Results obtained showed that ammonium carbonate led to high protease activity at 24 h but reduced at 48 and 72 h (Fig 2a). Growth was observed to be high in all the three sources of inorganic nitrogen. (Fig 2b). Urea did not enhance protease production at early stages of incubation but at later stages (48 and 72 h) protease production increased. Even though growth was stimulated, only moderate level of enzyme activities was obtained when ammonium nitrate was used as a nitrogen source. This was perhaps due to the inability of bacteria to utilize ammonium in the media. This effect of similar inorganic nitrogen sources was also observed for *Aeromonas hydrophila* (O'Reilly and Day, 1983, pp.1132-1135), *Bacillus cereus* strain 146. (Norazizah, *et al.*, 2005, pp.1-8).

Effect of amino acid on protease production

Alanine, glutamic acid and glycine were also tested as sources of amino acid for protease production in *Bacillus* sp. PS121. Results obtained showed various levels of growth and protease were produced in these different amino acid sources. In the presence of glycine, protease production was observed to be high when cultured at 48 h (Fig 3a). This observation is in agreement with previous report which showed that protease production in *Bacillus*

cereus strain 146 (Norazizah, *et al.*, 2005, pp.1-8) and *Pseudomonas* sp. B45 (Chakraborty & Srinivasan, 1992, pp.181-191) were enhanced considerably in the presence of glycine. Growth was observed to be high in all three sources of amino acid (Fig 3b).



(a) (b)

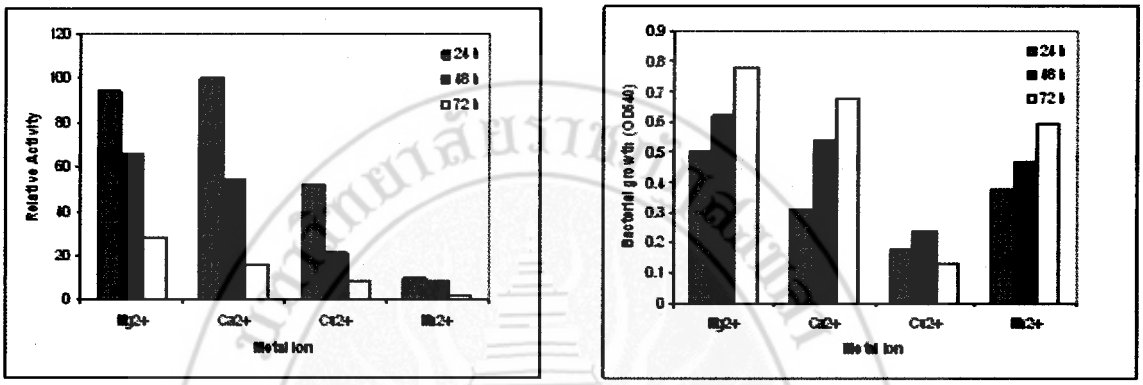
Fig 3 Effect of amino acids on protease production and bacterial growth. Alanine, glutamic acid and glycine were tested as sources of inorganic nitrogen for protease production in *Bacillus* sp. PS121 (a) : Various levels of protease activity were detected in the sample harvested after 24, 48 and 72 h incubation. (b) : Bacterial growth in response to each amino acid was also determined.

Effect of metal ions on protease production

Supplementation of culture medium with metal cations improved substantially the protease production and bacterial growth of *Bacillus* sp. PS121 (Fig 4a,b). This observation strongly suggested the requirement of some metal ions for protease production by organism. These results are in agreement with the earlier finding which showed enhancement of protease activity in the presence of metal ions. (Adinarayana, *et al.*, 2003, pp.1-9). Addition of Mg^{2+} and Ca^{2+} resulted in high protease production at 24 h incubation. Similarly, Mg^{2+} and Ca^{2+} were some of the best metal ions for protease production in *Bacillus* sp. SMIA2 (Wellingta and Meire, 2004, 91-96), *Bacillus polymyxa* B-17 (Matta & Punj, 1998, pp.139-145) and *Bacillus cereus* BG1. (Ghorbel-Frikha, *et al.*, 2005, pp.186-194).

CONCLUSION

In this study, it has been concluded that the screening of suitable medium ingredients plays an important role in the production of proteolytic enzyme by *Bacillus* sp.PS121. The presence of glucose, peptone, ammonium carbonate and Mg^{2+} in the growth medium has inducible effect on enzyme production.



(a) (b)

Fig 4 Effect of metal ions on protease production and bacterial growth. Several metal ions were added to culture media and incubated at 37°C, 150 rpm for 24, 48 and 72 h (a) : Quantitative analysis of alkaline protease activity was performed on samples harvested after the incubation periods. (b) : Bacterial growth in response to each metal ions was also determined.

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