ผลของพีเอชและอุณหภูมิต่อกัมมันตภาพของอะไมเลสที่ได้จากแบคทีเรีย *Vibrio harveyi*

The effect of pH and temperature on amylase activity from *Vibrio harveyi*

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บทคัดย่อ

อะไมเลส (amylase) เป็นเอนไซม์นอกเซลล์ที่สามารถย่อยโมเลกุลของแป้งให้เป็นมอโนแซ็กคาไรด์และหรือออลิโกแซ็กคาไรด์ อะไมเลสสามารถนำไปประยุกต์ใช้ในอุตสาหกรรมต่างๆ ได้มาก ดังนั้นงานวิจัยนี้จึงมุ่งศึกษากันมันตภาพของอะไมเลสที่พบในแบคทีเรียสายพันธุ์ *Vibrio harveyi* จากการทดสอบการเกิดวงใสบนวุ้นเลี้ยงเชื้อที่มีแป้งเป็นองค์ประกอบพบว่า *V. harveyi* สามารถย่อยแป้งบนวุ้นเลี้ยงเชื้อได้ เมื่อนำอาหารเหลวหลังจากบ่มกับแบคทีเรียเป็นเวลา 24 ชั่วโมง และแยกจุลินทรีย์ออกแล้ว ไปทดสอบผลของพีเอชและอุณหภูมิต่อกัมมันตภาพและเสถียรภาพของอะไมเลส จากผลการทดลองที่ได้พบว่า พีเอชและอุณหภูมิที่เหมาะสมกับอะไมเลสจากแบคทีเรีย *V. Harveyi* คือ pH 7.0 และอุณหภูมิ 30 ºC

คำสำคัญ: กันมันตภาพของอะไมเลส *Vibrio harveyi* พีเอช อุณหภูมิ

Abstract

Amylase is an extracellular enzyme which hydrolyzes starch molecules to monosaccharide and or small oligosaccharide. It has wide potential application in a number of industrial processes. In this study, *Vibrio harveyi* were used for investigating the amylase activity. From halo-forming zone test on starch agar medium, it was found that *V. harveyi* could hydrolyze soluble starch on the agar medium. In addition, the effect of pH and temperature on the amylase activity and its stability were studied using crude supernatant from broth medium after incubating with *V. harveyi* for 24 hours. The result showed that the optimal condition for this enzyme was at pH 7.0 and 30 ºC.

Keywords: Amylase activity, *Vibrio harveyi,* pH, temperature

Introduction

Amylase is an extracellular enzyme that hydrolyzes polysaccharides, starch and glycogen.[1] It is one important enzyme in industries such as food, paper, textiles, fruit juices, sweeteners and spot remover in dry cleaning.[2]

Amylase can be generally be divided into two types : endoamylases (α-amylase) and exoamylases (β-amylase, glucoamylase and α-glucosidase). Endoamylases randomly hydrolyze α-1,4 glucosidic linkages in starch to generate different length oligosaccharides. Exoamylases act at the non-reducing ends of polysaccharides by digesting α-1,4 glucosidic linkages and/or α-1,6 glucosidic linkages in starch and producing low molecular weight products, e.g., glucose and maltose.[3]

Although amylase is found in plants, animals and microorganisms,[4,5] those produced by microorganisms show more advantages over those produced by other sources, such as cost effectiveness, less time for production and ease of process improvement.[6] Exam-

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ples of amylase-producing microorganisms, there are Aspergillus sp.,[7] Penicillium sp.,[8] Pseudomonas sp.,[9] Streptomyces sp.,[10] Mucor sp.,[11] Rhizopus sp.,[12] Bacillus sp.[2] and Bifidobacterium.[13] In addition, few amylases from Vibrio sp. from marine sources are found such as Vibrio gazogenes[14] and Vibrio alginolyticus.[15,16] However, effects of temperature and pH on their amylase activity have never been reported. In this study, Vibrio harveyi were used for investigating those effects on amylase activity and its stability.

Materials and Methods

Halo zone test on TSA-starch agar

V. harveyi, isolated from the black tiger shrimp, were grown on a tryptic soy agar (TSA) plate supplemented with 2% (w/v) NaCl and 1% soluble starch and incubated at 30 ºC for 24 hours. The halo formation was observed after the iodine solution (0.02% (w/v) I_2 in 0.27% (w/v) KI) was poured onto the agar.

Production of amylase

V. harveyi on the TSA agar were inoculated into the tryptic soy broth (TSB) supplemented with 2% (w/v) NaCl and were incubated at 30 ºC for 24 hours. The overnight culture was diluted 1:100 into fresh TSB supplemented with 2% (w/v) NaCl and was incubated with shaking at 250 rpm for 24 hours at 30 ºC. After incubation, the supernatant of the culture after centrifugation (8,000 g, 15 minutes) at 4 ºC was used to determine amylase activity.

Amylase activity assay

Amylase activity was assayed using the procedure of Bernfeld.[17] Briefly, the reaction mixture contains 0.5 ml of supernatant and 0.5 ml (1.0%) solution of soluble starch in 0.1 M sodium phosphate buffer (pH 7.0). After incubation at 30 ºC for 5 minutes, the reaction was stopped by addition of 1.0 ml of 3,5-dinitrosalicylic acid reagent. Samples were then placed in a boiling water bath for 15 minutes and subsequently cooled down to room temperature, and 10.0 ml of distilled water was added. Absorbance was measured using a spectrophotometer at 540 nm. This was related to the glucose concentration by constructing a calibration curve, which gives the exact relationship between A_{540} and glucose concentration.

One unit (U) is defined as the amount of enzyme which releases 1 µg of reducing end groups per minute in 0.1 M sodium phosphate buffer (pH 7.0) with 1.0 % (w/v) soluble starch as substrate during 5 minutes incubation at 30 ºC.

Effect of pH on amylase activity and stability

The effects of pH on the amylase activity and stability were determined for a pH range of 3-11. The following buffer systems were used (0.1 M of each buffer): sodium citrate buffer (pH 3.0), sodium acetate buffer (pH 5.0), sodium phosphate buffer (pH 6.0 and 7.0), Tris-HCl buffer (pH 8.0 and 9.0), and glycine-NaOH buffer (pH 11.0). To measure the pH stability, the enzyme was incubated with the various buffers at 30 ºC for 60 minutes, and the residual activity was determined under the standard assay conditions.

Effect of temperature on amylase activity and stability

The effects of temperature on the enzyme activity and stability were determined at 30, 40, 50, 60, 70, 80 and 90 ºC at the optimum pH of 7.0. To determine thermal stability, the residual activity of the enzyme was determined under the standard assay conditions after incubation at different temperatures for 60 minutes.

Results and Discussion

The ability of Vibrio harveyi in producing extracellular amylase

V. harveyi producing amylase were screened by allowing them to grow for 24 hours on TSA agar with 1% soluble starch at 30 ºC. The plates were stained with the iodine solution, and a halo-forming zone was considered. The result showed that V. harveyi could hydrolyze soluble starch on the agar medium (Fig. 1). it was summarized that the amylase activity was found in V. harveyi.

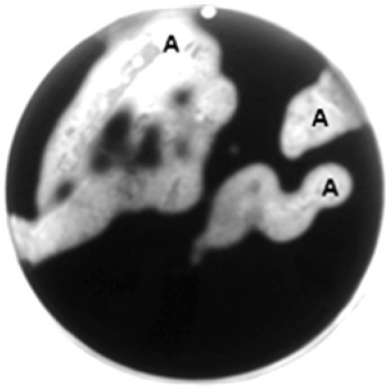

Figure 1 TSA agar containing 1% soluble starch with amylase activity from V. harveyi showing halo-forming zone formation (A)

Effects of pH on enzyme activity and stability

The optimal pH and amylase stability were examined in buffers pH 3-11 at 30ºC. The maximum activity of the enzyme was observed at pH 7.0 (Fig. 2), which is similar to that reported for the amylases from Bacillus sp strain SMIA-2[18] and Bacillus licheniformis.[19]

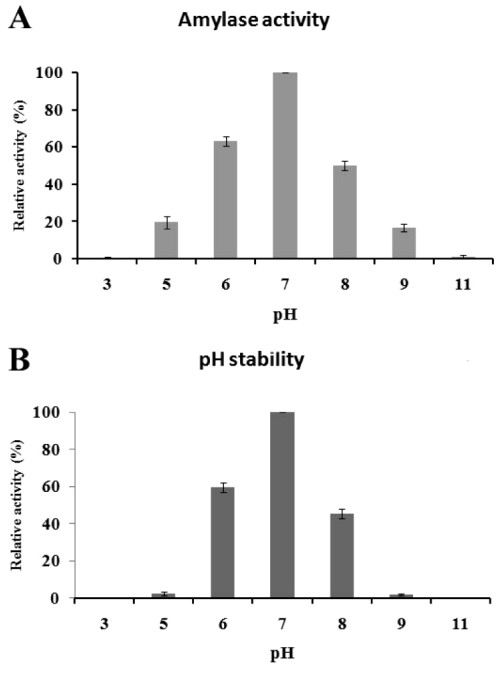

Figure 2 Effects of pH on the amylase activity (A) and stability (B) of the extracellular enzyme from V. harveyi. Values are the mean ± standard error from triplicate analyses

Effects of temparature on enzyme activity and stability

The optimal and stable activity of the amylase was investigated at different temperatures at 30-90 ºC and pH 7.0. The maximum enzyme activity was observed at 30 ºC (Fig. 3), which is similar to that reported for the amylases from Streptomyces strain 4ALGA[20] and Penicillium sp.[21]

Conclusion

The production of the extracellular amylase could be observed from V. harveyi. The optimal conditions for the maximum amylase activity and stability was at pH 7.0 at 30 ºC.

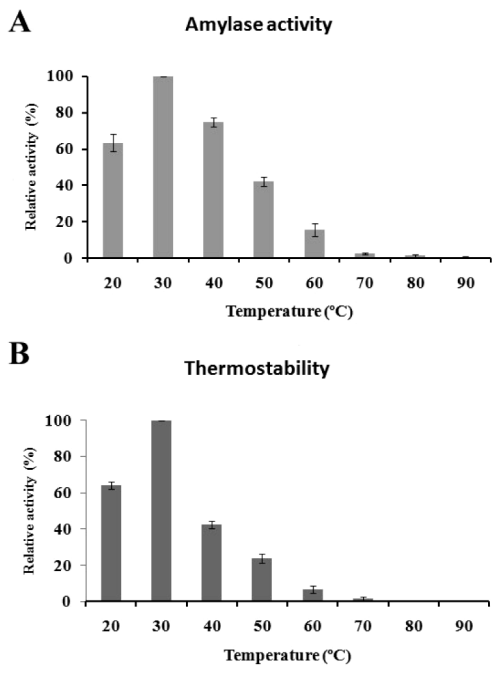

Figure 3 Effects of temperature on the amylase activity (A) and stability (B) of the extracellular enzyme from V. harveyi. Values are the mean ± standard error from triplicate analyses

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