Optimum conditions for isoamylase production by *Pseudomonas sp.*

تعيين الظروف المثلى لإنتاج إنزيم ايسواميليز بوساطة بكتريا *Pseudomonas sp.*

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Abstract

Different nutritional and cultural factors were studied to determine the optimum conditions for isoamylase production by *Pseudomonas* sp. in a batch culture of the production medium. These factors include carbon, nitrogen and phosphate sources and their concentrations, temperature and pH. Results showed that the optimum conditions for isoamylase production by *Pseudomonas sp.* were achieved when the production medium was supplemented with maltose 1%, peptone 0.4%, and K_2HPO_4 0.4% as a carbon, nitrogen, and phosphate sources respectively, at initial medium pH 6, and incubation at 28°C for 24 hours. Under these conditions isoamylase productivity reaches the maximum, at which enzyme specific activity was 0.85 U/mg proteins.

المستخلص

درست بعض العوامل التغذوية والمزرعية المؤثرة لتعيين الظروف المثلى لانتاج إنزيم ايسواميليز بوساطة بكتريا *Pseudomonas sp.* في الوسط الانتاجي باستخدام مزرعة الدفعة . وقد شملت هذه العوامل تعيين كل من نوع وتركيز المصدر الكربوني ، نوع وتركيز مصدر النيتروجين ، نوع وتركيز مصدر الفوسفات ، درجة الحرارة ، والرقم الهيدروجيني لوسط الإنتاج . أظهرت النتائج إن الظروف المثلى لانتاج إنزيم ايسواميليز تضمنت تدعيم وسط الإنتاج بالمالتوز 1% مصدرا وحيدا للكربون والطاقة ، والببتون 0.4 % مصدرا نايتروجينيا ، واستخدام فوسفات الهيدروجين ثنائية البوتاسيوم 0.4 % مصدرا فوسفاتيا ، وكان الرقم الهيدروجين الأمثل لوسط الإنتاج pH =6 ، ثم الحضن بدرجة 28م لمدة 24 ساعة . وقد ازدادت إنتاجية الانزيم المنتج من بكتريا *Pseudomonas sp.* لتصبح الفعالية النوعية للإنزيم الخام في رائق المزرعة 0.85 وحدة/ملغم بروتين .

Introduction

The use of enzymes is preferred as it offers a number of advantages including improved yields and favourable economics [1,2]. Isoamylase was first discovered in autolysed brewers' yeast. This intracellular enzyme has also been found in baker's yeast [3]. An extracellular yeast isoamylase has been reported by [4] from *Lypomyces kononenkoae* by identified an extracellular isoamylase produced by *Escherichia coli* then from *Pseudomonas amyloderamosa* which was then purified and characterized [5,6]. Isoamylase (amylopectin-6-glucanohydrolase or glycogen-6-glucanohydrolase, EC 3.2.1.68) catalyzes the hydrolysis of α-1, 6-glucosidic linkages of amylopectin and related polysaccharides [7,8]. Isoamylase is useful not only for the structural analysis of polysaccharides and derived oligosaccharides [9] but also for the starch industry [10] in producing glucose, maltose and higher oligosaccharides from starch with the action of exo-type hydrolases. Isoamylase also can be used in conjunction with

CGTase to enhance the production of cyclodextrins from starch [11,12] and to improve their solubility and hemolytic product through the reversed action of enzyme. The genus *Bacillus* has been in use in the biotechnology industry for a very long time with a number of new cultures exhibiting a variety of benefits to humans [13,14]. The main objective of this study is to determine the optimum conditions for enzyme production by a local isolate of *Pseudomonas sp.*

Materials and Methods

Microorganism and media

Pseudomonas sp. was isolated in a previous study [15]. The isolate was maintained on nutrient agar slants and stored at 4°C as a stock culture, while nutrient broth medium was used for the activation and preparation of bacterial inoculum. Isoamylase production was achieved in the production medium containing (per liter): 20g, maltose; 4g, sodium glutamate; 3g, Diammonium hydrogen phosphate [16].

Optimization of Isoamylase production

The optimization experiments were carried out aerobically under batch cultivation conditions in the production medium. Fifty ml of this medium was distributed in 250 ml Erlenmeyer flasks inoculated with 1% of mid-exponential phase culture of the *Pseudomonas sp.*, and incubated with shaking at 150rpm in a shaker incubator at 28C°. Optimization conditions include the type and concentration of carbon, nitrogen, and phosphate sources, effect of pH, and temperature.

Enzyme assay

Assay of isoamylase was achieved according to [17], by adding 1ml of the crude enzyme to the reaction mixture containing 5ml of 1% soluble glutinous rice starch solution, 1ml of 0.5M acetate buffer pH 3.5. After incubation at 40°C for 1 hr, 1ml of the reaction mixture was taken and mixed with 1ml of 0.01N of iodine solution, then the volume was completed to 25 ml with distilled water. The increase in the optical density at 610nm was measured using a photocell (1cm wide) and the enzyme activity was determined according to the following equation:

Enzyme activity (U/ml) = Abs (610nm)/1 x 0.01 x 60

Activity unit was defined as the amount of enzyme increase the absorbance up to 0.01 at 40°C under the reaction conditions [18].

Protein Concentration

Protein concentration in culture medium was determined according to [19] by using Coomassie blue G-250 and Bovine serum albumin standard solution.

Results and Discussion

Effect of carbon source

Six carbon sources (Lactose, sucrose, fructose, galactose, glucose and maltose) were used as a sole sources for carbon and energy to select the optimum for isoamylase production by *Pseudomonas sp*. H3. Results mentioned in Figure (1) showed that the maximum production of isoamylase was obtained when the production medium was supplemented with maltose as a soul source for carbon and energy. Enzyme specific activity in crude filtrate was 0.42 U/mg proteins.

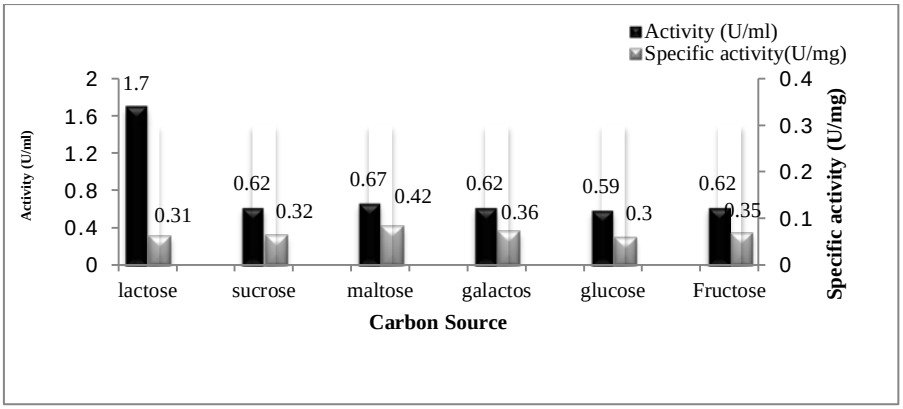

Fig (1): Effect of carbon source on isoamylase production by *Pseudomonas sp.* H3 after incubation in shaker incubator (150 rpm) at 28°C for 24 hr.

On the other hand [20, 21] were found that maltose was the optimum for isoamylase production by *Aerobacter aerugenes* .

Effect of carbon source concentration

Different concentrations of maltose were used to determine the optimum for isoamylase production by *Pseudomonas sp*. H3. Results indicated in Figure (2) showed that the maximum production of isoamylase was obtained when maltose was used in a concentration of 2% .The specific activity of isoamylase was 0.53 U/mg in culture filtrate. This indicates that this concentration of carbon source is the best for providing the microorganism with the needed energy for growth. This result was agreed with [22], who found that the maximum isoamylase production was obtained when maltose was used as a sole source for carbon and energy at concentration of 1.1%.

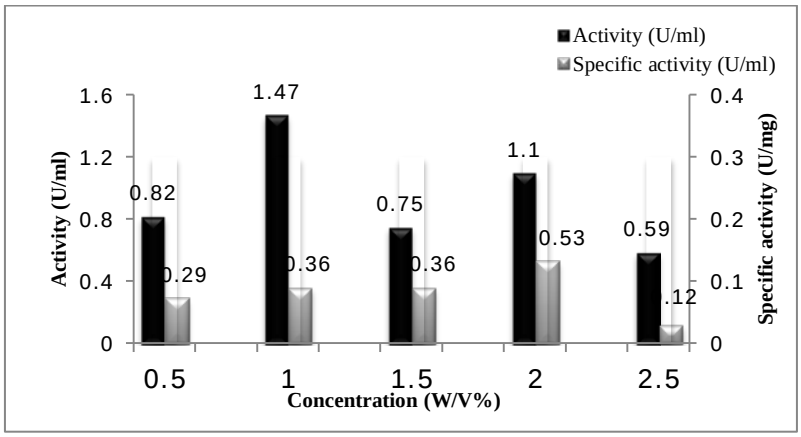

Fig (2): Effect of maltose concentration on isoamylase production by *Pseudomonas sp.* H3 after incubation in shaker incubator (150 rpm) at 28 °C for 24hrs.

Effect of nitrogen source

Different nitrogen sources (peptone, tryptone, yeast extract, NH4Cl and NH4NO3), were added to the production medium to determine the optimum for isoamylase production by *Pseudomonas sp*. H3. Results indicated in Figure (3) showed that peptone was the optimum nitrogen source for isoamylase production; the specific activity of the crude enzyme was 0.52 U/mg. This may be because that peptone was achieved the requirements for bacterial growth and cell division.

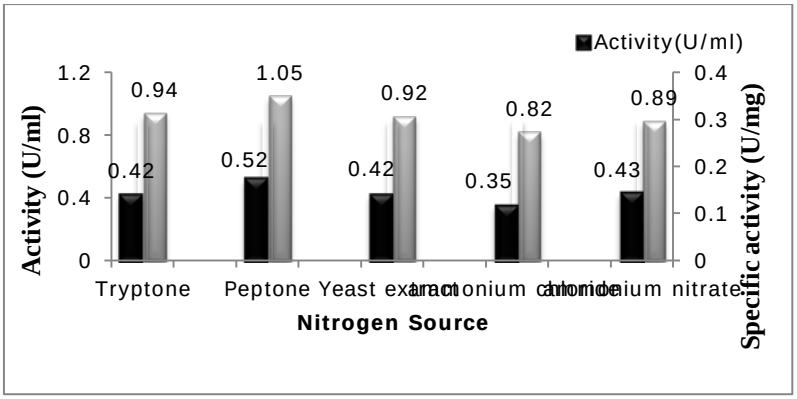

Fig (3): Effect of nitrogen source on isoamylase production by *Pseudomonas sp.* H3 after incubation in a shaker incubator (150 rpm) at 28 °C for 24 hr.

Effect of nitrogen source concentration

Five concentrations of the optimum nitrogen source (peptone) were used to determine the optimum for isoamylase production by *pseudomonas sp*. H3. Results indicated in Figure (4) showed that 0.4 % of peptone was the optimum for enhance enzyme production; the specific activity of crude isoamylase in culture filtrate was 0.55 U/mg. Another study found that 0.8% of peptone was the optimum for isoamylase production by *Escherichia intermedia* [20].

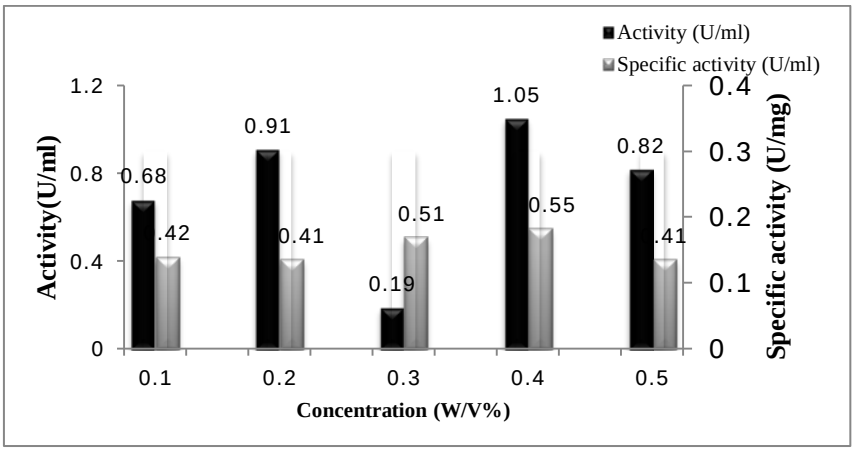

Fig (4): Effect of peptone concentration on isoamylase production by *Pseudomonas sp.* H3 after incubation in shaker incubator (150 rpm) at 28° for 24 hr.

Effect of Type of Phosphate source

Different phosphate sources (K2HPO4, KH2PO4, Na2HPO4 and NH4H2PO4) were also used to determine the optimum for isoamylase production by *Pseudomonsp sp*. H3. These sources were added to the production medium at a concentration of 0.3 %. Results indicated in Figure (5) showed that K2HPO4 was the optimum for isoamylase production, enzyme specific activity was 0.673 U/mg, this may be due to the effect of such concentration of phosphate source on buffering capacity that maintain the pH of the production medium which means more molecules of the buffer components present larger number of H+ and OH^- ions can be absorbed without changing the pH value [23]. Another study referred that 0.38% KH2PO4 was the optimum for isoamylase production by *P. amylodermosa* [24].

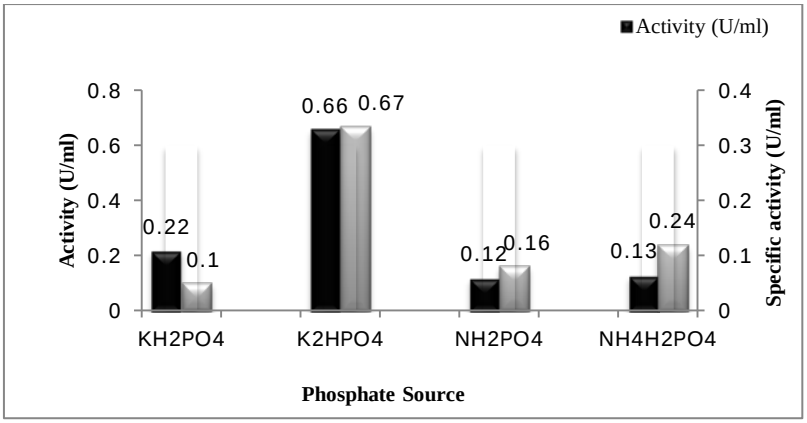

Fig (5): Effect of phosphate source on isoamylase production by *Pseudomonas sp*. H3 after incubation in shaker incubator (150 rpm) at 28°C for 24hr.

Effect of phosphate source concentration

Five concentrations of the optimum phosphate source (K2HPO4) were used to determine the optimum for isoamylase production by *pseudomonas sp*. H3. Results indicated in Figure (6) showed that 0.4 % of K2HPO4 was the optimum for enzyme production. Specific activity of crude isoamylase in culture filtrate was 0.69 U/mg. Another study showed that 0.5% of K2HPO4 was the optimum concentration for isoamylase production from *A. aerugenes* [25].

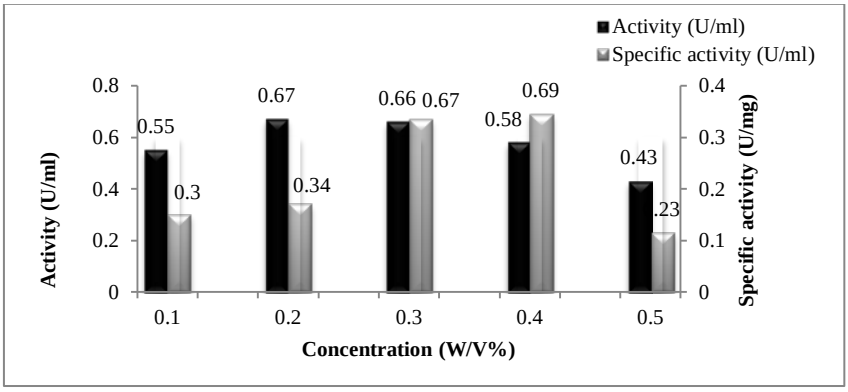

Fig (6): Effect of K2HPO4 concentration on isoamylase production by *Pseudomonas sp*. H3 in shaker incubator(150 rpm) after incubation at 28°C for 24 hr.

Effect of pH

Different pH values were used to determine the optimum for isoamylase production by *Pseudomonas sp*. H3. Results indicated in Figure(7) showed that the maximum production was obtained when the initial pH of the production medium was adjusted to 6.0 , enzyme specific activity in the crude filtrate was 0.843 U/mg. This result was agreed with that obtained by [9], who found that the optimum pH value for isoamylase production by *Flavobacterium odoratum* was 6.0. This may be because this pH value may achieve the optimum conditions for the bacterial growth and enzyme production due to its effect on the properties of the culture medium including the stability of the nutrients molecules transport and ionization. pH value also affect the stability and catalysis of the enzyme [26]. In other study, it was found that Isoamylase purified from *P. amyloderamosa* has a pH optimum at pH 3.0 – 4.0 [27].

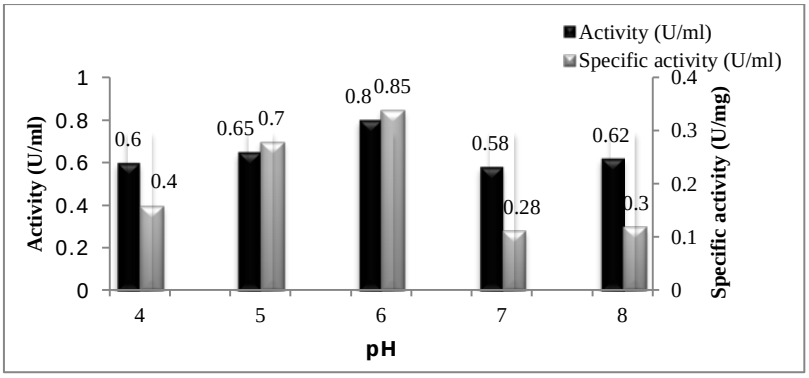

Fig (7): Effect of pH on isoamylase production by *Pseudomonas sp*. H3 after incubation in a shaker incubator (50 rpm) at 28°C for 24 hr.

Effect of temperature

In order to determine the optimum incubation temperature for isoamylase production by *Pseudomonas sp*. H3. Effects of different incubation temperatures were studied to determine the optimum for this purpose. Results indicated in Figure (8) showed that the maximum production of isoamylase in culture medium was obtained at 35°C. Specific activity of crude enzyme in culture filtrate at this temperature was 0.85 U/mg; this may be because that this temperature was the optimum for growth and propagation of the producing microorganism. [27] found that Isoamylase purified from *P. amyloderamosa* has a temperature optimum at 52^{o}C.

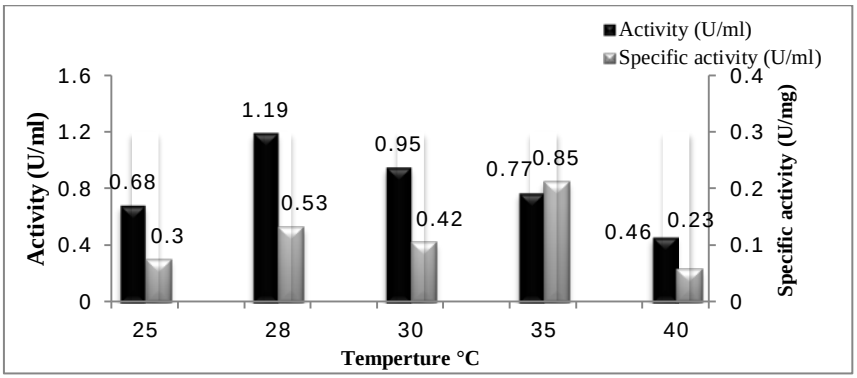

Fig (8): Effect of incubation temperature on isoamylase production by *Pseudomonas sp*. H3 after incubation in shaker incubator (150 rpm)at 28°C for 24 hr.

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