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Optimization of Pectinase Production from *Pesudomonas* sp. Isolated from Iraqi Soil

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Abstract

The isolates were screened according to their capability for pectinase production, screening process identified the best pectinolytic isolate and it was characterized by cultural and biochemical, as *Pesudomonas* sp. Pectinolytic enzyme producing bacterium *Pesudomonas* sp. was isolated from the Iraqi soil on nutrient agar plate. Optimiztion of process parameters were carried out by altering some of environmental conditions of chemo-physical environment for the production medium. The highest pectinase production was observed at 48 hrs of incubation at 35 °C with the initial pH of 6.0. Different nutrients and environmental conditions were investigated in terms of their effect on the production of extracellular pectinase using citrus pectin as substrate. The results exhibited that Yeast extract 0.15% with 0.5% of citrus pectin supported maximum pectinase production in the optimum conditions.

Keywords: Pectinase, *Pesudsomonas* sp., Optimization, Production.

تحديد الظروف المثلى لأنتاج البكتنيز من عزلة *Pesudomonas* sp. المعزولة من الترب العراقية

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الخلاصة

غربلت خمسة عشر عزلة بكتيرية على قدرتها انتاج انزيم البكتنيز، ثم حددت العزلة البكتيرية الافضل في انتاج البكتنيز. و شخصت العزلة الأكفأفي انتاج الانزيم بدراسة الظروف الزرعية والكيموحيوية، وبينت النتائج انها تعود الى *Pesudomonas* sp. حددت الظروف المثلى للانتاج بتغييربعض الظروف البيئية والكيموفيزيائية للوسط الانتاجي. اعلى انتاجية للآنزيم لوحظت خلال 48 ساعة وبدرجة حرارة 35 °م مع رقم هيدروجيني 6.0. درست تأثير المغذيات والظروف البيئية المختلفة على أنتاج أنزيم البكتنيز خارج الخلوية.أظهرت النتائج بان خميرة الخبز بتركيز0,15 مع 0,5% من بكتين الحمضيات دعمت اقصى انتاج لانزيم البكتنيز في الظروف المثلى.

Introduction

The plant cell wall is composed of pectin, hemicelluloses and lignin, which constitute the most plant polymers in the nature. Pectin is contains of carbohydrate group esterifies with methanol. It is an important component of the cell wall. If is find in most concentration in the middle lamella, which is acts as a cementing materials between the adjacent cells. Many of the microorganisms like *Bacillus* spp., *Pseudomonas* spp., *Streptomyces* spp. They can be degrading the polymers to simple sugars by secreting the enzymes like amylase, cellulase and pectinase [1]. Enzymes are protein molecules necessary in the life. The plant pathogens attack target cells by these enzymes of the plant cells, which

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facilitates the expansion of pathogen in the plant tissue [2]. Pectinases including depolymerizing enzymes. The pectinase enzymes are either polygalacturonase, or pectin-lyase [3]. The De-esterifying enzymes are include pectin-estrase [4]. Pectinases are industrially applied in the food industry and the clarification of fruit juices. Also, pectiolytic enzymes are being applied into the textile industry to release fibers from flax stems, and in conventional retting [5]. There are two types of pectinases in the industrially, acidic and alkalophilic, the first is used in the extraction and clarification, but the alkalophilic pectinases are finding immense use in the degummine of ramie fibers [6]. The current study was aimed to isolation, identification and study the optimum conditions for pectinase production.

Materials and Methods

Isolation of Bacterial Isolates.

Bacterial isolates for pectinase production were isolated of the soil by using a group of dilutions and pour plate technique. The bacteria were maintained on nutrient agar at 4 ºC and sub cultured weekly.

Identification of the Selected Isolate

The identification of the selected isolate was achieved according to cultural, morphological and biochemical tests. The selected isolate was identified depending on their morphological properties on macConkey agar medium, grams stain staining, motility test. In addition to oxidase test, catalase test, fermentation for glucose, lactose, arginine hydrolysis test, lysine hydrolysis test, nitrate reducing, ONPG test, esculin hydrolysis test, DNAase enzyme production test and H_2S production test [7].

Screening of Pectinase Producing Bacteria

Pectinase producing bacteria were induced and screened for pectinase production by using citrus pectin as substrate in agar plates. After incubation for 48 hrs at 30 ºC, colonies were flooded by iodine-potassium iodide solution. Clear zones were appeared around growing bacterial colonies indicating to the pectin hydrolysis [8].

Pectinase Production

The production medium was contained to the followings (g/L) Pectin 10, $MgSO_4$. $7H_2O$ 0.5, KH_2PO_4 1 and $NaNO_3$ 1. The pH was adjusted to 6.0, then 50 ml of the culture medium was autoclaved and cooled at 37 ºC and inoculated with 0.5 ml of the bacterial inoculums. The isolates were incubated for 48 h at 30 °C and 140 rpm. The supernatant was recovered by cooling centrifugation (6000 g 10 min. and 4 °C) and used for further analyses [9].

Effect of Carbon Source (%)

The optimum concentration of pectinase production was determined by addition of different concentrations (0.5, 1, 1.5 and 2.5%) of pectin in the production medium [10].

Effect of Initial pH

The optimum pH value for pectinase production was determined by preparing the production medium at different pH values (5, 6, 7 and 7.5).

Effect of Nitrogen Sources and Concentration for Pectinase Production

Different nitrogen sources were used for pectinase production, including organic nitrogen (Yeast extract, Peptone and Tryptone) and in organic (Sodium nitrate, Ammonium sulfate) nitrogen sources at 0.1%. Then the effect of best nitrogen source at different concentrations (0.05, 0.1, 0.15 and 0.25) % was studied for pectinase production.

Effect of Temperature

Optimum temperature for pectinase production by the selected, isolate was the medium at various temperatures of (25, 28, 30, 32 and 35 °C) were incubated.

Pectinase Assay

The assay was achieved by determining activity of pectinase in the culture filtrate after growth isolates in pectin broth medium at 30 ºC for 48 h. Pectinase activity was measured by mixing 1ml of 1% pectin in 0.1 M sodium acetate buffer solution pH 5.0 for 30 min at 37 ºC. The resulted reducing sugars were determined according to Miller by dinitrosalisylic acid (DNS) method [11].

Protein Assay

The protein assay was achived by addition 0.1 ml of crude enzyme to 0.4 ml of tris-HCl then 2.5 ml of comassie brilliant blue was added. After shaking for 2 min., absorbance at 595 nm was read, and protein concentration was determined from the standard curve for (BSA) Bovin serum albumine[12].

Results and Discussion

Isolation of Pectinolytic Bacteria

A total of 15 bacterial isolates were collected from different soil samples of Baghdad university fields. Then the isolates were examined to their ability for pectinase production.

Qualtitative and Quantitative Screening of Isolates for Pectinase Production

The pectinase production by local isolates was achieved through detecting the ability to grow on pectin agar medium. Results indicated that these isolates were growing on the pectin agar medium and the clear zones around of each bacterial isolate were detected table-1. The clear zone of isolates was ranged from 7 to 11 mm. The results showed that the isolate S2 was the most efficient in pectinase production with a clear zone of 11 mm and higher specific activity reached to 0.9 U/mg. Therefore, the isolate S2 was used for remaining studies.

Table 1- Ability of the isolated isolates for pectinase production by qualtitative method after incubation for 48 hrs at 30 ºC.

Number of isolate	Isolate symbol	Source	Diameter of clear zone (mm)	U/mg
1	S1	Soil	9	0.30
2	**S2**	**Soil**	**11**	**0.90**
3	S3	Soil	0	0.00
4	S4	Soil	0	0.00
5	S5	Soil	0	0.00
6	S6	Soil	0	0.00
7	S7	Soil	7	0.21
8	S8	Soil	8	0.22
9	S9	Soil	0	0.00
10	S10	Soil	0	0.00
11	S11	Soil	0	0.00
12	S12	Soil	6	0.17
13	S13	Soil	7	0.15
14	S14	Soil	0	0.00
15	S15	Soil	8	0.19

Identification of Selected Isolate

The cultural and biochemical results showed that the isolate pale colony, 3 mm in diameter, gram negative bacilli, motile , Oxidase and catalase positive, glucose fermented, arginine hydrolysed, nitrate reducer, DNAase positive, esculin hydrolysis, H2S production and non sporulated bacteria, non fermented to lactose, non lysine hydrolysed, negatively for each of ONPG test,. So the isolate was belonged to *Pesudomonas* spp. The results were agreed with Winn *et al*. (2006).

Optimization for Pectinase Production

Effect of Pectin Concentration

The optimum concentration of pectin for pectinase production by *Pesudomonas* sp. was determined using the concentrations 0.5, 1, 1.5 and 2.5%. Results in figure 1 showed that specific activity of pectinase in the filtrate by this isolate was varied from one concentration to other in the medium. The highest specific activity was achieved when the medium was contained 0.5% pectin, with specific activity of 1.0 U/mg protein. The results were agreed with that the [Wilson, 1953] mentioned that the production was related with pectin concentration, therefore it is represent important factor for decrease and increase of the specific activity as appeared in the figure -1, Which may be belonging to the scaling up of pectinase production at 0.5%.

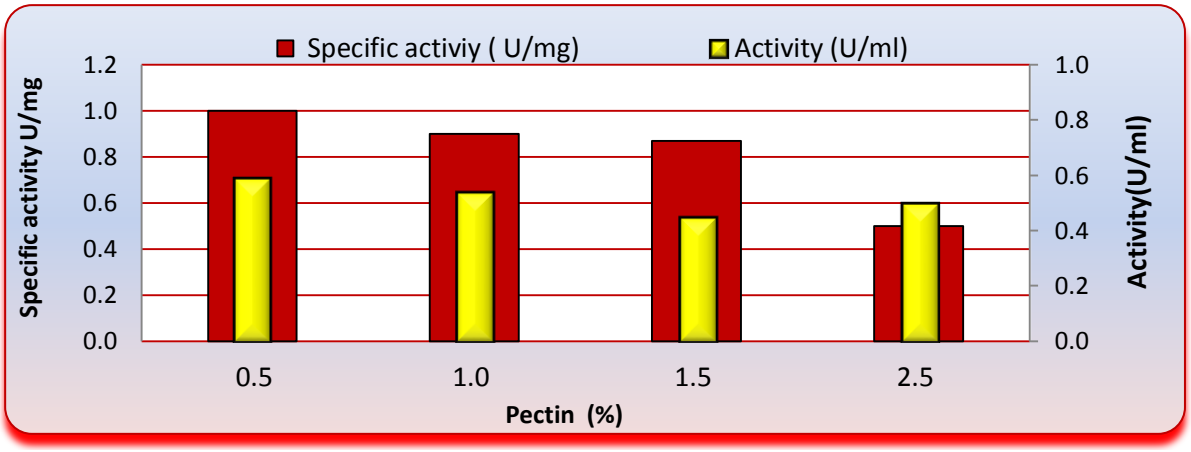

Figure 1- Effect of pectin on pectinase production by *Pesudomonas* sp. after incubation for 48 h at 30 ºC.

Effect of Nitrogen Source

The best nitrogen source for pectinase production was determined by the selected isolate *Pesudomonas* sp.**,** with five nitrogen sources including (potassium nitrate, tryptone, peptone, yeast extract and ammonium sulfate) at a concentration of 0.1% in the medium. Results indicated in figure-2 that the production was varied according to the nitrogen sources, and the best source was ammonium sulfate with 1.5 U/mg proteins. The results also indicated that there are crucial effects by using different sources of nitrogen, which represent the important factor for the protein synthesis. The result was agreed with Kashyap *et al*. [2000] who reported that the concentration 0.1% of nitrogen source is optimal for the pectinase production, while Qureshi *et al*. [2012], who reported that the best nitrogen source for pectinase production was yeast extract in culture of *Bacillus subtilis* EFRL 01. Also Kumar *et al*. [2012] reported that the best source of nitrogen for pectinase production was peptone in *Bacillus* sp. MFW7.

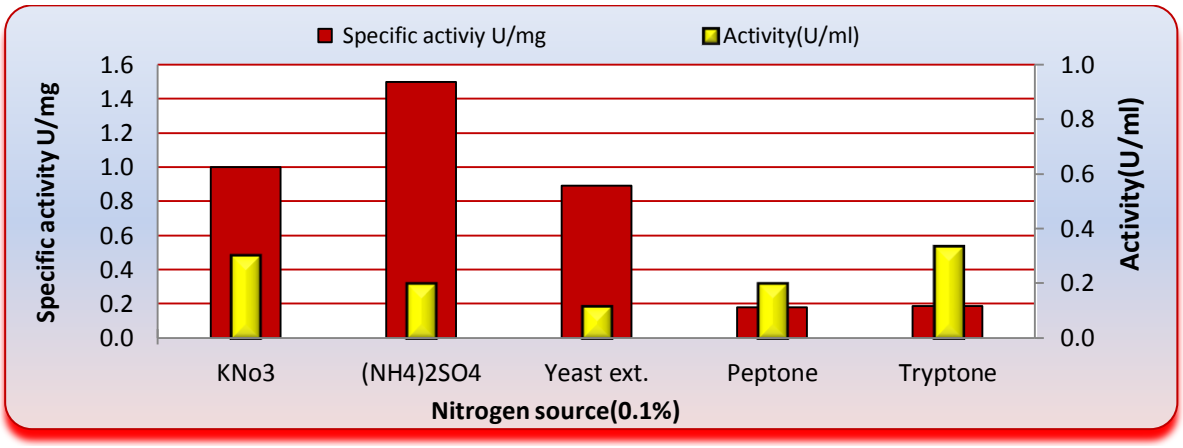

Figure 2-Effect of nitrogen source on pectinase production by *Pesudomonas* sp. after incubation for 48 hrs at 30 ºC.

Effect of Ammonium Sulfate Concentration (%)

The optimal concentration of the ammonium sulfate in the pectinase production was determined by the selected isolate with different concentrations of 0.05, 0.1, 0.15, 0.2 and 0.25%. Results showed that the best concentration of ammonium sulfate was 0.1% which achieved 1.5 U/mg proteins as specific activity figure -3. The result was similar to that reported by Holt *et al*. [1994] and Soares *et al*. [1999], those indicated that the best concentration of ammonium sulfate for the production was 0.1%.

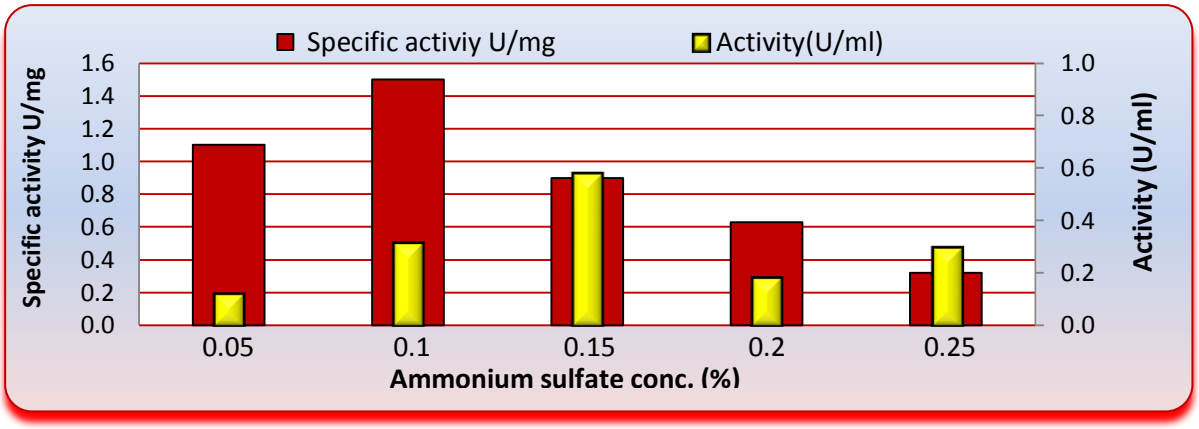

Figure 3-Effect of ammonium sulfate concentration on pectinase production by *Pesudomonas* sp. after incubation for 48 hrs at 30 ºC.

Effect of the pH

To determine the best pH value for pectinase production, different pH values were used (5, 6, 7 and 7.5). Results illustrated in figure-4 showed that the highest production of pectinase was obtained at pH 6.0; the specific activity was reached to 1.5 U/mg proteins. Results were accordance with Soares *et al*. [1999], who reported that the best pH value for pectinase production was 6.0. While Qureshi *et al*. and Kumar *et al*. [2012] reported that the best pH value for pectinase production by isolate *Bacillus* sp. was 6.5 and 7.0 respectively. While Patil *et al*. [2012] reported that the best pH for pectinase production was 9.0.

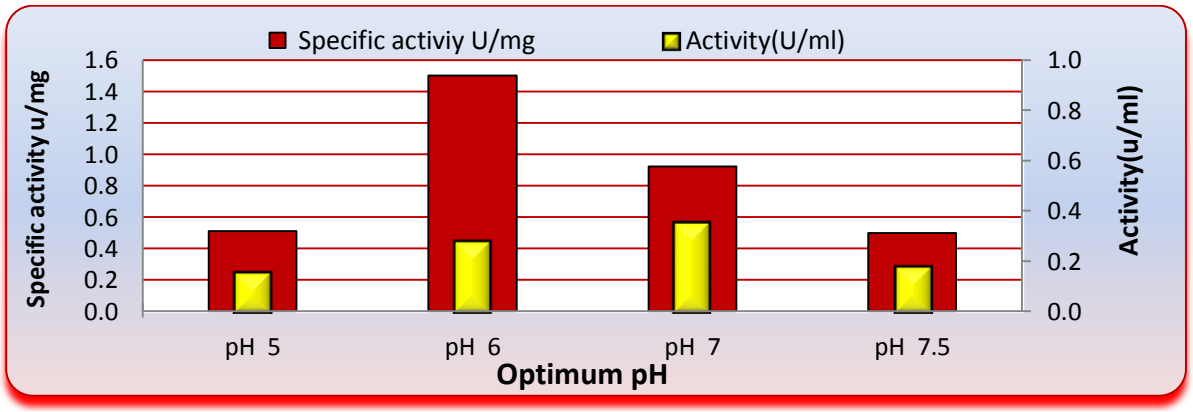

Figure 4- Effect of pH value on pectinase production by *Pesudomonas* sp. after incubation for 48 hrs at 30 ºC.

Effect of Temperature

To determine the best temperature degree for pectinase production, different temperature degrees were used (25, 28, 30, 32 and 35) ºC. Results showed that the highest production of pectinase was obtained at 30 ºC, the specific activity was reached 5 U/mg protein as specific activity figure -5. Results were accordance with Fernandes *et al*. [1996], who reported that the best temperature degree for pectinase production was 30 ºC.

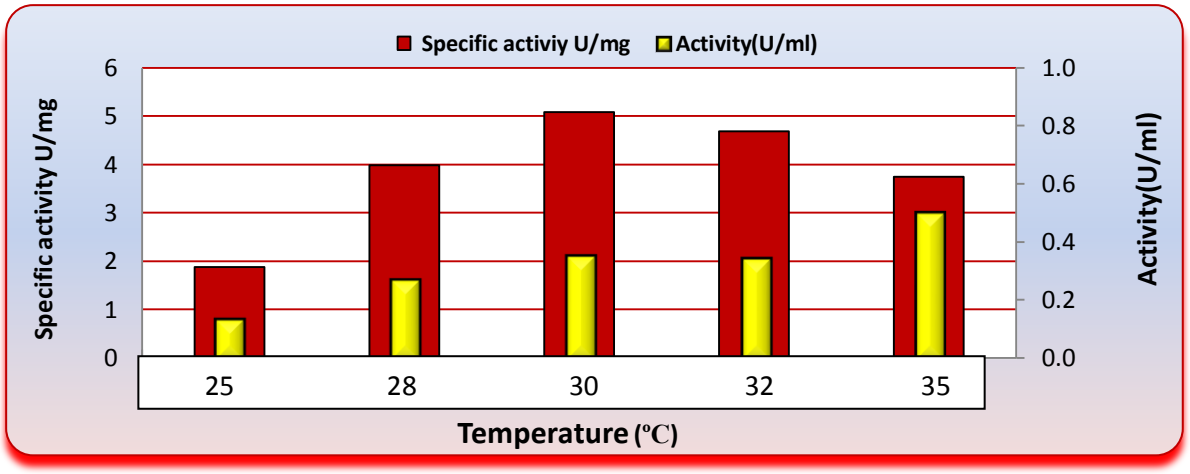

Figure 5- Effect of temperature degree on pectinase production by *Pesudomonas* sp. after incubation for 48 hrs at 30 ºC.

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