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Optimum Conditions for Alginaseby *Bacilllus Circulans* R Isolate

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Abstract: *Fourteen isolated bacteria same species of Bacillus which were taken from hospital Ramadi and Fallujah sample were collected during the period from October to December 2013 and cultured on blood agar to test the ability to lysis to measure the inhibition zone . six isolate were selected for production of alginase enzyme when cultured in alginate yeast extract broth then only one which have high efficient in the production of alginase enzyme was selected after diagnosed depending on phenotypic, microscopic and biochemical tests which show that the isolate was Bacillus circulans R. The optimal conditions for alginase enzyme production were determined; the optimum incubation period was 24 hrs which gave enzymatic activity (106.1U/ml). 6x10^7 cell/g dry weight was the optimum inoculums percentage which gave activity (111 Unit/ml), the optimum carbon source which gave activity (212.86 U/ml), when used sodium alginate while peptone was the optimum nitrogen sources with enzymatic activity (275 U/ml). pH 7.5 gave maximum activity (211.63 U/ml) , the shaking incubator was best in the production of the enzyme which gave enzymatic activity (210 U/ml).*
Key words: alginase, optimum condition, Bacillus circulans R,

I. Introduction

Alginate is a gelling polysaccharide found in great abundance as part of the cell wall and intracellular material in brown seaweeds . Alginate is a linear hetero-polyuronic acid composed of 1,4 linked α-L-guluronic acid (G) and β-D-mannuronic acid (M). These two residues are arranged in block structures comprising homopolymeric G blocks, M blocks, alternating MG (GM) blocks, and heteropolymeric MG (GM) blocks .Alginate is widely used as a stabilizer, viscosifier, and gelling agent in the food and beverage, paper and printing, biomaterials, and pharmaceutical industries [1].

Alginate produced by *Pseudomonas* species ,*Azotobactervinelandii* and several species of brown seaweed .In bacteria, alginate is modified by the addition of O-acetyl groups on some D-mannuronate residues. The sugar residues of alginate do not show repeating subunits characteristic of other bacterial exopolysaccharides [2].This acetylation is always present in bacterial alginates, but the degree of acetylation varies over a wide range among different species, and also in different strains of the same species .The role of acetylation has been proposed to be to protect the polymer against degradation and epimerization .Acetylation has been most thoroughly investigated for *P. aeruginosa* due to its role in cystic fibrosis**.** [3].

The aim of is study tothe optimal condition for the production of the alginase enzyme from high efficient *Bacillus circulans* local strain.

II. Materials and Methods

Diagnosis of efficient isolate in the production of alginase enzyme

Diagnostic tests performed on the efficient isolate producing alginase enzyme as in the simplified diagnostic key proposed by Betty*et al* .[4].

a- Phenotypic Characteristics: The bacteria characterized with long coli or moderate length, dimensions of vegetative cells ranging from (0.5 ×12 μm) to (2.5×10μm). Its response to Gram stain is positive, the form of spores elliptical spores site in sub-terminal of the cell or to the terminal

b. Nature of growth on a solid medium: isolates formed creamy-mucous, transparent convex colonies, with even edges and smooth surface.

c. Biochemical Characteristics:

Confirmed some of the biochemical tests Table (1), as well as phenotypic characteristics previously mentioned ownership of these isolates to the genus *Bacillus circulans*. As all were product of the alginase enzyme, and this isolate to genus *Bacillluscirculans*. As all were product of the alginase enzyme, and this isolate was lcethinaseproductive, grow an aerobically, gelatin liquefied, Catalase production, Voges - Proskauer test, Starch hydrolysis test , Urease test and Mannitol fermentation medium.

From microscopic and biochemical tests of the isolates belonging to the local bacteria *Bacillus circulans*selected from the quality and quantity screening processes produced alginase enzyme according to the. Macfaddin,[5] .

Table (1):Thebiochemical test of the *Bacillus circulans*R isolate

Isolate	Species	Gelatin Liquefact	Anaerobic growth	Lecithinase production	mannitol	Voges - Proskauer	Starch hydrolysis	Catalase	Urease
R	*B.circulans*	+	+	-	+	-	+	+	-

(+) positive test , (-) negative test

d .Screening the isolates depending on the diameter of inhibition zone

A total number of 14 samples were collected from different area of hospital. after the screening phenotypic ,morphology ,biochemical tests the isolates screening the ability to hydrolysis blood agar and selected according the inhibition zone diameter whereas appear inhibition zone (type ß)around the colonies on blood agar medium and it variation between 0.9 -2.5 cm as show in table (2).

Table (2):TheScreening of isolates of *Bacilluscirculans*hemolysis of blood agar

Isolates	Diameter of inhibition zone(mm)
A1	1
A2	0.9
A3	1.2
F	1.1
CR	2
R	2.5

The ability of *Bacillus circulans*isolates to produce alginase enzyme

Screening of alginase enzyme was using alginate yeast extract broth.sixisolate screening their ability to produce alginase enzyme in the media containing sodium alginate as a substrate stimulator.*Bacillus circulans*R isolate characterized with its optimum production for alginase enzyme activity was 106.1 Unit /ml.(Table 3).

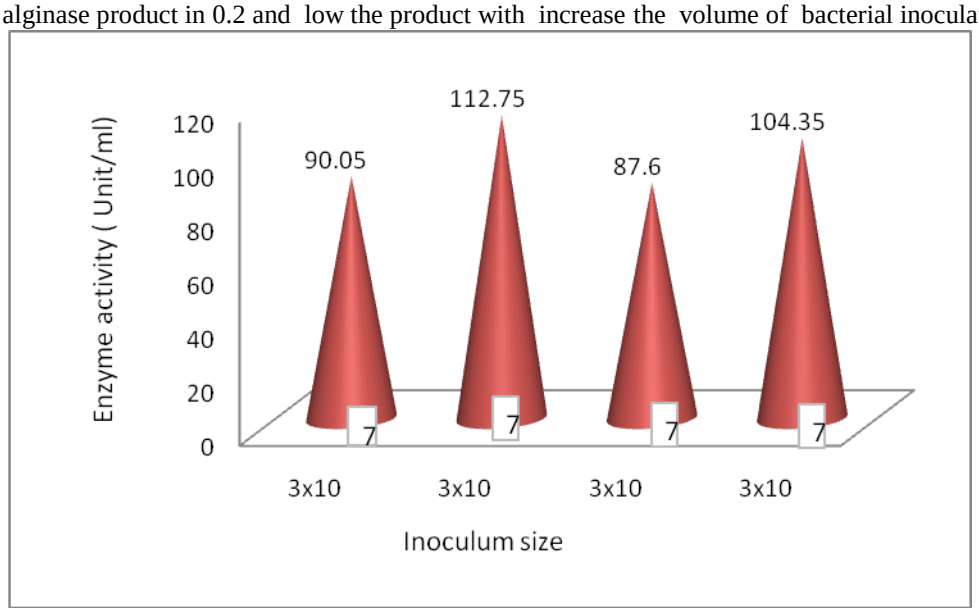
Table (3): The efficient *Bacilluscirculans*isolates producing alginase enzymeusing alginate yeast extract broth.

Isolates	Enzyme activity Unit /ml
A1	81.4
A2	36.8
A3	13
F	81.7
CR	85.2
R	106.1

The optimum conditions for alginase enzyme produced by *B. circulans* R

III. Screening the optimum inoculum volume for enzyme production

The maximum enzyme activity was (111 Unit/ml) when the inoculum volume 1ml . Activity decreased when the amount of inoculums increased(figure 1). This can be explained as follows the components of the medium consume efficiently and on increasing the amount of the inoculums increase the number of cells that secrete enzymes which analyzed the components of the medium. decreasing the activity due to competes of cells for nutrients, increases the amount of toxic metabolic products, and rapid consumption of oxygen. The optimization of the inoculums size depends mainly on the growth period allowed (age of culture) for the applied culture, thus while the best inoculums age for production of alginase enzyme by *Bacillus circulans* was 24 hrs.this results similar to [6] they used different volume of inoculum (0.1 ,0.2 ,0.3 ,0.4, 0.5%)and showed maximum alginase product in 0.2 and low the product with increase the volume of bacterial inoculate.



Figure(1):The effect of inoculums volume on the enzyme production by *B. circulans R*.

IV. Determination the optimal temperature for enzyme production

Bacillus circulans R incubating in different temperatures at 24hrs to obtained the maximum production of alginaseenzyme. the results showed in figure (2) that the production of the enzyme is very low at temperature 30^{0}Cwhile increased productivity and reached a maximum at temperature 37^{0}C it was 167 U/ml. These results agree with Ashour*et al*.[7] found when incubated the medium at different temperature (25,30 ,35,40) ^{0}C for 48 hrs the maximum product at 30 ^{0}C was 69 U/ml and low product at 40 ^{0}C [8].The best temperature for alginase production from marine bacterium at 30^{0}C for 24 hrs. An *et al*.[9]which found that *Flavobacterium*sp maximum alginase production at 30^{0}C for 24 hrs. The maximum alginaseproduct from*P. aeruginosa* was (0.335 U/ml) at 30 °C for 48hrs [10]. Ueno [11]show 25 ^{0}C for 72 hrs best optimal condition for alginase product. Muslim *et al*.[6] found the optimal temperature for production at 35 ^{0}C for 18 hrs.

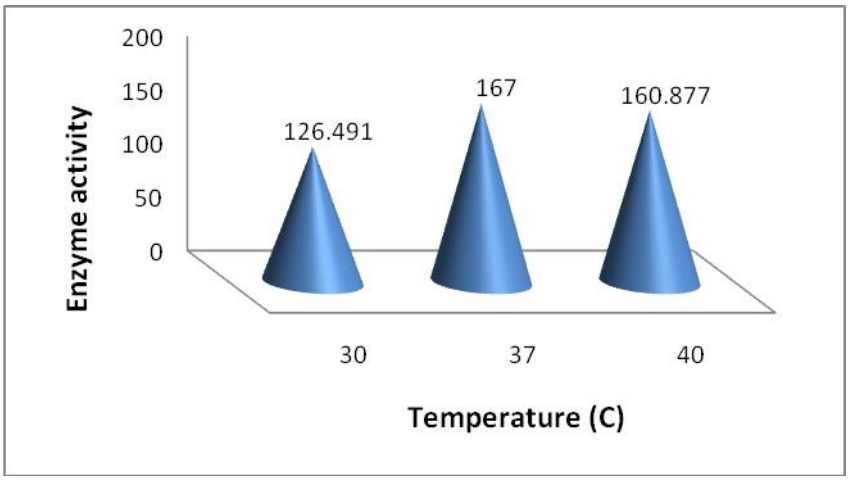


Figure (2): The effect ofthe different temperatures on the enzyme production by *B.circulans* R.

V. The effect of different concentration carbon sources on production of alginase enzyme

Fourconcentration of sodium alginate as carbon sources were investigated (0.25 ,0.5 ,0.75 , 1g /100ml) . This concentration of carbon sources had all been reported to be the best in respect of alginase production by strains *B. circulans*.The results showed that, based on alginase production,in 0.5% was the optimal carbon source (212 U /ml). and 0.25 % was activity (207 U/ml). while the concentration(0.75 %) had different trivial in activity (194 and191 U/ml) respectively .So concentration 0.5 was chosen as carbon source for the following investigations figure (3).Ma *et al*.,[12]mention that high concentration of sodium alginate 0.3% to production maximum alginase enzyme. (Ashour*et al*.,2014).Also An*et al*. [9]found that the concentration 0.8% was the best for alginaseenzyme production .

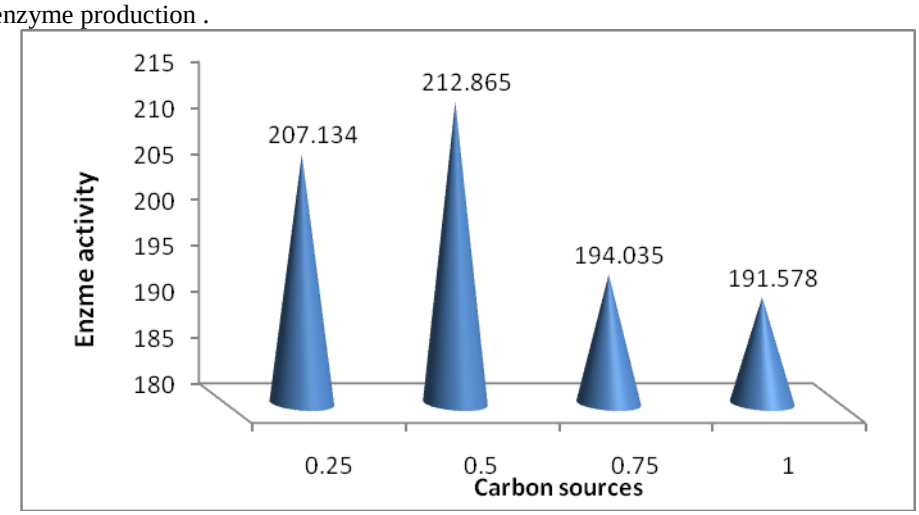


Figure (3):**The effect of different carbon sources on the enzymeproduction by *B.circulansR*.**

VI. The effects of different nitrogen sources on production of alginase enzyme:

Five kinds of nitrogen sources were examined (NH_4Cl, KNO_3, NH_3PO_4 , peptone and soya peptone)of all the nitrogen sources tested,peptone was found to be the most promising one, and the corresponding alginase activity is (275 unit/ml) Figure(4).

When NH_4Cl inorganic nitrogen sources were used, very low enzyme activities were obtained. While much higher enzyme activity were obtained by using the peptone organic nitrogen sources. The optimal nitrogen source was peptone, which was used as nitrogen source in the following investigations.

These results similar to the mentioned by [14] Who study the effect of different nitrogen sources on alginase enzyme production , such as peptone ,NH_4Cl, soya bean ,ammonium sulphate and others showed that Ammonium nitrate gave higher activity alginase (0.335 U/ml) and lower activity when using peptone was (0.037 U/ml) produced by *Pseudomonas aeruginosa*[10].

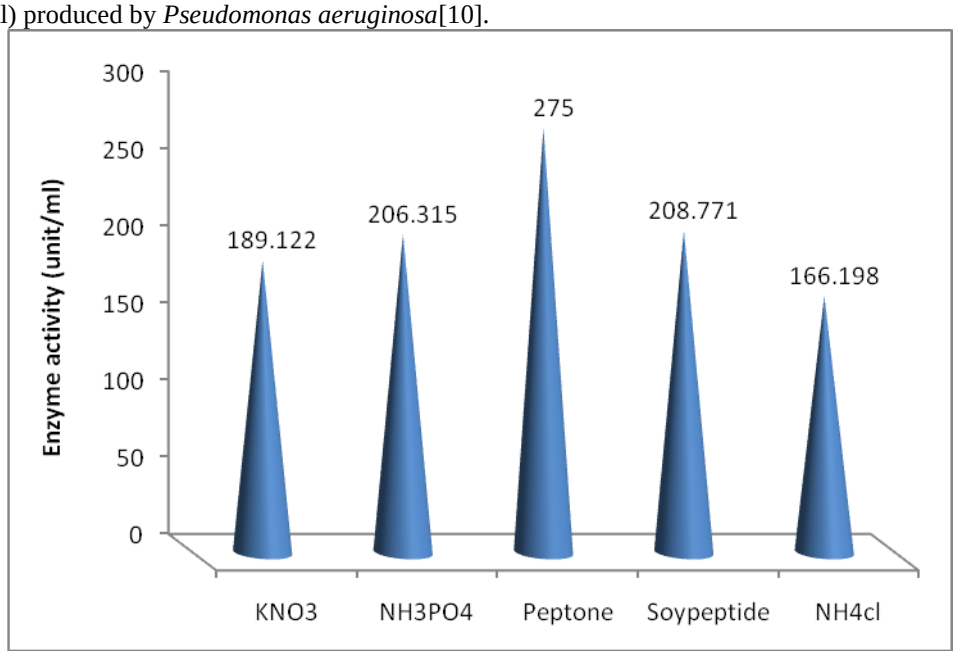


Figure (4): The effect of different nitrogen sources on the enzymeproduction by*B.circulans R.*

VII. Estimate the optimal pH for enzyme production

The ability of the *Bacillus circulans R* isolate to produce alginaseenzyme using the medium of alginate yeast extract in different pH (6, 6.5,7, 7.5)the results showed that the production of the enzyme is very low at pH 7 while increased productivity and reached a maximum at pH values 7.5as it was enzymatic activity 211.63 U/ml while there was a decrease in activity at higher pH values 7 the activity 137.53 U/ml Which indicates that the optimum pH for the production of the enzyme is 7.5 Figure. (5).

These results were agreed with [14] who mentioned that the optimum pH for cell growth and alginase enzyme production was 7.5 -8 when produced that enzyme from Altramonas sp. But Wang *et al.* [15] showed that the optimal pH 7.0 when produced the alginate lyase from marine vibrio sp.YWA.

Song *et al*.,[16] mentioned that alginate lyase from vibrio sp. YWA was most active at pH 7.5.The alginase enzyme production affected by the change in the pH by effect on the nature of the media, solubility of the nutrient and transfer it all these effect on the stability of the enzyme.

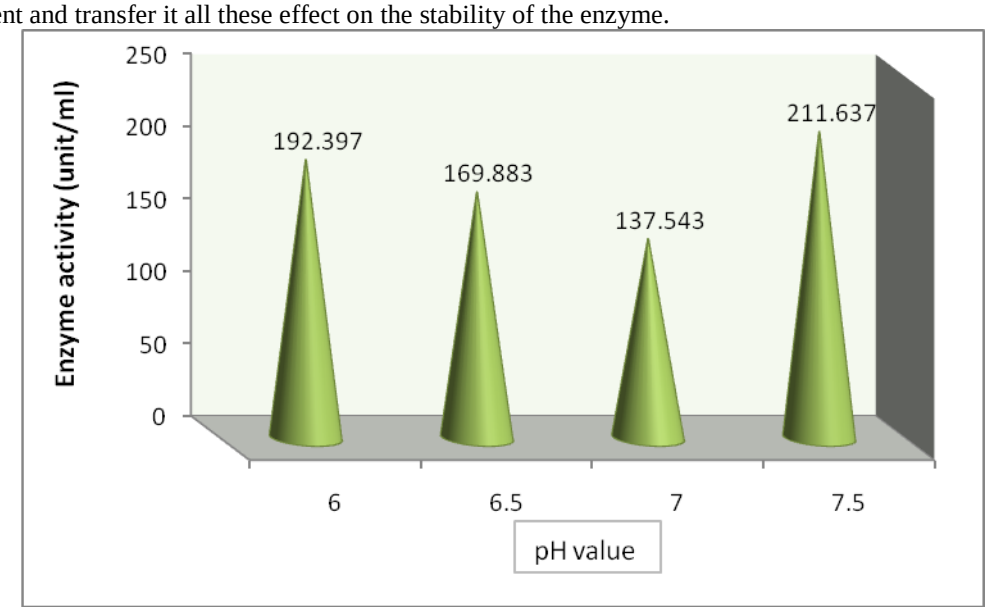


Figure (5): The effect of pH on the enzyme production by *B. circulans R*

VIII. Screening the optimal incubator for alginase enzyme production

The microorganism growth affected by the amount of oxygen in the culture media the increase in amount of oxygen due to increase in the multiplication and metabolism of microorganism.Shacking of the culture led to enhancement of enzyme production, and the highest enzyme activity was 210 U/ml gained at 200 rpm figure(6).Higher rotating speed gave richer air for aerophilic bacteria, which is advantageous to enzyme production compared with static incubation when used gave (81.87U/ml).

Muslim *et al*.[6] Showed the highest production when incubating with shacking 150 rpm at 18 hr.[12].*Pseudoalteromonas*sp was producing alginase enzyme that best shacking was 150 rpm for 24 hrs. [15] the optimal shacking for *Bacillus*sp was 250 rpm 24hrs. [7]the best shacking for production 200 rpm at 48 hrs.[10] the alginase product from *Pseudomonas aeruginosa* when incubating in shacking 170 rpm at 48 hrs. Ueno [11]found the best product incubated shacking 120 rpm for 72hrs.

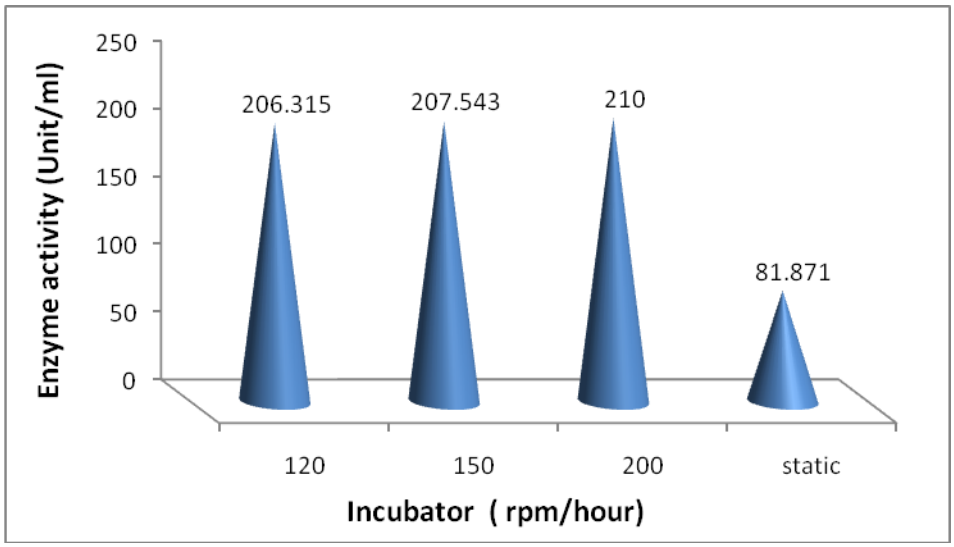


Figure (6):The effect of the shackingincubator on the enzyme production by *B.circulans R*

References

[1] Li, J.W.; Dong, S. ; Song, J.; Li, C.B.; Chen, X.L.; Xie, B.B.and Zhang, Y.Z.(2011).Purification and characterization of a bifunctional alginate lyase from *Pseudoalteromonas* sp. SM0524.Mar Drugs. 9(1):109-123.

[2] Steigedal,M.(2006).The *Azotobactervinelandii*mannuronan C5-epimerases: their biological functions and new tools useful for their future in vivo biotechnological application.PhD thesis.Department of Biotechnology.University of Science and Technology.Norwegian.

[3] Hay,I.D.;Rehman,Z.U.;Ghafoor,A. and Rehm,B.H.A.(2010).Bacterial biosynthesis of alginates.J Chem. TechnolBiotechnol ; 85: 752–759.

[4] Betty, A.F.; Daniel, F.S. and Alice S.W. (2007).Bailey and Scotts diagnostic microbiology. Ed12. Mosby Inc.USA.

[5] Macfaddin, J.F (2000). Biochemical test for identification of medical bacteria .The Williams and wilkinsCo.USA.

[6] Muslim, S .N.; Abdul Hamid, A . M. and Hamid, E .H.(2013). Designate the optimum conditions for the production of alginase enzyme from *Bacillus sp*. isolated from soil. Journal of the Science of Mustansiriya.Vol 24,No.1.

[7] Ashour,S.M ;Kheiralla ,Z .M; Eldiwany , A .I. and Maany, D .A.(2014).Production ,Purification and characterization of polysaccharide lytic enzymes of marine isolate, *Bacillus cereus* NRC-20 and their application in biofilm removal.African Journal of Microbiology Research . vol 8 (6) pp 2492-2504.

[8] El-Ahwany, A. M. D. and Elborai ,A. M. (2012). Optimization of medium composition for extra cellular alginate lyases of a marine bacterium. African Journal of Microbiology Research Vol. 6(10), pp. 2403-2409.

[9] An, Q.-D.; Zhang, G.-L.; Wu, H.-T.; Zhang, Z.C.; Zheng, G.-S.; Zhang, Y.-L.; Li, X. and Murata, Y. (2008).Properties of an alginate-degrading *Flavobacterium*sp.LXA isolated from rotting algae from coastal China. Can J Microbiol 54, 314–320

[10] Koti, B. A ; Manohar ,S. andLalitha,J. (2014). Media Optimization for Depolymerization of Alginate by *Pseudomonas aeruginosa* AG LSL-11. International Letters of Natural Sciences.(14):30-39.

[11] Ueno,R.(2009).Method of *in situ* screening for bacteria capable of producing alginate-degrading enzyme from aquatic fields.Journal of Food, Agriculture & Environment Vol.7 (2) : 731 -735.

[12] Ma , Y.; Ji, T.; Li, H. ; Chen, W. ; Gao, S. and Liu, S.(2009).Culture optimization and characterization of an alginate-lyase from Pseudoalteromonas sp. LJ1.Wei Sheng Wu XueBao .49(8):1086-94.

[13] Iwamoto, Y.;Araki, R.;Iriyama, K. ; Oda, T.;Fukuda, H.; Hayashida, S.andMuramatsu, T .(2001).Purification and characterization of bifunctional alginate lyase from *Alteromonas*sp. strain no. 272 and its action on saturated oligomeric substrates. Biosci.Biotechnol.Biochem., 65*:* 133–142.

[14] Wang ,Y .H. ; Yu, G.L. ; Wang , X .M.; Lv, Z.H.; Zhao,X. ; Wu,Z.H. and Ji, W.S.(2006).Purification and characterization of alginate lyase from marine Vibrio sp. YWA.ActaBiochimBiophys Sin (Shanghai). 38(9):633-8.

[15] Song ,K. ; Yu, W.G.; Han ,F.; Han,W.J. and Li ,J.B.(2003).Purification and characterization of alginate lyase from marine bacterium Vibrio sp. .QY101.Sheng Wu Hua Xue Yu Sheng Wu Wu Li XueBao (Shanghai). (5):473-7.

[16] Nakagawa, A. ; Ozaki ,T. ; Chubachi ,K. ; Hosoyama, T. ; Okubo, T. ; Iyobe ,S . and Suzuki, T.(1998). An effective method for isolating alginate lyase-producing *Bacillus* sp. ATB-1015 strain and purification and characterization of the lyase. Journal of Applied Microbiology, 84. 328–335.