Research Paper 研究报告

微生物学报 *Acta Microbiologica Sinica*
48(6)：757~764; 4 June 2008
ISSN 0001-6209; CN11-1995/Q
http://journals.im.ac.cn

# Optimization of κ-carrageenase production by *Pseudoalteromonas* sp. AJ5

Yuexin Ma[1,2], Shuanglin Dong[1], Shuanglian Liu[2], Haijin Mou[3], Xiaolu Jiang[3]*

([1] *Key Laboratory of Mariculture, Ministry of Education, Ocean University of China, Qingdao* 266003, *China*)
([2] *Key Laboratory of Mariculture and Biotechnology Agriculture Ministry, Dalian Fisheries University, Dalian* 116023, *China*)
([3] *College of Food Science and Engineering, Ocean University of China, Qingdao* 266003, *China*)

**Abstract: [Objectives]** To optimize the culture conditions of *Pseudoalteromonas* sp. AJ5 for a higher production of extracellular κ-carrageenase. **[Methods]** A κ-carrageenan-degrading bacterium AJ5, capable of utilizing κ-carrageenan as sole source of carbon and energy, was isolated from the intestine of holothurian *Apostichopus japonicus* by enrichment culture technique. The strain was identified as the genus *Pseudoalteromonas* sp. according to its morphological and physiological characterization and 16S rRNA gene analysis. Culture conditions for the bacterium were standardized for the maximal productivity of the extracellular κ-carrageenase by the single factor and orthogonal tests. **[Results]** According to the single factor test, the optimal culture conditions were: 75 mL medium in 250 mL Erlenmeyer flask, shaking speed of 150 r/min, inoculum's volume 7%, and pH 8.0. Based on the single factor and orthogonal tests the optimal medium components were: κ-carrageenan (1 g/L), beef extract (2 g/L ), NaCl (20 g/L), $K_2HPO_4 \cdot 3H_2O$ (1 g/L), $MgSO_4 \cdot 7H_2O$ (0.5 g/L), $MnCl_2 \cdot 4H_2O$ (0.2 g/L), $FePO_4 \cdot 4H_2O$ (0.01 g/L), with the incubation temperature and time of 28°C and 28 h. **[Conclusions]** *Pseudoalteromonas* sp. AJ5 secreted an extracellular κ-carrageenase. Under the optimal culture conditions, four-fold increase in κ-carrageenase activity was achieved as compared to the control.

**Keywords:** κ-carrageenase; optimization; fermentation condition; medium component; *Pseudoalteromonas*
**CLC number:** Q939.3 **Document code:** A **Article ID:** 0001-6209(2008)06-0757-08

## 1 INTRODUCTION

Carrageenans are cell wall polysaccharides found in several members of the Rhodophyceae. The carrageenans are notated κ-, λ-, ι-, etc. according to their structure. κ-Carrageenans are alternating galactan of 1,3-linked β-D-galactopyranose 4-O-sulfate and 1,4-linked 3,6 anhydro-α-D-galactopyranose [1].

κ-Carrageenases have been found in several genera of marine bacteria, such as *Pseudoalteromonas*[1–3], *Pseudomonas*[4], *Cytophaga*[5–6], *Zobellia*[7–8] and *Vibrio*[9].

κ-Carrageenases degrade κ-carrageenan by hydrolyzing the β-1, 4 linkages to a series of homologous, even numbered oligosaccharides, which is a useful tool for the structural analysis of the cell walls and protoplast isolation from red algae[9]. Carrageenan and its depolymerized products, oligosaccharides, have been shown to have biological activities such as anti-viral and anti-tumor activities[10–15].

Studies regarding the optimization of culture conditions for the production of carrageenase are still few in the scientific literature [4]. In this paper we describe the isolation of a new potent κ-carrageenan-degrading bacterium AJ5 capable of utilizing carrageen as sole source of carbon and energy by enrichment culture technique. The optimal culture conditions for the production of κ-carrageenase were studied. These studies led to a significant improvement in the κ-carrageenase activity.

## 2 MATERIALS AND METHODS

### 2.1 Screening of microorganism

Samples of holothurian *Apostichopus japonicus*

Supported by the National Natural Science Foundation of China (40506027) and the Open Project of Program of Key Laboratory of Mariculture and Biotechnology Agriculture Ministry (K2006-12)
* Corresponding author. +86-532-82032290; Fax: +86-532-82032389; E-mail: jiangxl@ouc.edu.cn
Received: 14 September 2007/Revised: 28 January 2008

and marine algae were collected from a holothurian farm and sea area in Dalian, China. For isolation of κ-carrageenase producing organism, the intestine of holothurian and the pieces of alga washed with aseptic seawater were inoculated into 25 mL of enrichment medium containing: κ-carrageenan (commercial carrageenan, Yantai Seaweed Industry Co., Yantai, China) (2 g/L), $NaNO_3$ (2 g/L), inorganic salt solution 100 mL [salt solution containing: $K_2HPO_4 \cdot 3H_2O$ (10 g/L), $MgSO_4 \cdot 7H_2O$ (5 g/L), $FePO_4 \cdot 4H_2O$ (0.1 g/L ),$CaCl_2$ (1 g/L)], dissolved in 900 mL seawater, pH 8.0. Successive transfers were made in the same medium and finally 0.1 mL diluted enriched sample was plated out on first-screened medium containing: κ-carrageenan (12 g/L), $NaNO_3$ (2 g/L), NaCl (15 g/L), inorganic salt solution 100 mL, dissolved in 900mL distilled water, pH 8.0. The colonies of bacteria which hydrolyzed κ-carrageenan were surrounded by an area of depression or liquefaction. A pure culture of a strain was obtained by repeated subculture on the above κ-carrageenan plate. A total of 33 isolates that actively degraded κ-carrageenan were screened for κ-carrageenase production by shaking culture. The fermentation medium contained the same composition as first-screened medium except 2 g/L κ-carrageenan was used. Strain AJ5 which showed maximum activity was selected for optimization of culture conditions for enzyme production and maintained in the same medium containing 30%(V/V) glycerol at −80°C and on slants at 4°C containing: κ-carrageenan (12 g/L), peptone (5 g/L), yeast extract (1 g/L), $FePO_4 \cdot 4H_2O$ (0.01 g/L), dissolved in 800 mL seawater and 200 mL distilled water, pH 7.6.

### 2.2 Identification of κ-carrageenase-producing bacterium

The morphological and physiological characterization of the strain AJ5 was identified according to Bergey's Manual of Determinative Bacteriology [16]. The 16S rRNA gene from strain AJ5 was amplified using the F27 and R1492 primer set [17] and sequenced from both primers (ABI377 DNA analyzer). Sequence was aligned by using BLAST analysis [18]. For comparison with currently available sequences, several sequences were retrieved from NCBI (http://www.ncbi.nlm.nih.gov/BLAST) and multiple alignment was performed by using Clustal X 1.83 and phylogenetic tree was constructed by PHYLIP 3.56 [19]. Distance matrices were generated by the DNADIST program, based on Kimura's two-parameter model [20]. Neighbour-joining analysis of the data sets was carried out with the program Neighbour of the PHYLIP package. *Vibrio fumissii* was used as outgroup during the construction of consensus tree of the strain AJ5 based on 16S rDNA sequence.

### 2.3 Production of κ-carrageenase

For enzyme preparation, 250 mL Erlenmeyer flasks containing 100 mL fermentation medium were inoculated with 5 mL of 24 h seed culture of AJ5, which was prepared in the same medium (50 mL) inoculated with active slant culture. The flasks were shaken at 150 r/min and 25°C in an incubator shaker for 36 h. The bacterial supernatant obtained by centrifuging the culture at 7,000×*g* for 10 min was assayed for κ-carrageenase activity.

### 2.4 κ-Carrageenase assay

κ-Carrageenase activity was determined by measuring the increase in the concentration of reducing sugar ends formed by enzymic reaction, by applying the dinitrosalicylic method of Miller [21]. Standard incubation conditions were 5 g/L κ-carrageenan in 25 mmol/L Tris-HCl (pH 8.0) at 32°C for 30 min. One unit (U) of κ-carrageenase activity is defined as the amount of enzyme needed to liberate 1 μmol of D-galactose equivalents per minute under the given conditions. All assays were conducted in triplicates and the standard errors of the means were calculated.

### 2.5 Optimization of culture conditions for κ-carrageenase production

The optimization of fermentation conditions and medium compositions were carried out based on the stepwise modification of the governing parameters for κ-carrageenase production. They include: the culture volume, shaking speed, inoculum volume, pH, sole carbon source, different concentrations of optimum carbon source, sole nitrogen source, different concentrations of optimum nitrogen source, inorganic salts. The optimum culture condition obtained in previous step was used in next step.

### 2.6 Orthogonal test

NaCl, κ-carrageenan, beef extract in fermentation medium and culture temperature were chosen to design four factors three levels orthogonal test L9 ($4^3$) to examine the effects of them on κ-carrageenase production. Other conditions were optimized as above mentioned.

### 2.7 Cell growth and κ-carrageenase activity curves

For cell growth and κ-carrageenase activity curves measurement, 250 mL Erlenmeyer flasks containing 75 mL fermentation medium optimized by orthogonal test were inoculated with 7% seed culture. The flasks were shaken at 150 r/min and 28°C in an incubator shaker for 36 h. Growth was estimated by taking cell counts and enzyme activity by the reducing assay.

## 3 RESULTS

### 3.1 Isolation and identification of κ-carrageenan-degrading bacterium

By enrichment culture technique and first-screened by carrageenan plate containing κ-carrageenan as sole carbon source 33 isolates which hydrolyzed κ- carrageenan were obtained. The strain AJ5 showed maximum κ-carrageenase activity by second-screened of shaking culture. Electron micrograph of the strain AJ5 showed a polarly flagellated and rod-shaped cell (data not shown). It was Gram-negative, oxidase-and catalase-positive, urease, indole, and arginine dihydrolase negative. It required sodium ion for growth, had an oxidative metabolism, and did not accumulate poly-β-hydroxybutyrate (PHB) as an intracellular reserve product. The preliminary identification results showed that the morphologic and physiological characteristics of this strain were in accordance with *Alteromonas* according to Bergey's Manual of Determinative Bacteriology [16] or *Pseudoalteromonas* [3] (Table 1). Total 1285 bp sequence of the 16S rRNA gene was determined for strain AJ5 (GenBank accession number: EF208037). The 16S rDNA sequence of strain AJ5 was compared to sequences available from public databases. Fig. 1 shows a consensus tree of the *Pseudoalteromonas* species. Thus, the strain AJ5 was identified as *Pseudoalteromonas* sp.

**Table 1** Morphologic and physiological characteristics of *Pseudoalteromonas* sp. AJ5

| Characteristics tested | Results |
|---|---|
| Cell shape | rod |
| Endospores | – |
| Polar flagellum | + |
| Production of pigments | – |
| Growth at 4°C | – |
| Growth at 35°C | – |
| Growth at 40°C | – |
| Anaerobic growth | – |
| Organic growth factors required | – |
| Oxidase reaction | + |
| Catalase reaction | + |
| Urease [a] | – |
| Indole [a] | – |
| Arginine dihydrolase [a] | – |
| Accumulation of PHB | – |
| O/F test | O |
| Reduction of $NO_3^-$ | + |
| Production of $H_2S$ | – |
| Requirement of sodium for growth | + |
| Hydrolysis of starch, κ-carrageenan, and gelatin | + |
| Hydrolysis of alginate | – |
| Utilization of D-glucose, sucrose, maltose, cellobiose, D-mannitol, L-arabinose, N-acetyl-D-glucosamine [b], citrate, L-glutamic acid [b] and L-tyrosine | + |
| Utilization of salicin and succinic acid [b] | W |
| Utilization of α-D-lactose [b], D-galactose [b], D-fructose [b], D-mannose [b], D-melibiose [b], glycerol, α-ketoglutaric acid [b], cis-aconitic acid [b] and L-threonine [b] | – |

w: weak; [a] API 20E; [b] Biolog.

### 3.2 Effect of culture volume, shaking speed, inoculum volume and initial pH on κ-carrageenase production

*Pseudoalteromonas* sp. AJ5 with initial κ-carrageenase activity 1.274 U/mL was used to optimize the culture conditions to increase the enzyme activity. Varying the amount of medium (50–150 mL) during the fermentation indicated that 250 mL Erlenmeyer flasks containing 75 mL fermentation medium was optimal for the synthesis of κ-carrageenase (Fig.2). Increase or decrease of the amount of medium was found to adversely affect the enzyme production. Fig.3 shows the effect of shaking speed (0–200 r/min) on κ-carrageenase production. κ-Carrageenase activity enhanced gradually when shaking speed was increased from zero to 150 r/min but reduced over 150 r/min. Different inoculum volume of bacterial cell [3%–9% (V/V)] inoculating fermentation medium resulted in changes of carrageenase activities. Maximum enzyme activity was observed with 7% (V/V) inoculum volume (Fig.4). Fermentation medium with different initial pH(6–10) had remarkable effect on κ-carrageenase production (Fig.5). The optimum pH for production of κ-carrageenase by *Pseudoalteromonas* sp. AJ5 was around 8.

### 3.3 Effect of carbon, nitrogen sources and inorganic salts on κ-carrageenase production

The effect of different carbon sources (2–5 g/L) on κ-carrageenase production was determined and the data are shown in Table 2. κ-Carrageenan was the dominant carbon source, yielding 2.384 U/mL of enzyme activity, although agar, alginate, starch, lactose, glucose, fructose and sucrose could be used as the sole carbon source, the κ-carrageenase production was lower. Therefore we studied the effect of various concentrations of κ-carrageenan on the enzyme production (Table 2), and found that 2 g/L κ-carrageenan was optimal for the synthesis of the κ-carrageenase. Addition of beef extract to the medium resulted in the

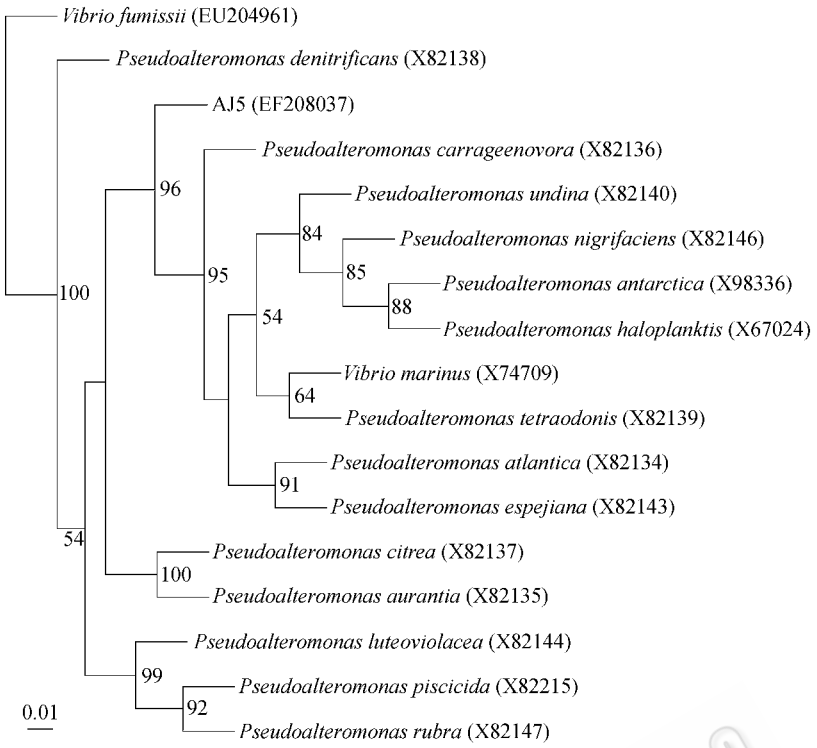


**Fig. 1** Phylogenetic tree of the strain AJ5 based on 16S rDNAs used in this study and 16 previously published from Genbank. The outgroup we used was *Vibrio fumissii*. Numbers in parentheses represent the sequences' accession number in GenBank. The number at each branch points is the percentage supported by bootstrap. Bar, 1% sequence divergence.

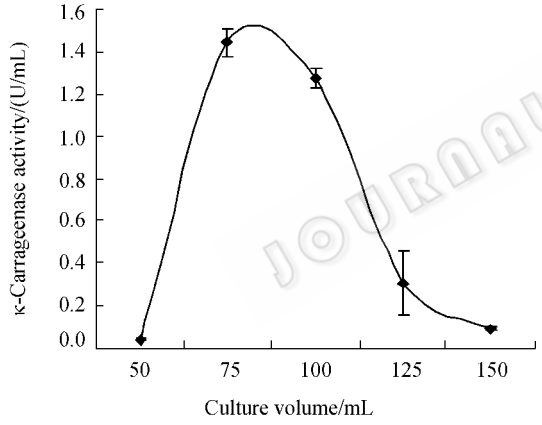


**Fig. 2** Effect of culture volume on κ-carrageenase production by *Pseudoalteromonas* sp. AJ5. Bars denote standard deviation (n=3).

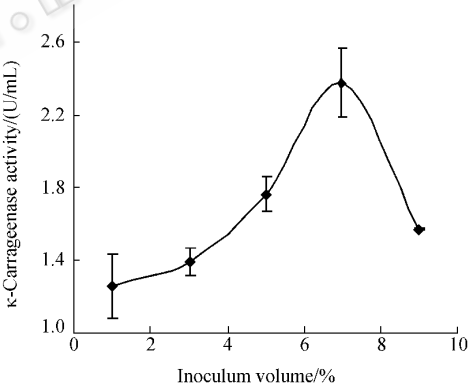


**Fig. 4** Effect of inoculum volume on κ-carrageenase production by *Pseudoalteromonas* sp. AJ5. Bars denote standard deviation (n=3).

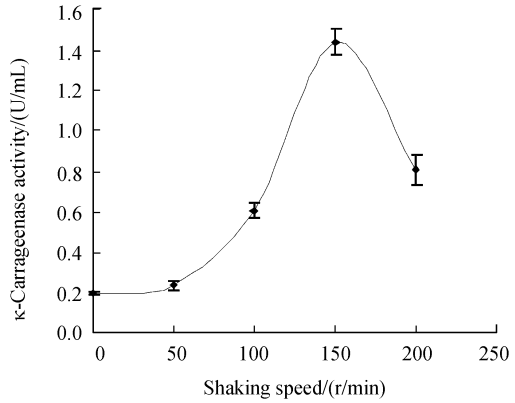


**Fig. 3** Effect of shaking speed on κ-carrageenase production by *Pseudoalteromonas* sp. AJ5. Bars denote standard deviation (n=3).

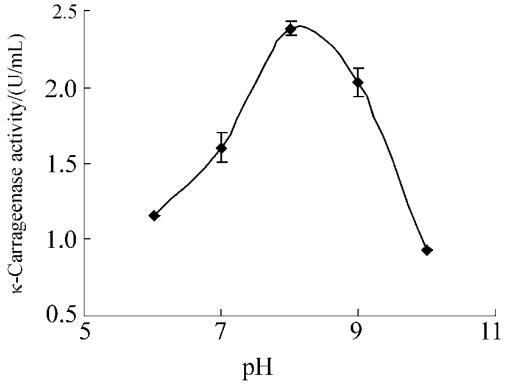


**Fig. 5** Effect of initial pH of medium on κ-carrageenase production by *Pseudoalteromonas* sp. AJ5. Bars denote standard deviation (n=3).

maximal κ-carrageenase yield among the eight nitrogen sources supplemented (Table 2). Different concentrations of beef extract supplemented during the fermentation showed that 2 g/L beef extract was optimal for the enzyme production. The fermentation medium supplemented with complex inorganic salts compared with single salt led to the higher κ-carrageenase activity (Table 3). Of those tested, the best combination for the enzyme production was $K_2HPO_4{\cdot}3H_2O$ +$MgSO_4{\cdot}7H_2O$ +$FePO_4{\cdot}4H_2O$ + $MnCl_2{\cdot}4H_2O$.

**Table 2** Effect of carbon sources and nitrogen sources on κ-carrageenase production by *Pseudoalteromonas* sp. AJ5

| Carbon source /(g/L) | κ-Carrageenase activity ±SD/(U/mL) | Nitrogen source /(g/L) | κ-Carrageenase activity ±SD/(U/mL) |
|---|---|---|---|
| Lactose, 5 | 0.950±0.005 | $NaNO_3$, 2 | 1.239±0.111 |
| Glucose, 5 | 1.192±0.346 | $KNO_3$, 2 | 0.597±0.043 |
| Fructose, 5 | 1.159±0.023 | $NH_4Cl$, 2 | 0.579±0.048 |
| Sucrose, 5 | 0.080±0.009 | $(NH_4)_2SO_4$, 2 | 0.439±0.018 |
| Starch, 2 | 0.620±0.037 | $NH_4NO_3$, 2 | 0.146±0.007 |
| Agar, 2 | 0.682±0.115 | Yeast extract, 2 | 1.305±0.213 |
| Sodium alginate, 2 | 0.245±0.058 | Peptone, 2 | 0.922±0.009 |
| κ-Carrageenan, 0.5 | 0.522±0.153 | Beef extract, 0.5 | 1.907±0.125 |
| κ-Carrageenan, 1 | 1.421±0.111 | Beef extract, 1 | 1.974±0.188 |
| κ-Carrageenan, 2 | 2.384±0.043 | Beef extract, 2 | 2.100±0.063 |
| κ-Carrageenan, 3 | 2.227±0.266 | Beef extract, 3 | 1.803±0.254 |
| κ-Carrageenan, 4 | 1.593±0.177 | Beef extract, 4 | 1.575±0.354 |

**Table 3** Effect of inorganic salts on κ-carrageenase production by *Pseudoalteromonas* sp. AJ5

| Inorganic salts/(g/L) | κ-Carrageenase activity ±SD/(U/mL) |
|---|---|
| $K_2HPO_4{\cdot}3H_2O$, 1 + $MgSO_4{\cdot}7H_2O$, 0.5 + $FePO_4{\cdot}4H_2O$, 0.01 + $CaCl_2$, 0.1 | 2.100±0.063 |
| $K_2HPO_4{\cdot}3H_2O$, 1 + $MgSO_4{\cdot}7H_2O$, 0.5 + $FePO_4{\cdot}4H_2O$, 0.01 + $MnCl_2{\cdot}4H_2O$, 0.2 | 2.817±0.133 |
| $K_2HPO_4{\cdot}3H_2O$, 1 + $MgSO_4{\cdot}7H_2O$, 0.5 + $CaCl_2$, 0.1 | 2.073±0.049 |
| $K_2HPO_4{\cdot}3H_2O$, 1 + $MgSO_4{\cdot}7H_2O$, 0.5 | 1.905±0.116 |
| $K_2HPO_4{\cdot}3H_2O$, 1 | 1.772±0.049 |
| $FePO_4{\cdot}4H_2O$, 0.01 | 1.274±0.063 |

### 3.4 Orthogonal test

Nine group tests were conducted based on the design of the orthogonal test and the κ-carrageenase activities are shown in Table 4. The primary and secondary order for the effect of NaCl, κ-carrageenan, beef extract and temperature on the enzyme production was temperature>NaCl>beef extract>κ-carrageenan. The best combination for the strain AJ5 to produce κ-carrageenase was NaCl 20 g/L, κ-carrageenan 1 g/L, beef extract 2 g/L, and temperature at 28°C(Table 4). ANOVA of orthogonal test data also indicated that temperature affected the production of κ-carrageenase significantly (Table 5).

**Table 4** Data of orthogonal test

| Run No. | NaCl/(g /L) | κ-Carrageenan/(g /L) | Beef extract/(g /L) | *T*/°C | κ-Carrageenase activity/(U/mL) |
|---|---|---|---|---|---|
| 1 | 15 | 1 | 1 | 25 | 2.026 |
| 2 | 15 | 2 | 2 | 28 | 2.194 |
| 3 | 15 | 3 | 3 | 32 | 0.125 |
| 4 | 20 | 1 | 2 | 32 | 1.138 |
| 5 | 20 | 2 | 3 | 25 | 2.005 |
| 6 | 20 | 3 | 1 | 28 | 2.047 |
| 7 | 30 | 1 | 3 | 28 | 1.870 |
| 8 | 30 | 2 | 1 | 32 | 0.406 |
| 9 | 30 | 3 | 2 | 25 | 1.775 |
| $K_1$ | 4.344 | 5.034 | 4.479 | 5.086 | |
| $K_2$ | 5.190 | 4.604 | 5.106 | 6.110 | |
| $K_3$ | 4.051 | 3.947 | 4.000 | 1.669 | |
| R | 1.139 | 1.087 | 1.106 | 4.441 | |

**Table 5** ANOVA of orthogonal test data

| Source of variation | Sum of squares | df | Mean square | F value | Critical value | Significance |
|---|---|---|---|---|---|---|
| NaCl | 0.700 | 2 | 0.350 | 5.959 | $F_{0.01}(2, 18)=6.01$ | 0.010 |
| κ-Carrageenan | 0.598 | 2 | 0.299 | 5.088 | | 0.018 |
| Beef extract | 0.616 | 2 | 0.308 | 5.241 | | 0.016 |
| Temperature | 12.316 | 2 | 6.158 | 104.861 | | 0.000 |
| Error | 1.057 | 18 | 0.059 | | | |

### 3.5 Cell growth and κ-carrageenase activity curves

Fig.6 illustrates the growth curve of *Pseudoalteromonas* sp. AJ5 and the associated rise in κ-carrageenase activity under the culture conditions optimized by single factor and orthogonal tests. The highest level of κ-carrageenase (6.543 U/mL ) was reached during the stationary phase, which was four-fold higher than that under unoptimized conditions.

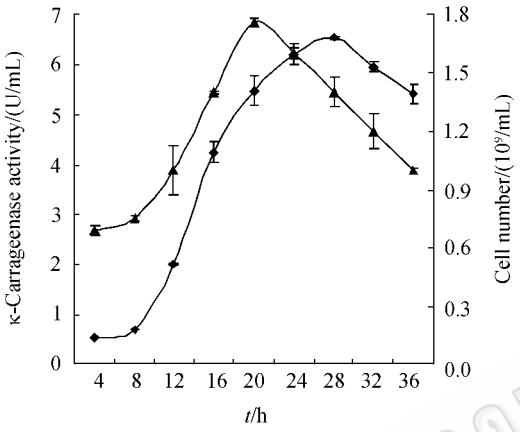


**Fig. 6** Growth (cell number▲)and κ-carrageenase activity (◆) of *Pseudoalteromonas* sp. AJ5. Bars denote standard deviation (n=3).

## 4 DISCUSSION

Several species of carrageenan-degrading bacteria have been isolated from marine algae [7,9,22]. In addition, bacteria isolated from the abalone gut can hydrolyze carrageenan[23]. The κ-carrageenan-degrading bacterium AJ5, identified as *Pseudoalteromonas* sp., was isolated from the intestine contents of holothurian *Apostichopus japonicus.* Alginic acid and fucoidan-degrading bacteria have also been isolated from the coelomic liquid of the holothurian *Apostichopus japonicus*[24] and the gut contents of the sea cucumber *Stichopus japonicus*[25].

The morphologic and physiological characteristics of the strain AJ5 were in accordance with *Alteromonas* based on Bergey's Manual of Systematic Bacteriology, however, the differences between the bacterium and other 12 species in this genus were observed. *Pseudoalteromonas* is a new genus created from *Alteromonas* according to phylogenetic comparisons performed by Gauthier *et al*. in 1995[3]. Using 16S rRNA gene analysis, the strain AJ5 could be identified as *Pseudoalteromonas* sp., which was different from the *P. carrageenovora* in production of amylase and alginase and utilization of D-fructose, melibiose, lactose, N-acetyl-D-glucosamine and glycerol. *Pseudoalteromonas* sp. CL19 can degrade λ-carrageenan[26]. *Alteromonas fortis* produced ι-carrageenase [27].

The optimization of culture conditions for enzyme production indicated that the culture volume, shaking speed, inoculum volume, pH and temperature significantly affected the production of κ-carrageenase by *Pseudoalteromonas* sp. AJ5. 250 mL Erlenmeyer flask containing 75 mL medium and 150 r/min were optimum for the enzyme production. The lower culture volume and higher shaking speed result in increased shear stress, which might decrease the growth of the bacterium and the κ-carrageenase production. The optimum pH and temperature for enzyme production were around 8 and 28°C, respectively, and this might because the bacterium was adapt to the alkaline and low temperature marine environment for the growth of holothurian. The pH of the culture strongly affects enzymatic processes and transport of compounds across the cell membrane. Several carrageenan-degrading bacteria were found to produce carrageenases at pH 7–8 [4, 6–7, 22, 28]. However, various bacteria produced carrageenases at different temperatures, ranging from 25°C [5, 9] to 37°C [4, 22]. The mechanism of temperature control in carrageenase production needs to be elucidated.

κ-Carrageenan can be used as the dominant carbon source by *Pseudoalteromonas* sp. AJ5. As to the effect of κ-carrageenan concentrations on enzyme production, the results of this study were comparable to those reported earlier [6, 29]. The κ-carrageenases production were optimal when bacteria *Pseudomonas elongata* and *Zobellia galactanovorans* were grown in the presence of 2 g/L κ-carrageenan and 3 g/L non-purified λ-carrageenan, respectively [4,7–8]. When different carrageenan concentrations were directly compared in parallel cultures, all concentrations within 0.5–1 g/L gave maximal activities at 90 U/mL, but the activity dropped rapidly at higher carrageenan levels [29]. This effect appeared to be related to the increasing viscosity of the medium [29]. Addition of certain concentration of NaCl to the fermentation medium affected significantly on the κ-carrageenase production by *Pseudoalteromonas* sp. AJ5. The results revealed that 20 g/L NaCl supplemented was optimal for the enzyme pro-

duction. The media used for κ-carrageenase production in previous studies contained 25–30 g/L NaCl [2, 4] or seawater [7]. Artificial seawater components were necessary for carrageenase production by a marine *Cytophaga* 1k-C783[5], and only NaCl and $MgCl_2$ were utilized by the organism to produce carrageenase among artificial seawater salts [30]. These findings suggest NaCl or (artificial) seawater is important to the carrageenase production by marine bacteria.

Under optimized condition the cell density of *Pseudoalteromonas* sp. AJ5 reached its maximum within 20 h (Fig. 6), followed by a decrease. The κ-carrageenase production continued after growth had ceased, reaching the highest level after 28 h of incubation. This observation was in agreement with the report for *P. carrageenovora* growth and κ-carrageenase production in shaking flask cultures [29]. Furthermore, the maximal κ-carrageenase activities of AJ5 were higher than, and comparable to those of *P. carrageenovora* in batch cultures, and in fermentor studies [29].

## REFERENCES


[1] Weigl J, Yaphe W. The enzymatic hydrolysis of carrageenan by *Pseudomonas carrageenovora*: Purification of a κ-carrageenase. *Canadian Journal of Microbiology*, 1966, 12: 939–947.

[2] McLean MW, Williamson FB. κ-Carrageenase from *Pseudomonas carrageenovora*. *European Journal of Biochemistry*, 1979, 93: 553–558.

[3] Gauthier G, Gauthier M, Christen R, *et al*. Phylogenetic analysis of the genera *Alteromonas*, *Shewanella*, and *Moritella* using genes coding for small-subunit rRNA sequences and division of the genus *Alteromonas* into two genera, *Alteromonas* (emended) and *Pseudoalteromonas* gen. nov., and proposal of twelve new species combinations. *International Journal of Systematic Bacteriology*, 1995, 45: 755–761.

[4] Khambhaty Y, Mody K, Jha B, *et al*. Statistical optimization of medium components for κ-carrageenase production by *Pseudomonas elongate*. *Enzyme and Microbial Technology*, 2007, 40: 813–822.

[5] Sarwar G, Sakata T, Kakamoto D. The production and characteristics of carrageenase from marine *Cytophaga*. *Bulletin of the Japanese Society of Scientific Fisheries*, 1983, 49: 1689–1694.

[6] Sarwar G, Matayoshi S, Oda H. Purification of a κappa-carrageenase from marine *Cytophaga* species. *Microbiology and Immunology*, 1987, 31: 869–877.

[7] Potin P, Sanseau A, LeGall Y, *et al*. Purification and characterization of a new κappa-carrageenase from a marine *Cytophaga*-like bacterium. *European Journal of Biochemistry*, 1991, 201: 241–247.

[8] Barbeyron T, L'Haridon S, Corre E, *et al*. *Zobellia galactanovorans* gen. nov., sp. nov., a marine species of Flavobacteriaceae isolated from a red alga, and classification of [*Cytophaga*] *uliginosa* (ZoBell and Upham 1944) Reichenbach 1989 as *Zobellia uliginosa* gen. nov., comb. nov.. *International Journal of Systematic and Evolutinary Microbiology*, 2001, 51: 985–997.

[9] Araki T, Higashitomo Y, Morishita T. Purification and Characterization of κ-carrageenase from a marine bacterium *Vibrio* sp.CA-1004. *Fisheries Science*, 1999, 65: 937–942.

[10] Nakashima H, Kido Y, Kobayashi N, *et al*. Purification and characterization of an avian myeloblastosis and human immunodeficiency virus reverse transcriptase inhibitor, sulfated polysaccharides extracted from sea algae. *Antimicrobial Agents Chemotherapy*, 1987, 31: 1524–1528.

[11] Coombe DR, Parish CR, Ramshaw IA. Analysis of the inhibition of tumor metastasis by sulfated polysaccharides. *International Journal of Cancer*, 1987, 39: 82–88

[12] Noda H, Amano H, Arashima K, *et al*. Studies on the anti-tumor activity of marine algae. *Nippon Suissan Gakkaishi*, 1989, 55: 1259–1264.

[13] Yamada T, Ogamo A, Saito T, *et al*. Preparation and anti-HIV activity of low-molecular-weight carrageenans and their derivatives. *Carbohydrate Polymers*, 1997, 32, 51–55.

[14] Mou HJ, Jiang XL, Guan HS. A κ-carrageenan derived oligosaccharide prepared by enzymatic degradation containing anti-tumor activity. *Journal of Applied Phycology*, 2003, 15: 297–303.

[15] Hu XK , Jiang XL, Aubree E, *et al*. Preparation and in vivo anti-tumor activity of kappa-carrageenan oligosaccharides. *Pharmaceutical Biology*, 2006, 44: 646–650.

[16] Holt JG, Krieg NR, Sneath PHA, et al. Bergey's Manual of Determinative Bacteriology. 9th edition. Battimore: Williams & Wilkins, 1994.

[17] Lane DJ. 16S/23S rRNA sequencing. In: Stackebrandt, E., Goodfellow, M. Nucleic acid Techniques in Bacterial Systematics. New York: John Wiley & Sons, 1991.

[18] Altschul SF, Gish W, Miller W, *et al*. Basic local alignment search tool. *Journal of Molecular Biology*, 1990, 215: 403– 410.

[19] Felsenstein J. PHYLIP (Phylogenetic Inference Package), Version 3.75. Distributed by author, Department of Genetics, Univerity of Washinton, Seattle, WA. 1995.

[20] Kimura M. A simple method for estimating evolutionary rate of base substitution through comparative studies on nucleotide sequence. Journal of Molecular Evolution, 1980, 16: 111–120.

[21] Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugars. *Analytical Chemistry*, 1959, 31: 426–428.

[22] 牟海津, 江晓路, 蒋萱, 等.卡拉胶降解菌 M-2 的筛选与产酶性质.中国水产科学(*Journal of Fishery Sciences of China*), 2002, 9: 251–254.

[23] Erasmus JH, Cook PA, Coyne VE. The role of bacteria in the digestion of seaweed by the abalone *Haliotis midae*. *Aquaculture*, 1997, 155: 377–386.

[24] Alekseeva SA, Bakunina IY, Nedashkovskaya OI, *et al*. Introcelluar alginolytic enzymes of the marine bacterium *Pseudoalteromonas citrea* KMM3297. *Biochemistry* (*Moscow*), 2004, 69: 262–269.

[25] Sakai T, Ishizuka K, Kato I. Isolation and characterization of a fucoidan-degrading marine bacterium. *Marine Biotechnology*, 2003, 5: 409–416.

[26] Ohta Y, Hatada Y. A novel enzyme, λ-carrageenase, isolated from a deep-sea bacterium. *Journal of Biochemistry* (*Tokyo*), 2006, 140: 175–181.
[27] Michel G, Flament D, Barbeyron T, *et al*. Expression, purification, crystallization and preliminary x-ray analysis of the iota-carrageenase from *Alteromonas fortis*. *Acta Crystallographica* (*Section D*), 2000, 56: 766–768.
[28] Yaphe W, Baxter B. The enzyme hydrolysis of carrageenan. *Applied Microbiology*, 1955, 3: 380–383.
[29] Østgaard K, Wangen BF, Knutsen SH, et al. Large-scale production and purification of κappa-carrageenase from *Pseudomonas carrageenovora* for applications in seaweed biotechnology. Enzyme and Microbial Technology, 1993, 15: 326 –333.
[30] Sarwar G, Oda H, Sakata T, *et al*. Potentiality of artificial sea water salts for the production of carrageenase by a marine *Cytophaga* sp.. *Microbiology and Immunology*, 1985, 29: 405 –411.

# 假交替单胞菌 *Pseudoalteromonas* sp. AJ5 产κ-卡拉胶酶的培养条件优化

马悦欣[1, 2]，董双林[1]，刘双连[2]，牟海津[3]，江晓路[3*]

([1] 中国海洋大学，教育部海水养殖重点实验室，青岛 266003)
([2] 大连水产学院，农业部海洋水产增养殖学与生物技术重点开放实验室，大连 116023)
([3] 中国海洋大学食品科学与工程学院，青岛 266003)

摘要：【目的】本研究的目的是优化 *Pseudoalteromonas* sp. AJ5 菌株的培养条件使之产生高活性的胞外κ-卡拉胶酶。【方法】通过富集培养技术从刺参肠道分离出一株卡拉胶降解菌 AJ5，该菌株能利用卡拉胶作为惟一碳源和能源。依据形态学和生理学特征及 16S rRNA 基因序列分析，将该菌株鉴定为假交替单胞菌属（*Pseudoalteromonas*）。通过单因素试验和正交试验对 *Pseudoalteromonas* sp. AJ5 菌株产胞外κ-卡拉胶酶的培养条件进行了优化。【结果】单因素试验结果表明，*Pseudoalteromonas* sp. AJ5 菌株的最佳培养条件为 250 mL 三角瓶装入 75 mL 发酵培养基、摇床转速 150 r/min、接种量 7%、pH8.0。单因素试验和正交试验结果显示该菌株的最佳培养基组成为κ-卡拉胶 1 g/L、牛肉膏 2 g/L、 NaCl 20 g/L、$K_2HPO_4{\cdot}3H_2O$ 1 g/L、 $MgSO_4{\cdot}7H_2O$ 0.5 g/L、$MnCl_2{\cdot}4H_2O$ 0.2 g/L、 $FePO_4{\cdot}4H_2O$ 0.01 g/L；培养温度为 28℃，培养时间为 28 h。【结论】*Pseudoalteromonas* sp. AJ5 菌株分泌胞外κ-卡拉胶酶，在最佳培养条件下，该菌株的κ-卡拉胶酶活力比优化前提高了 4 倍。

关键词：κ-卡拉胶酶；优化；发酵条件；培养基组成；假交替单胞菌属

中图分类号： Q939.3 文献标识码：A 文章编号：0001-6209(2008) 06-0764-08

基金项目：国家自然科学基金(40506027)；农业部海洋水产增养殖学与生物技术重点开放实验室开放课题(K2006-12)
*通讯作者。Tel: +86-532-82032290; E-mail: jiangxl@ouc.edu.cn
作者简介：马悦欣(1963–)，女，山东招远人，主要从事应用微生物学研究。Tel: +86-411- 84763522; E-mail: mayuexin@dlfu.edu.cn
收稿日期：2007-09-14；修回日期：2008-01-28