The Effect of Potassium Phosphate as a pH Stabilizer on the Production of Gellan by *Sphingomonas paucibilis* NK-2000

Nam-Kyu Lee, Young-Bae Jo, Il-Hyuck Jin[1], Chang-Woo Son[2] and Jin-Woo Lee[3]*

Marine-Bioindustry Development Center, Busan 619-912
[1]*Gene Bioscience Co., Busan 612-020,* [2]*Korea Bio-Solution Co., Busan 612-020*
[3]*Department of Biotechnology, Dong-A University, Pusan, 604-714, the Republic of Korea*

Received April 13, 2009 / Accepted May 28, 2009

Maximal productions of gellan by *Sphingomnas paucibilis* NK2000 from 20 g/l glucose and 10 g/l soybean pomace were 7.46 g/l in a flask and 7.35 g/l in a 7 l bioreactor, when the initial pH of media was 6.8. Maximal production of gellan in a 7 l bioreactor under pH control by sodium hydroxide was 8.42 g/l, whereas that under control by potassium phosphate was 8.50 g/l. The optimal concentration of potassium phosphate in a medium for production of gellan by *S. paucibilis* NK2000 was found to be 5.0 g/l. Maximal production of gellan in a medium containing 5.0 g/l potassium phosphate without pH control was 8.93 g/l in a 7 l bioreactor. In this study, a simple process without pH control was developed to enhance the production of gellan, with optimized concentration of potassium phosphate in the medium.

Key words : Gellan, *Sphingomnas paucibilis*, production, pH control, potassium phosphate

Introduction

The heteropolysaccharide-60, commercially known as gellan, was produced by *Sphingomnas paucibilis* (formerly *Pseudomonas elodea*) [17,23]. Gellan consists of linear repeating tetrasaccharides [→3)-β-D-Glc-(1→4)-β-D-GluA-(1→4)-β-D-Glc-(1→4)-α-L-Rha-(1→] composed of D-glucose (Glc), D-glucuronic acid (GlcA), and L-rhamnose residues (Rha) [12,28]. Due to the diversity of its structure and properties, gellan has a wide range of applications in the food, pharmaceutical and other industries as texturizing, stabilizing, thickening, emulsifying and gelling agents [9,25]. Gellan exhibits good stability, which is a distinct advantage in fruit-based products [18]. Furthermore, gellan has also been used for enzyme and cell immobilization as well as gel electrophoresis.

Factors that affect the conversion of gellan are carbon and nitrogen sources, temperature, the initial pH of the medium, and oxygen supply [2,4]. The conversion carbon source to curdlan by *Agrobacterium* sp. is about 50% if the pH of the culture medium is maintained at around 6.0 [16]. The pH of the culture medium influences not only production of pullulan by *Aureobasidum pullulan*, but also the morphology of this strain [19]. The initial pH of medium as well as pH control during cultivation is considered to be significant factors affecting the production of microbial metabolites [1,7]. The cost of an equipment for pH control in a bioreactor is expensive, and there is a possibility of contamination during cultivation in the process for pH control. Production of gellan with soybean pomace as a nitrogen source was previously reported [13]. The aim of this study was to examine effects of initial pH of the medium with a cheap nitrogen source and pH control during a batch culture on the production of gellan, and to develop an economic process to enhance its productivity.

***Corresponding author**
Tel : +82-51-200-7593, Fax : +82-51-200-7505
E-mail : jwlee@dau.ac.kr

Materials and Methods

Bacterial strain and medium

Sphingomnas paucibilis NK2000 (formerly *Pseudomonas elodea*) is a UV-induced mutant of *S. paucibilis* ATCC 31461. The medium used for cell growth and production of gellan contained 20.0 g/l glucose, 10.0 g/l soybean pomace, 0.5 g/l K_2HPO_4, 0.1 g/l $MgSO_4 \cdot 7H_2O$, and 0.1 ml mineral salt solution [13]. The mineral salt solution contained 1.8 mg/l $MnCl_2 \cdot 4H_2O$, 2.487 mg/l $FeSO_4 \cdot 7H_2O$, 0.285 mg/l H_3BO_3, 27.0 mg/l $CuCl_2$, 21.0 mg/l $ZnCl_2$, 74 mg/l $CoCl_2 \cdot 6H_2O$, 23.0 mg/l $MgMoO_4$, and 2.1 g/l sodium tartrate (dihydrate). The pH of the medium was adjusted to 6.5~6.8 before sterilization. The carbon source was autoclaved separately for 15 min at 121 and added to the medium under aseptic conditions.

Production of gellan

Starter cultures were prepared by transferring cells from agar slants to 100 ml of the medium with 20 g/l glucose and 2.5 g/l yeast extract in 500 ml Erlenmeyer flasks. These cultures were incubated for 1 day at 30°C and 200 rpm. These starter cultures were used as inoculum for a 5 l medium with 20 g/l glucose and 10 g/l soybean pomace in a 7 l fermentor (Ko-Biotech Co., Korea). Working volumes of the 7 l bioreactors was 5 l and inoculum size of batch fermentations for production of gellan by *S. paucibilis* NK2000 was 5.0% (v/v). Agitation speed and aeration rate were 400 rpm and 1.0 vvm, respectively. Samples were periodically withdrawn from the culture to determine cell growth and production of gellan.

To isolate gellan, the culture broth was heated at 95°C for 15 min in a boiling water bath. The pH of heated culture broth was adjusted to 10.0 by 2.0 N sodium hydroxide and then neutralized with 2.0 N sulfuric acid. The pretreated broth was centrifuged at 12,000× *g* for 20 min to separate the cells. The supernatant was mixed with 2 vol. of isopropanol and then kept overnight at 4°C. This precipitated gellan was centrifuged at 8,000× *g* for 30 min and dried at 100~105°C until constant weight [21].

Analytical methods

Cell growth was determined by directly weighing biomass after drying to a constant weight at 100~105°C. Reducing sugar was determined by the DNS method [24].

Results and Discussion

The effect of initial pH on production of gellan

The effect of initial pH on cell growth and the production of gellan by *S. paucibilis* NK2000 was investigated. Carbon and nitrogen sources for the production of gellan were 20 g/l glucose and 10 g/l soybean pomace. The initial pH of medium ranged from 4.3 to 8.8. The production of gellan increased with increased initial pH of medium as shown in Table 1. Maximal production of gellan was 7.46 g/l when the initial pH of the medium was 6.8, whereas the optimal pH for cell growth of *S. paucibilis* NK2000 was 5.8. The initial pH of the culture medium is a vital factor for cell growth and production of gellan by *S. paucimobilis* ATCC 31461 [2]. Optimal pH for the production of gellan by *S. paucibilis* NK2000 was also different from that for its cell growth as in production of other microbial metabolites [5,8,13].

Production of gellan without pH control

Gellan was produced by *S. paucibilis* NK2000 in a 7 l bioreactor without pH control. The carbon and nitrogen sources for the production of gellan were 20 g/l glucose and 10 g/l soybean pomace and the initial pH of the medium was 6.8. The agitation speed and aeration rate were 400 rpm and 1.0 vvm, respectively. The pH of medium decreased with culture time and reached around 4.9 after 72 hr as shown in Fig. 1. Dissolved oxygen decreased with cell growth and production of gellan and it was maintained at about 40% after cell growth ceased. The reducing sugars in the medium gradually decreased and production of gellan rapidly increased until 36 hr of culture. Maximal production of gellan in a 7 l bioreactor without pH control was 7.35 g/l, and the conversion rate of gellan from 20 g/l glucose was 38%.

Production of gellan under pH control by sodium hydroxide

Gellan was produced by *S. paucibilis* NK2000 in a 7 l

Table 1. The effect of initial pH on cell growth and production of gellan by *S. paucibilis* NK2000

Initial pH	Final pH	DCW[a] (g/l)	Gellan (g/l)	Yp/s[b]	Yx/s[c]	Yp/x[d]
4.3	3.9	1.94±0.19	3.24±0.21	0.16	0.10	1.67
4.8	4.1	2.67±0.24	4.76±0.36	0.24	0.13	1.78
5.3	4.3	3.43±0.31	5.21±0.41	0.26	0.17	1.52
5.8	4.6	3.86±0.32	6.57±0.53	0.33	0.19	1.70
6.3	4.8	3.31±0.28	7.12±0.48	0.36	0.17	2.15
6.8	5.6	3.15±0.30	7.46±0.51	0.37	0.16	2.35
7.3	5.9	2.73±0.28	7.11±0.63	0.36	0.14	2.60
7.8	6.1	2.32±0.22	6.53±0.54	0.33	0.12	2.81
8.3	6.3	1.94±0.21	5.58±0.47	0.28	0.10	2.87
8.8	6.5	1.76±0.19	4.86±0.37	0.24	0.09	2.76

a: dry cells weight, b: yield of product per substrate, c: yield of cell mass per substrate, d: yield of product pre cell mass

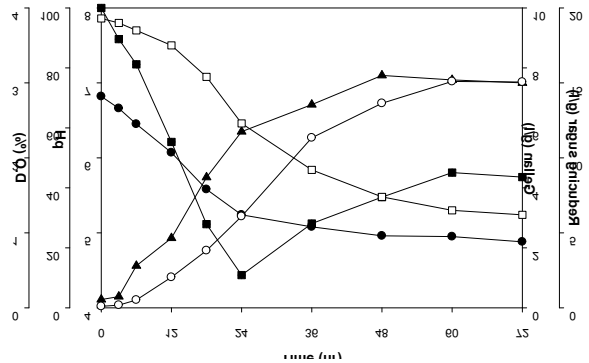

Fig. 1. Cell growth and production of gellan by *S. paucibilis* NK2000 without pH control (●, pH; ■, dissolved oxygen; ▲, DCW; ○, gellan and □, reducing sugar).

bioreactor under pH control by 0.2 N sodium hydroxide. The pH of medium was maintained at around 6.8 during cultivation as shown in Fig. 2. Dissolved oxygen rapidly decreased with cell growth and production of gellan and increased gradually after cell growth ceased. The reducing sugars in the medium gradually decreased and production of gellan increased until 60 hr of culture. The concentration of reducing sugars was lower than that in a culture without pH control. Maximal production of gellan in a 7 l bioreactor under pH control was 8.42 g/l and the conversion rate of gellan from 2.0% (w/v) glucose was 42%.

The highest level of ammonium lactate was obtained when the pH of the medium was maintained at a constant 6.5 [3]. Production of human lysozyme by recombinant *S. cerevisiae* was increased by performing pH controlled fermentation, as compared to production with pH uncontrolled fermentation [6]. Production of astaxanthin by *Xanthophyllomyces dendrorhous* under pH control using 2.0 N sodium hydroxide was 24.1% higher than that without pH control [10]. Production of gellan under pH control in this study was found to be 14.6% higher than that without pH control.

Production of gellan under pH control by potassium phosphate

Gellan was produced by *S. paucibilis* NK2000 in a 7 l bioreactor under pH control by 2.0 N potassium phosphate. The pH of the medium, as that under pH control by sodium hydroxide, was maintained around 6.6 during cultivation. Decrease in dissolved oxygen and increase in the production of gellan in culture were also similar to that in which pH was controlled by sodium hydroxide as shown in Fig. 3. Maximal production of gellan in a 7 l bioreactor under pH control by potassium phosphate was 8.50 g/l. The conversion rate of gellan from 20 g/l glucose was 43%. Potassium phosphate is one of major salts in a medium for the production of microbial polysacchrides and enzymes [14,15,29] as well as a well-known ingredient in buffer solutions [20,26]. Production of gellan by *S. paucibilis* NK2000 under pH control by potassium phosphate as well as sodium hydroxide was higher than that without pH control.

The effect of potassium phosphate in the medium on production of gellan

The effect of potassium phosphate on cell growth and the production of gellan by *S. paucibilis* NK2000 was investigated. Initial concentration of potassium phosphate ranged from 0.0 g/l to 10.0 g/l and the initial pH of the medium was adjusted to 6.8. Production of gellan increased with increased concentration of potassium phosphate in the medium. Maximal production of gellan was 8.33 g/l when the concentration of potassium phosphate was 5.0 g/l as

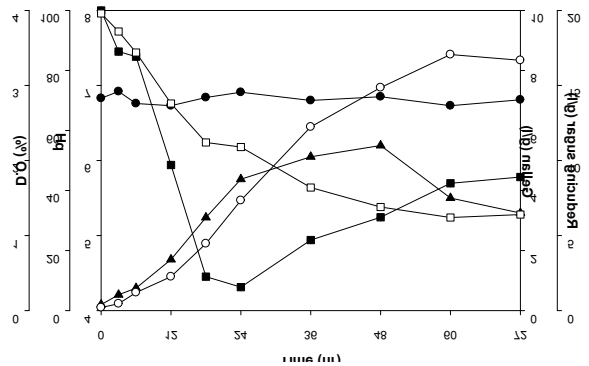

Fig. 2. Cell growth and production of gellan by *S. paucibilis* NK2000 with pH control by 2.0 N sodium hydroxide (●, pH; ■, dissolved oxygen; ▲, DCW; ○, gellan and □, reducing sugar).

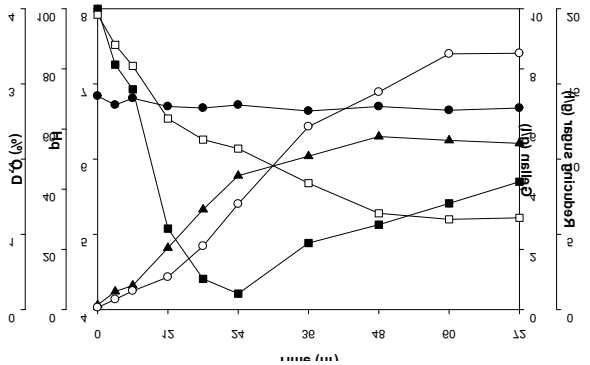

Fig. 3. Cell growth and production of gellan by *S. paucibilis* NK2000 with pH control by 2.0 N potassium phosphate (●, pH; ■, dissolved oxygen; ▲, DCW; ○, gellan and □, reducing sugar).

Table 2. The effect of postassium phosphate on cell growth and production of gellan by *S. paucibilis* NK2000

K_2HPO_4 (g/l)	Final pH	DCW (g/l)	Gellan (g/l)	Yp/s	Yx/s	Yp/x
0.0	3.9	2.12±0.10	4.33±0.32	0.22	0.11	2.04
0.5	4.2	3.23±0.13	7.21±0.46	0.36	0.16	2.23
1.0	4.4	3.46±0.28	7.47±0.41	0.37	0.17	2.16
1.5	4.6	3.21±0.25	7.60±0.55	0.38	0.16	2.36
2.0	4.8	3.05±0.24	7.87±0.48	0.39	0.15	2.58
3.0	5.1	2.85±0.19	8.03±0.51	0.40	0.14	2.82
4.0	5.3	2.67±0.18	8.17±0.55	0.41	0.13	3.06
5.0	5.6	2.43±0.15	8.33±0.46	0.42	0.12	3.43
7.5	6.2	2.23±0.21	7.22±0.45	0.36	0.11	3.23
10.0	6.8	1.82±0.17	5.94±0.38	0.30	0.09	3.26

shown in Table 2. The final pH of the medium containing 5.0 g/l potassium phosphate was 5.6. The 5 g/l potassium phosphate in the medium was not enough to maintain the pH of the medium for the production of gellan by *S. paucibilis* NK2000, but it resulted in improved production of gellan. The ranking of factors affecting production of compactin by *P. brevicompactum* in batch cultures using an orthogonal array method was glycerol> $MgSO_4$> glucose> $(NH_4)_2SO_4$> K_2HPO_4> maltose [30]. This suggested that potassium phosphate in a medium was more effective than maltose on the production of compactin. The potassium phosphate among all the different phosphorus sources was the most effective for production of β-cyclodextirn glucanotransferase by *Bacillus* sp. [10]. The quadratic effect of potassium phosphate ($p<0.0001$), which was more pronounced than the linear effect, showed that it significantly influenced the production of γ-hirudin by *S. cerevisiae* [27].

Production of gellan in a medium containing potassium phosphate

Cell growth and production of gellan by *S. paucibilis* NK2000 in a medium containing 5.0 g/l potassium phosphate without pH control in a 7 l bioreactor were shown in Fig. 4. The pH of the medium gradually decreased with culture time and reached around 5.6 after 72 hr as shown in Fig. 4. Dissolved oxygen rapidly decreased and reached 0 after 24 hr of cultivation, afterwards it gradually increased. The reducing sugars in the medium gradually decreased and reached around 4.2 g/l after 48 hr of cultivation. Maximal production of gellan in a 7 l bioreactor without pH control was 8.93 g/l and the conversion rate of gellan from 20 g/l glucose was 45%. Production of gellan in a medium containing 5.0 g/l potassium phosphate without pH control was higher than that under pH control by potassium phosphate as well as sodium hydroxide.

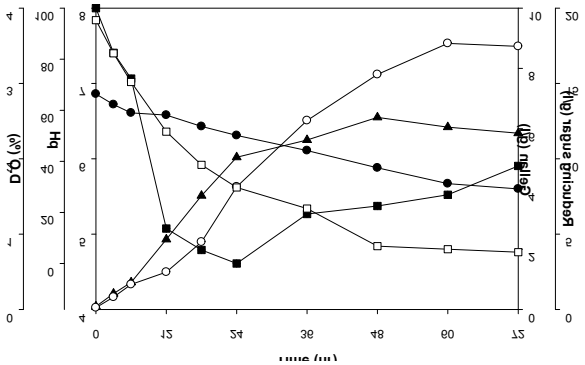

Fig. 4. Cell growth and production of gellan by *S. paucibilis* NK2000 cultured in a medium containing 5.0 g/l potassium phosphate as a pH stabililizer (●, pH; ■, dissolved oxygen; ▲, DCW; ○, gellan and □, reducing sugar).

Production of gellan by *S. paucibilis* NK2000 under pH control was higher than that without pH control, as in production of other microbial exopolysaccharides [14,16,31]. But the cost of equipment for pH control in a batch culture, as well as in a continuous culture, is expensive and there is a possibility of contamination in the pH control process [22]. In this study, a simple process without pH control to enhance production of gellan by *S. paucibilis* NK2000 was developed with optimized concentration of potassium phosphate in the medium. Production of gellan by this process was 21.5% higher than that without pH control.

Acknowledgment

This study was supported by research funds from Dong-A University, Busan, Korea.

References

1. Ahn, Y. H., J. H. Park, S. H. Go, and H. K. Jun. 2009. Characterization of bacterial cellulose production by

Gluconacetobater sp. JH232. *J. Life Science* **17**, 1582-1586.
2. Arockiasamy, S. and R. M. Banik. 2008. Optimization of gellan gum production by *Sphingmonas paucimobilis* ATCC 31461 with nonionic surfactants using central composite design. *J. Biosci. Bioeng.* **105**, 203-210.
3. Bai, D. M., Z. H. Yan, Q. Wei, X. M. Zhao, X. G. Li, and S. M. Xu. 2004. Ammonium lactate production by *Lactobacillus lactis* BNE5-18M in pH-controlled fed-batch fermentations. *Biochem. Eng. J.* **19**, 47-51.
4. Banik, R. M., A. Santhiagu, and S. N. Upadhyay. 2006. Optimization of nutrients for gellan gum production by *Sphingmonas paucimobilis* ATCC-31461 in molasses based medium using response surface methodology. *Bioresource Technol.* **98**, 792-797.
5. Cha, J. Y., S. H. Park, J. S. Heo, B. K. Park, J. W. Lee, J. W. Kim, and Y. S. Cho. 2008. Culture conditions for gulutathione maximum production by *Saccharomyces cerevisiae* FF-8 in bioreactor. *J. Life Science* **18**, 620-624.
6. Choi, S. U., H. D. Paik, S. C. Lee, T. Nihira, and Y. I. Hwang. 2004. Enhanced productivity of human lysozyme by pH-controlled batch fermentation of recombinant *Saccharomyces cerevisiase*. *J. Biosci. Bioeng.* **98**, 132-135.
7. Cokgor, E. U., S. Oktay, D. O. Tas, G. E. Zengin, and D. Orhon. 2009. Influence of pH and temperature on soluble substrate generation with primary sludge fermentation. *Bioresouce Technol.* **100**, 380-386.
8. Fang, Q. H. and J. J. Zhong. 2002. Effect of initial pH on production of ganoderic acid and polysaccharide by submerged fermentation of *Ganoderma lucidum*. *Process Biochem.* **37**, 769-774.
9. Harding, N. E., Y. N. Patel, and R. J. Coleman. 2004. Organization of genes required for gellan polysaccharide biosynthesis in *Sphingomonas elodea* ATCC 31461. *J. Ind. Microbiol. Biotechnol.* **31**, 70-82.
10. Hu, Z. C., Y. G. Zheng, Z. Wang, and Y. C. Shen. 2006. The pH control strategy in astaxanthin fermentation bioprocess by *Xanthophyllomtces dendrorhous*. *Enzyme Microb. Technol.* **39**, 586-590.
11. Ibrahim, H. M., W. M. W. Yusoff, A. A. Hamid, R. M. Illias, O. Hassan, and O. Omar. 2005. Optimization of medium for the production of β-cyclodextrin glucanotransferase using central composite design (CCD). *Process Biochem.* **40**, 753-758.
12. Jansson, R. E., B. Lindberg, and P. I. A. Sandford. 1983. Structural studies of gellan gum, an extracellular polysaccharide elaborated by *Pseudomonas elodea*. *Carbohydr. Res.* **124**, 135-223.
13. Jin, H., N. K. Lee, M. K. Shin, S. K. Kim, D. L. Kaplan, and J. W. Lee. 2003. Production of gellan gum by *Sphingomanas paucimobilis* NK200 with soybean pomace. *Biochem. Eng. J.* **16**, 357-360.
14. Jin, H. J. K. Yang, K. I. Jo, C. H. Chung, S. K. Kim, S. W. Nam, and J. W. Lee. 2006. Mass production of heteropolysaccharide-7 (PS-7) by *Beijerinckia indica* HS-2001 with soybean pomace as a nitrogen source. *Process Biochem.* **41**, 270-275.
15. Jo, K. I. Y. J. Lee, B. K. Kim, B. H. Lee, C. H. Chung, S. W. Nam, S. K. Kim, and J. W. Lee. 2008. Pilot-scale production of carboxymethylcellulase from rice hull by *Bacillus amyloliquefaciens* DL-3. *Biotechnol. Bioprocess Eng.* **13**, 182-188.
16. Jung, D. Y., Y. S. Cho, C. H. Chung, D. I. Jung, K. Kim, and J. W. Lee. 2001. Improved production of curdlan with concentrated cells of *Agrobacterium* sp. *Biotechnol. Bioprocess Eng.* **6**, 107-111.
17. Kang, K. S. and G. T. Veeder. 1982. Polysaccharide S-60 and bacterial fermentation process for its fermentation. US patent 4,326,053.
18. Kang, K. S., G. T. Veeder, P. J. Mirrasoul, T. K. Kaneko, and L. W. Cottrell. 1982. Agar-like polysaccharide produced by a *Pseudomonas* species: production and basic properties. *Appl. Environ. Mircobiol.* **43**, 1086-1091.
19. Lee, J. H., J. H. Kim, I. H. Zhu, X. B. Zhan, J. W. Lee, H. D. Shin, and S. K. Kim. 2001. Optimization of conditions for the production of pullulan and high molecular weight pullulan by *Aureobasidium pullulans*. *Biotechnol. Lett.* **23**, 817-820.
20. Lee, Y. J., B. K. Kim, B. H. Lee, K. I. Jo, N. K. Lee, C. H. Chung, Y. C. Lee, and J. W. Lee. 2008. Purification and characterization of cellulase produced *Bacillus amyloliquefaciens* DL-3 utilizing rice hull. *Bioresourec Technol.* **99**, 378-386.
21. Lim, S. M., J. R. Ru, J. W. Lee, and S. K. Kim. 2003. Optimization of culture condition for the gellan production by *Pseudomonas elodea* ATCC 31461. *J. Life Science* **13**, 705-711.
22. Liu, Y. C., F. S. Wang, and W. C. Lee. 2001. On-line monitoring and controlling system for fermentation process. *Biochem. Eng. J.* **7**, 17-25.
23. Martin, L. O., A. M. Fialho, P. L. Rodrigues, and I. Sa-Correia. 1996. Gellan gum production and activity of biosynthetic enzymes in *Sphingomonas paucimobilis* mucoid and non-mucoid variants. *Biotechnol. Appl. Biochem.* **24**, 47-54.
24. Miller, G. L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.* **31**, 426-428.
25. Nampoothiri, K. M. R. R. Singhania, C. Sabarinath, and A. Pandey. 2003. Fermentative production of gellan using *Sphingomonas paucimobilis*. *Process Biochem.* **38**, 1513-1519.
26. Parente, E., M. Moles, and A. Ricciardi, 1996. Leucocin F10, a bactericin from *Leucinostic carnosum*. *Int J. Food Microbiol.* **33**, 231-243.
27. Rao, K. J., C. H. Kim, and S. K. Rhee. 2000. Statistical optimization of medium for the production of recombinant hirudin from *Saccharomyces cerevisiae* using response surface methodology. *Process Biochem.* **35**, 639-647.
28. Sá-Correia, I., A. M. Fialho, P. Videria, L. M. Moreira, A. R. Marques, and H. Albano. 2002. Gellan gum biosynthesis in *Sphingomonas paucimobilis* ATCC 31461: genes, enzymes and exopolysaccharide production engineering. *J. Ind. Microbiol. Biotechnol.* **29**, 170-176.
29. Seo, H. P., C. H. Chung, S. K. Kim, R. A. Gross, D. L. Kapaln, and J. W. Lee. 2004. Mass production of pullulan with optimized concentrations of carbon and nitrogen sources by *Aureobasidium pullulans* HP-2001 in 100 l bioreactor

with the inner pressure. *J. Microbiol. Bitechnol.* **14**, 237-242.
30. Shakigram, N. S., S. K. Singh, R. S. Singhal, G. Szakacs, and A. Pandey. 2008. Compactin production in solid-state fermentation using orthogonal array method by *P. brevicompactum*. *Biochem. Eng. J.* **41**, 295-300.
31. Shu, C. H. and M. Y. Lung. 2004. Effect of pH on the production and molecular weight distribution of exopolysaccharide by *Antriodia camphorata* in batch cultures. *Process Biochem.* **39**, 931-937.

초록 : *Sphingomonas paucibilis* NK-2000에 의한 젤란의 생산에 미치는 pH 안정제로서의 인산칼슘의 영향

이남규 · 조영배 · 진일혁[1] · 손창우[2] · 이진우[3]*
(해양생물산업육성센터, [1]진바이오사이언스, [2]한국바이오솔루션, [3]동아대학교 생명공학과)

포도당 및 간장박의 농도가 각각 20 g/l 및 10 g/인 배지에서 *Sphingomnas paucibilis* NK2000가 생산하는 젤란의 최대 생산성은 배지의 초기 pH를 6.8로 하였을 경우, 플라스크 규모에서 7.46 g/l이었으며, 7 l 생물배양기에서는 7.35 g/l이었다. 배지의 pH를 6.8로 유지하면서 7 l 생물배양기에서 젤란을 생산할 때, 젤란의 최대 생산성은 pH 조절제로 수산화나트륨을 사용하였을 경우에 8.42 g/l이었으며, 인산칼슘을 사용하였을 경우에 8.50 g/l이었다. *Sphingomnas paucibilis* NK2000를 배양하여 젤란을 생산할 경우에 배지에 첨가되는 인산칼슘의 최적 농도는 5.0 g/l이었다. 인산칼슘의 농도가 5.0 g/l인 배지를 사용하여 7 l 생물배양기에서 젤란을 생산하였을 때, 젤란의 최대 생산성은 8.93g/l이었다. 본 연구를 통하여 배지의 pH를 조절하지 않고 인산칼슘의 농도를 최적화한 배지를 사용하여 젤란의 생산성을 향상시킬 수 있는 경제적인 방법을 개발하였다.