J. Korean Agric. Chem. Soc.
한국농화학회지 32(3), 309—314(1989)

Cultural Condition of *Enterobacter agglomerans* U-1 for Polysaccharide Production

Jin-Young Yoo, Young-Jo Koo, Dong-Hwa Shin* and Dong-Hyo Chung**

Microbiology Laboratory, Korea Food Research Institute, Hwasung 445-820, Korea
*Department of Food Science and Technology, Chonbuk National University, Chonju 560-756, Korea
**Department of Food Science and Technology, Chungang University, Seoul 156-756, Korea

Enterobacter agglomerans U-1의 다당류 생산을 위한 배양조건

유진영 · 구영조 · 신동화* · 정동효**

한국식품개발연구원 미생물연구실, *전북대학교 식품가공학과, **중앙대학교 식품가공학과

초 록

점질성 물질을 생산하는 세균인 *Enterobacter agglomerans*의 다당류 생산조건을 검토하였다. 최적 조건은 배지로서 sucrose(23.75g/L), peptone(2.06g/L), yeast extract(0.5g/L), KH_2PO_4 (1.0g/L) 및 $MgSO_4 \cdot 7H_2O$(1.0g/L)를 함유하며 배양 pH와 온도는 6.5와 30°C이었다. 이 조건에서 3일 배양후 8.5g/L의 다당류가 생산되며 점도는 240 mPa.s.를 나타내었다. 이때의 다당류 생산수율 및 다당류 생산 속도는 각각 36%와 142.07 mg/g/h이었다.

Introduction

Polysaccharides are very important stuffs in industry because of their valuable properties that they thicken, suspend or stabilize aqueous systems and sometimes produce gel or act as emulsifiers, flocculants, binders, film formers, lubricants and friction reducers[1,2]. These polysaccharides, traditionally, have been produced from algae and plants[2], but recently, some are derived from microbial sources[3~7], i.e., dextran and xanthan, for example.

In searching new microbial polysaccharide producers of potentiality, several bacteria were screened as candidates. One of those strains was found to produce viscous and somewhat sticky polysaccharides[8]. The strain was identified as *Enterobacter agglomerans* and the polymer was β-glucan composed of glucose and galactose. In this paper, we report on the cultural condition of *E. agglomerans* for polysaccharide production.

1988년 4월 11일 수리
Corresponding author : J.Y. Yoo

Materials and Methods

Microorganism used in this study was *E. agglomerans* U-1[8]. Culture condition and apparatus was as used in the previous paper[9]. Polysaccharide recovery and analysis were as reported by Yoo et al.[9]. Viscometry was conducted by Brabender viscotron viscometer(Model 80241, System E-17, West Germany).

Results and Discussion

1. Cultivation temperature

Flask cultures of the microorganism were undertaken to find the optimum temperature for polysaccharide production by *E. agglomerans* U-1(Table 1). The growth at 40°C was negligible and also polysaccharide could not be detected by isopropanol precipitation. The polysaccharide was synthesized more at the lower temperature. This is similar to the case of *Aerobacter aerogenes*[10]. However, Goto et al.[11] reported that *Pseudomonas aeruginosa* produced more slime at high temperature. The polysaccharide production at 25, 30 and 35°C were 6.16, 6.56 and 5.09 g/L. The optimum temperature was concluded as 30°C, at which 26.24% of glucose was converted to polysaccharide. The culture broth was the most viscous at the optimum cultivation temperature. The apparent viscosities of culture broths grown at 25, 30, 35°C were 56, 134 and 117 mPa.s. at 70sec^{-1}.

Table 1. Effect of temperature on the polysaccharide production by *Enterobacter agglomerans* U-1 after 72 hours of incubation

Temperature [°C]	Polymer [g/L]	Yield [%]	η_{app} [mPa.s.]	pH
25	6.16	24.64	56	3.4
30	6.56	26.24	134	6.6
35	5.09	20.36	117	7.5
40	—	—	—	—

Viscosity was measured at 70sec^{-1}.
Medium : Glucose 25g/L, peptone 2.06g/L, KH_2PO_4 1g/L, $MgSO_4 \cdot 7H_2O$ 1g/L, yeast extract 0.5g/L and $CaCO_3$ 2.5g/L.
η_{app} : Apparent viscosity.
— : Means not detected or not tested.

2. Effect of carbon source

Sugar sources are known to affect the formation of polysaccharide for some microorganisms[12~14]. Table 2 shows the effect of sugar sources on the polysaccharide production by *Enterobacter agglomerans* U-1. Maltose and sucrose were found to be proper carbon sources for the polysaccharide synthesis. The polysaccharide productions were 8.51 and 8.49 g/L. Over 35% of sugars added were converted to polysaccharide on the media. Glucose and fructose were less satisfactory and lactose was worse carbon source. The growth and polysaccharide formation was not observed in xylose medium. The culture broths were fairly viscous when using glucose, maltose and sucrose as substrate. The viscosities were 100, 267 and 240 mPa.s. at 70sec^{-1} on the respective medium. They seemed to be somewhat related with the amount of recovable polysaccharide in the culture broth. Normally, sucrose[15] and glucose[12,13] were recommended for polysaccharide production.

Table 2. Effect of sugar source on the polysaccharide production by *Enterobacter agglomerans* U-1 at 30°C after 72 hours of incubation

Sugar source	Polymer [g/L]	Yield [%]	η_{app} [mPa.s.]	pH
Glucose	6.56	26.24	100	6.5
Lactose	3.83	16.13	45	6.9
Maltose	8.51	35.83	268	6.5
Sucrose	8.49	35.75	240	7.0
Fructose	6.80	27.20	95	6.5
Xylose	—	—	—	—

Basal medium : Peptone 2.06g/L, KH_2PO_4 1g/L, $MgSO_4 \cdot 7H_2O$ 1g/L, yeast extract 0.5g/L and $CaCO_3$ 2.5g/L.
Sugar source : Glucose, fructose, xylose ; 25g/L, Sucrose, lactose, maltose ; 23.75g/L.

3. Effect of sucrose concentration

Concentration of carbon source is important because the polysaccharide such as xanthan[16] and alginate[17] are produced under carbon-limited culture whereas *Pseudomonas* sp. does not produce[18] at the condition. Therefore, optimum sucrose concentration for the polysaccharide production by *Enterobacter agglomerans* U-1 was examined (Table 3). The higher initial level of sucrose concentration at below 23.75 g/L raised the polysaccharide production. However the product yield was high when the initial sucrose concentration was lower, i.e. 69.80% of $Y_{p/s}$ at 10 g/L of concentration. The increasing trend in viscosities of culture broths was

Table 3. Effect of sucrose concentration on the polysaccharide production by *Enterobacter agglomerans* U-1 at 30°C after 72 hours of incubation

Concentration [g/L]	Polymer [g/L]	Yield [%]	η_{app} [mPa.s.]
10	6.98	69.8	73
15	7.51	50.1	78
20	7.54	37.7	100
23.75	8.31	35.0	95
30	7.58	25.3	112
40	5.78	14.5	28

Basal medium : KH_2PO_4 1g/L, $MgSO_4 \cdot 7H_2O$ 1g/L, yeast extract 0.5g/L and $CaCO_3$ 2.5g/L.
Nitrogen source : Peptone, C/N ratio 30.

observed with the increased sucrose concentration up to 30 g/L above which the culture broth became drastically thin. Therefore, it was concluded that the optimum sucrose concentration was 23.75 g/L.

4. Effect of nitrogen source

For the production of polysaccharide, organic and inorganic nitrogen source can be used[9,12,13]. Table 4 shows the result in glucose media when using different nitrogen sources. It was surprising that *Enterobacter agglomerans* U-1 grew very well on the media containing various nitrogen sources. Especially, the dry cell weight of culture broth grown in ammonium sulfate medium was tremendously high(4.32g/L). Urea, ammonium nitrate and peptone also promoted the growth. The optimum nitrogen sources for U-1 polysaccharide production were peptone and ammonium chloride. The polysaccharide productions at the respective nitrogen source were 6.4 and 5.67g/L which were amount to 25.60 and 22.68% of product yield. The Q_p at the respective nitrogen source were 42.94 and 60.12 mg/g/h. The other nitrogen sources were found to be less satisfactory. The viscosities of the culture broths were dependent upon the nitrogen sources used and were 318, 184 and 122 mPa.s. at $70sec^{-1}$, when using ammonium chloride, ammonium phosphate dibasic and peptone.

5. Effect of C/N ratio

Table 5 shows the effect of C/N ratio on the polysaccharide production in glucose medium. The optimum C/N ratio was found to be 30, below and above which the polysaccharide production decreased. The amount of polysaccharide formed at the C/N ratio of 10, 30, 50, 100 and 200 were 2.86, 6.56, 5.81, 3.06 and 2.21 g/L.

6. Effect of KH_2PO_4 concentration

Potassium and phosphorus are important minerals involving the cell structure[19,20]. The effect of KH_2PO_4 concentration on the growth and polysaccharide production in sucrose medium was examined(Table 6). The dry cell we-

Table 4. Effect of nitrogen source on the polysaccharide production by *Enterbacter agglomerans* U-1 at 30°C after 72 hours of incubation

Nitrogen source	DCW [g/L]	Polymer [g/L]	Yield [%]	Qp [mg/g/h]	η_{app} [mPa.s.]
NH_4Cl	1.31	5.67	22.68	60.12	318
Urea	1.83	2.57	10.28	19.51	25
$(NH_4)_2SO_4$	4.32	4.50	18.00	14.47	95
$(NH_4)_2HPO_4$	1.95	1.18	4.72	8.40	184
NH_4NO_3	2.19	3.96	15.84	25.11	11
Peptone	2.07	6.40	25.60	42.94	122

Basal medium: Glucose 25g/L, yeast extract 0.5g/L, $MgSO_4 \cdot 7H_2O$ 1g/L, KH_2PO_4 1g/L, $CaCO_3$ 2.5g/L and C/N ratio 30.
DCW : Dry cell weight.
Q_p : Specific productivity.

Table 5. Effect of C/N ratio on the polysaccharide production by *Enterobacter agglomerans* U-1 at 30°C after 72 hours of incubation

C/N ratio	5	10	30	50	100	200	300
Polymer[g/L]	1.80	2.86	6.56	5.81	3.06	2.21	2.48
Yield[%]	7.20	11.44	26.24	23.24	12.24	8.84	9.92
pH	6. 8	6. 6	6. 4	6. 4	6. 5	6. 5	6. 6

Basal medium : Glucose 25g/L, KH_2PO_4 1g/L, $MgSO_4 \cdot 7H_2O$ 1g/L, yeast extract 0.5g/L and $CaCO_3$ 2.5g/L. Nitrogen source : Peptone.

Table 6. Effect of KH_2PO_4 concentration on the polysaccharide production by *Enterobacter agglomerans* U-1 at 30°C after 72hours of incubation

Concentration [g/L]	DCW [g/L]	Polymer [g/L]	Yield [%]	Qp [mg/g/h]	η_{app} [mPa.s.]
1	0.83	8.49	35.67	142.07	136
2	0.94	8.81	35.75	130. 1	215
3	1.02	8.40	35.30	114.38	179
4	1.43	8.40	35.30	81.58	146
5	1.29	8.32	34.98	89.58	231

Basal medium : Sucrose 23.75g/L, peptone 2.06g/L, yeast extract 0.5g/L, $MgSO_4 \cdot 7H_2O$ 1g/L and $CaCO_3$ 2.5g/L.

ight of culture broth rose up with the increase in the KH_2PO_4 concentration up to 4 g/L and then slightly decreased at the higher concentration, whereas polysaccharide production was not so much affected. The amount of polysaccharide formed and yield were more than 8.4g/L and 35% respectively. Therefore, the Qp was higher when the KH_2PO_4 concentration was low since small amount of cell was produced. It was not necessary to add more than 1 g/L of KH_2PO_4. The viscosity of culture broth increased as the concentration became higher. The Qp at 1 g/L KH_2PO_4 concentration was 142.07 mg/g/h.

7. Fermentation profile

Table 7 shows the time course of U-1 polysaccharide production. The non-fibrous isopropanol precipitate of polysaccharide which might be low in degree of polymerization emerged

Table 7. Time course of polysaccharide production by *Enterobacter agglomerans* U-1 at 30°C

Elapsed fermentation time [hrs]	Polymer [g/L]	Glucose [g/L]	K [mPa.s.]	n
16	—	19.1	0.12	1.60
22	—	17.9	1.15	1.35
26.5	—	10.8	32.02	0.85
40	7.17	3.7	108.00	0.69
48	6.54	—	55.00	0.78
64	6.33	—	43.00	0.78
72	6.30	—	36.00	0.81

Agitation : 400rpm, air : 1vvm and working volume : 1.2L.
Medium : Glucose 25g/L, peptone 2.06g/L, yeast extract 0.5g/L, $MgSO_4 \cdot 7H_2O$ 1g/L, K_2PO_4 1g/L, $CaCO_3$ 2.5g/L.
n : Flow behaviour index.
K : Consistency coefficient.

after 26.5hours of elapsed fermentation time. However, copious amount of fibrous polysaccharide could be recovered after 40 hours. The polysaccharide concentration in the culture broth then slightly decreased by subjecting to the prolonged fermentation time. The consistency index (K-value) of fermentation broth showed maximum at 40hours and then also decreased drastically with the prolonged fermentation time. The culture broth behaved like dilatant system at the earlier stage of fermentation. The flow behaviour index (n-value) decreased slowly with the elapsed time and then again increased after 40hours, losing pseudoplasticity. This kind of change could be caused by exposure to prolonged shear stress or by the possible synthesis of degrading enzyme[20,21]. The maximum production was 7.17 g/L.

Abstract

The cultural condition of *Enterobacter agglomerans* U-1, a polysaccharide producing soil bacterium, was examined. The optimal medium composition was that contains the following components per liter of distilled water; sucrose (23.75 g/L), peptone(2.06 g/L), yeast extract (0.5 g/L), KH_2PO_4(1.0 g/L) and $MgSO_4 \cdot 7H_2O$(1.0 g/L). The optimum temperature for polysaccharide production was 30°C, where 8.5 g/L of polysaccharide was produced. The apparent viscosity of fermentation broth after 3 days was 240 mPa.s. at 70sec^{-1}. The product yield and specific productivity were 36% and 142.07 mg/g/h.

References

1. Glicksman, M.: in "Polysaccharides in Food (Blanshard, J.M. V., J.R. Mitchell, ed.), Butterworth, p.185(1978)
2. Glicksman, M.: in "Food Hydrocolloids(Glicksman, M., ed.), CRC Press, 1 : 3(1982)
3. Cadmus, M.C., Knutson, C.A., Lagoda, A.A. Pittsley, J.E. and Burton, K.A.: Biotechnol. Bioeng., 20 : 1003(1978)
4. Kang, K.S., Veeder, G.T., Mirrasoul, P., Kaneko, J.T. and Cottrell, I.W.: Appl. Environ. Microbiol., 43(5) : 1086(1982)
5. Harada, T. and Yoshimura, T.: Agric. Biol. Chem., 29(11) : 1027(1965)
6. Jeanes, A.: in "Methods in Carbohydrate Chemistry" (Whistler, R.L., BeMiller, J.N. and Wolfrom, M.L. ed.), Academic Press, 5 : 118(1965)
7. Kang, K.S. and Kovacs, P.: Proc. IV Int. Conv. Fd. Sci. Technol., 1 : 518(1974)
8. Yoo, J.Y. and D. H. Chung: J. Korean Agric. Chem. Soc., 32 : 303(1989)
9. Yoo, J.Y., Koo, Y.J., Shin, D.H. and Chung, D.H.: Kor. J. Microbiol. Bioeng., 16 (2) : 98(1988)
10. Duguid, J.P. and Wilkinson, J.F.: J. Gen. Microbiol., 9 : 174(1953)
11. Goto, S., Enemoto, S., Takahashi, Y. and Motomatsu, R.: Jap. J. Microbiol., 15 : 317 (1941)
12. Sakar, J.M., Hennebert, G.L. and Mayaudon, J.: Biotechnol. Letters, 7(9) : 631(1985)
13. Michel, M., Seviour, R.J. and Pethica, L. M.: Biotechnol. Letters, 9(10) : 741(1987)
14. Linton, J.D., Gouldney, D. and Woodard, S.: J. Gen Microbiol., 134 : 1913(1988)
15. Ueda, S., Momii, F., Osajima, K. and Ito, K.: Agric. Biol. Chem., 49(9) : 1977(1981)
16. Ellwood, D.C., Righelato, R.C. and Smith, C.E.: Brit. Pat. 2008138(1978)
17. Jarman, T.R.: in "Microbial Polysaccharides and Polysaccharides" (Berkeley, R. C.W., Gooday, G.W. and Ellwood, D.C. ed.), Academic Press, p. 35(1979)
18. Williams, A.G. and J.W.T. Wimpenny: J. Gen. Microbiol., 104 : 47(1978)
19. Evans, C.G.T., Yeo, R.G. and Ellwood, D.C.: in "Microbial Polysaccharides and Polysaccharase"(Berkeley, R.C.W., Gooday, G.W. and Ellwood, D.C. ed.), Academic Press, p. 51(1979)
20. Lawford, H.G., Keenan, J., Phillips, K. and Orts, W.: Biotechnol. Letters, 8(3) :

145(1986)
21. Sutherland, I.W. and Ellwood, D.C.: in "Microbial Technology" (Bull, A.T., Ellwood, D.C. and Ratledge, C. ed.), Cambridge University Press, SGM Symposium Series, 29 : 107(1979)