P215

Production of curdlan with agro-industrial byproduct by *Agrobacterium* sp. ATCC 31749

정대영, 김현숙, 서형필, 이남규, 김지모 이진우*
Faculty of Natural Resources and Life Science, College of Natural Resources and Life Science, Dong-A University. Pusan, 604-714, Korea.* Tel (051) 200-6995

Abstract

Effect of carbon sources including agro-industrial byproduct on cell growth and production of curdlan by *Agrobacterium* sp. ATCC 31749 was investigated. Maximal production of curdlan was obtained when the carbon source was sucrose. The conversion rate of curdlan from 2% (w/v) sucrose was 59%. Glucose, mannose and maltose were also found to be good carbon sources for production of curdlan. Production of curdlan increased up to 3% (w/v) glucose as the carbon source and then decrease as the concentration of glucose increased. The major components of agro-industrial byproduct (AIB) were glucose, maltose, and maltose, and maltotriose. *Agrobacterium* sp. ATCC 31749 utilized up to 25% (v/v) AIB and produced curdlan with 29.8g/l.

Introduction

Agrobacterium sp. ATCC 31749 produced three different types of exopolymers. Major product is curdlan, which is extracellular, unbranched homo-β-(1-3)-glucan, and minor ones are the water-soluble non curdlan type exopolymer-A and B (WSNCE-A and B)[3,4]. Curdlan is a water-insoluble polysaccharide and can be used in food such as jelly-like foods, dietary fibers and films[7]. Curdlan production is associated with the poststationary phase of a nitrogen depleted aerobic batch culture[11]. Curdlan yield from glucose is usually 50% if the cultivation pH is maintained at 6[11]. The purpose of this study was to study on production of curdlan by *Agrobacterium* sp. with agro-industrial byproduct.

Materials and methods

Microorganism and medium *Agrobacterium* sp. ATCC 31749 was grown and maintained on plates containing 2% glucose, 0.5% yeast extract, 1.0% calcium carbonate, and 1.5% agar[3].

Growth medium The mineral salt medium (MSM) was used for the production of exopolymers. The MSM contained the following components (g/l): KH_2PO_4, 1.74; K_2HPO_4, 0.49; $Na_2SO_4 \cdot 10H_2O$, 3.7; $MgCl_2 \cdot 6H_2O$, 0.25; $FeCl_3 \cdot 6H_2O$, 0.024; $CaCl_2 \cdot 2H_2O$, 0.015; $MnCl_2 \cdot 4H_2O$, 0.01; citrate, 0.21; NH_4Cl, 2.1; and 2% (w/v) carbon source[7]. Carbon source autoclaved separately for 20 min at 120°C and added to media under aseptic conditions.

Production of exopolymers Main cultures were incubated for 5 days with inoculation of high density cell and nitrogen limitation[6)] The culture after 5 days was centrifuged at 12,000 x g for 30 min. The pellet was added to an equivalent volume of 0.5 N sodium hydroxide at 3°C, the mixture was stirred for 10 min, and then left to stand for 3 hr at the same temperature. The resulting viscous solution was centrifuged at 12,000 x g for 40 min, and the supernatant was neutralized with 10% acetic acid, repeatedly washed with water, acetone, and ether.

Analytical methods Dry cells weight (DCW) was determined by direct weighting of the cell fraction after drying to constant weight at 100 - 105°C. The yield of the curdlan was determined by the same procedure. Gas chromatographic analysis after methanolysis of the polysaccharides and subsequent trimethylsilylation was used to determine the composition of carbohydrates in the exopolymers[1)]. Preparation of samples for GC analyses was carried out as was described previously.[9)]

Results and discussions

Effect of carbon source To establish the optimal carbon source for the production of curdlan, *Agrobacterium* sp. ATCC 31749 was cultivated in a medium containing various carbon sources. Maximal production of curdlan was obtained when carbon source is 2% (w/v) glucose. The conversion rate of curdlan from 2% sucrose was 59%. Glucose, maltose, and mannose were also found to be good carbon sources for production of curdlan (Table 1).

(Table 1) Effect of carbon sources on production of curdlan by *Agrobacterium* sp.

Carbon[1)] source	Final status				
	pH	O.D	DCW(g/l)	Curdlan(g/l)	Yield(Yp/s)
Glucose	6.36	18.8	1.98	10.9	0.55
Mannose	6.38	16.2	1.75	9.4	0.47
Sucrose	6.30	24.1	1.98	11.8	0.59
Maltose	6.26	15.1	2.23	10.0	0.50
Dextrin	6.56	6.0	1.73	1.4	0.07

1) The concentration of the carbon source was 2% (w/v).

Effect of glucose concentration The effect of glucose on cell growth and production of curdlan was investigated (Table 2). The production of curdlan increased up tp a glucose concentration of 3%, and then decreased as the concentration of glucose increased. It seems that production of curdlan by *Agrobacterium* sp. ATCC 31749 si affected by the catabolite repression.

(**Table 2**) Effect of glucose on production of curdlan by *Agrobacterium* sp.

Glucose (%;w/v)	Final status				
	pH	OD	DCW(g/l)	Curdlan(g/l)	Yield(Yp/s)
0.5	6.63	10.7	1.52	2.7	0.55
1.0	6.58	11.6	1.62	4.3	0.43
1.5	6.54	13.2	1.51	6.9	0.46
2.0	6.45	16.9	1.63	10.5	0.52
3.0	6.33	15.4	2.13	15.2	0.51
4.0	6.22	13.9	2.84	14.5	0.36
5.0	6.11	14.0	2.26	13.4	0.27
7.5	5.89	13.8	2.25	14.2	0.19
10.0	5.75	8.4	1.93	14.4	0.14

The composition of agro-industrial byproduct (AIB). The composition of AIB was determined by HPLC. The AIB contained the following components (%): glucose, 6; maltose, 25; maltotriose, 25; maltotetraose, 9; maltopentaose, 12; dextrin, 24; and else 0.6. AIB was used for the carbon source for the production of curdlan because it has a relatively high amount of glucose and maltose which was found to be a good carbon source.

The effect of agro-industrial byproduct (AIB) concentration. The effect of AIB on production of curdlan was investigated (Table 3). Maximal production of curdlan was obtained when carbon source is 5% AIB. The conversion rate of curdlan from 2% AIB was 22%. As AIB concentration increased to 25%(w/v), production of curdlan was increased to 29.8g/l.

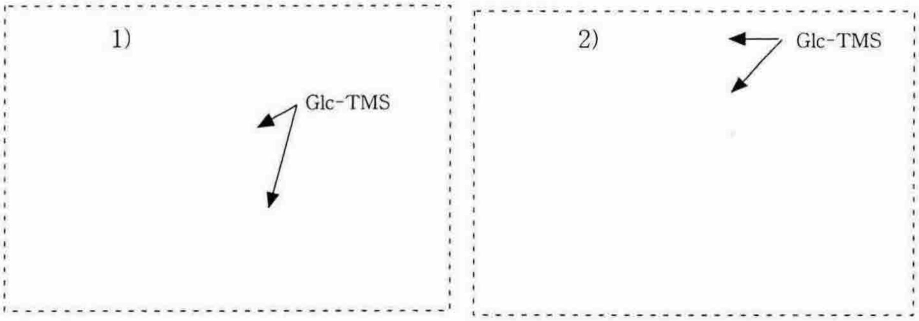
(**Table 3**) The effect of AIB concentration on curdlan production by *Agrobacterium* sp.

AIB (%;w/v)	Final status				
	pH	OD	DCW(g/l)	Curdlan(g/l)	Yield(Yp/s)
2	6.58	13.3	2.23	4.5	0.22
5	6.51	17.6	2.83	10.9	0.22
10	6.39	20.7	2.78	19.1	0.19
15	6.32	26.2	2.93	22.5	0.15
20	6.28	24.7	3.08	24.0	0.12
25	6.28	31.4	3.48	29.8	0.12
30	6.23	34.6	3.50	26.9	0.09

GC analysis A gas chromatogram of curdlan purified from culture grown in MSM with AIB showed that the major components were glucose identified by the peak retention times and the relative peak area for the corresponding α and β anomers. It showed exopolymer produced by *Agrobacterium* sp. ATCC 31749 with AIB consisted of glucose such as curdlan (Figure 1).

(**Figure 1**) Gas chromatogram of trimethylated (TMS) sugar components of curdlan purified from cultures grown in MSM with AIB as carbon source.

2) Gas chromatogram of trimethylated (TMS) glucose. Glucose is Glu.



Reference

1. Chaplin M., "A rapid and sensitive for the analysis of carbohydrate components in glycoproteins using gas-liquid chromatography", (1982), *Anal. Biochem.*, 123, 336-341.

2. Harada T., "Succinoglucan 10C3: a new acidic polysaccharide of *Alcaligenes faecalis* var. *myxogenes*", (1965), *Arch. Biochem. Biophy.*, 112, 65-69.

3. Harada T., Masada M., Fujimori K. and Maeda M., "Production of firm, resilient gel-forming polyscchraide by a mutant of *Alcaligenes faecalis* var. myxogenes 10C3", (1966), *Agr. Biol. Chem.*, 30, 196-198.

4. Harada T., Masada M., Hidaka H., and Takada M., "Production of film, resilientgel-forming polyscchraide in natural medium by a mutant of *Alcaligenes faecalis* var. *myxogenes* 10C3" (1966), *J. Ferment. Technol.*, 44, 20-24.

5. Harada T., Misaki A. and Saito H., "Curdlan: a bacterial gel-forming β-1,3-glucan" (1968), *Arch. Biochem.*, 124, 292-298.

6. Jung D. Y., Jo Y. S., Jung H. J. and Lee J. W., "Improved Production of Curdlan with Concentrated Cells of *Agrobacterium* sp. ATCC 31749" (in process)

7. Kimura H., Sato S., Nakagawa T., Nakatani H., Matsukura A., Suzuki T., Asai T, Kanamaru M., Shibata S. and Yamamoto S., "New thermo-gelable polysaccharide-contaning foodstuffs" (1973), *U.S Pat.* 3,754,925.

8. Lawford H. G. and Pillips K. R., "A two stage continuous process for the production of themogellable curdlan-type exopolysaccharide" (1982), *Biotechnol. Lett.*, 4, 689-694.

9. Lee J. W., Yeomans W. G., Allen A. L., Kaplan D. L., Deng F., and Gross, R. A., "Exopolymers from curdlan Production: incorporation of glucose-related sugar by *Agrobacterium sp.* strain ATCC 31749." (1997), *Can. J. Microbiol.*, 43, 149-156.

10. Lee J. W., Yeomans W. G., Allen A. L., Gross R. A., and Kaplan D. L., "Microbial production of water-soluble non curdlan type exopolymer-B with controlled composition by *Agrobacterium sp.*" (1997), *Biotechnol. Letters*, 19, 75-80.

11. Pillips K. R., Lawford H. G., Lavers B., Kligerman A., and Lawford G. R.,"Production of curdlan-type polysaccharide by *Alcaligenes faecalis* in batch and continuous culture" (1983), *Can. J. Microbiol.*, 29, 1331-1338.