Research Journal of Microbiology

ISSN 1816-4935

Academic Journals Inc.

www.academicjournals.com

Research Journal of Microbiology, 4 (5): 202-207, 2009
ISSN 1816-4935

Effect of Yeast Extract Supplementation on Curdlan Production from Condensed Corn Distillers Solubles

Thomas P. West
Department of Biology and Microbiology,
South Dakota State University, Brookings, SD 57007, USA

Abstract: The effect of yeast extract supplementation on bacterial curdlan production using a medium containing corn syrup and the corn-based ethanol coproduct condensed corn distillers solubles was determined. Curdlan was produced by *Agrobacterium* sp. ATCC 31749 on a medium containing selected solubles concentrations as a source of nitrogen and corn syrup as a carbon source. The presence of yeast extract in the medium was found to enhance bacterial curdlan production at all three concentrations of solubles tested after 120 h of growth. Bacterial biomass production was also noted to be higher after 120 h when the cells were supplemented with yeast extract. It was concluded that the observed increase in curdlan production by the yeast extract-supplemented ATCC 31749 cells was due to the yeast extract stimulating biomass formation.

Key words: Polysaccharide, biomass, corn syrup, ethanol coproduct, *Agrobacterium* sp.

INTRODUCTION

The high molecular weight polysaccharide curdlan is an extracellular, non-toxic polysaccharide synthesized by the species *Agrobacterium* (Harada *et al*., 1968; Phillips and Lawford, 1983a; Spicer *et al*., 1999). With respect to structure, the unbranched polysaccharide is composed of approximately 135 glucosyl residues linked by β-D-(1→3) bonds (Harada *et al*., 1968). Curdlan is alkali-soluble but insoluble in water or acidic solutions (Harada *et al*., 1968). The polysaccharide has a number of applications including food and pharmaceutical uses (Nakao *et al*., 1991; Kanke *et al*., 1992). The polysaccharide can be synthesized on a number of carbon sources including glucose, a high maltose corn syrup, maltose and sucrose (Phillips and Lawford, 1983b; Lee *et al*., 1997; Saudagar and Singhal, 2004; Portilho *et al*., 2006; West, 2006; West and Nemmers, 2008).

During dry milling production of ethanol from corn, one of the major coproducts produced is condensed corn distillers solubles (Rausch and Belyea, 2006). At present, the solubles is mixed with the corn distillers grains to produce corn distillers grains with solubles that is used as a protein supplement in animal feeds (Ham *et al*., 1994). The low value condensed corn distillers solubles could be better utilized to produce a high value polysaccharide gum. It has been shown that curdlan can be produced from the ethanol processing coproduct condensed corn distillers solubles (West and Nemmers, 2008). Unfortunately, relatively low curdlan concentrations were produced by *Agrobacterium* sp. ATCC 31749 when the strain was grown on a solubles-containing medium (West and Nemmers, 2008). A possible way to stimulate curdlan production is by the addition of a growth supplement such as yeast extract to the culture medium. Prior studies showed that the supplementation of yeast extract to the culture medium was capable of stimulating production of the bacterial gum gellan (West and Fullenkamp, 2000; Bajaj *et al*., 2006) or xanthan (Lo *et al*., 1997). Therefore, it was of interest to learn whether the addition of yeast extract to the medium could stimulate curdlan production by *Agrobacterium* sp. ATCC 31749. In this investigation, the influence of yeast extract supplementation on curdlan production by the bacterial strain was examined using a medium containing corn syrup and 50, 100 and 200 g L^{-1} condensed corn distillers solubles.

MATERIALS AND METHODS

Strain and Medium

The known curdlan-producing strain, namely *Agrobacterium* sp. ATCC 31749, was utilized in this study (Phillips and Lawford, 1983a). The strain was grown in a minimal medium (pH 6.8) consisting of 1.74 g L^{-1} KH_2PO_4, 0.49 g L^{-1} K_2HPO_4, 3.70 g L^{-1} Na_2SO_4•2H_2O, 0.25 g L^{-1} $MgCl_2$•$6H_2O$, 0.024 g L^{-1} $FeCl_3$•$6H_2O$, 0.015 g L^{-1} $CaCl_2$•$2H_2O$, 0.010 g L^{-1} $MnCl_2$•$4H_2O$, 0.21 g L^{-1} sodium citrate•$2H_2O$ and filtered condensed corn distillers solubles (West and Nemmers, 2008). The source of the condensed corn distillers solubles was Dakota Ethanol, LLC (Wentworth, SD USA) and the concentrations of solubles used in the medium was 50 g L^{-1}, 100 g L^{-1} or 200 g L^{-1}. Higher concentrations of solubles could not be tested due to precipitate formation in the medium. Following autoclaving, 3% (v/v) corn syrup was added to the medium. When supplemented in the medium, 0.5% (w/v) yeast extract was added. The project was conducted from January 2005 to June 2006 at South Dakota State University in Brookings, South Dakota, USA.

Growth Conditions

To prepare an inoculum, a batch minimal medium culture of ATCC 31749 was grown for 48 h at 30°C. After inoculation with the 48 h minimal medium culture of ATCC 31749 grown in the same medium (2.5 mL), each batch culture (50 mL) in 250 mL erlenmeyer flasks was shaken at 200 rpm at 30°C for 120 h.

Curdlan and Biomass Determinations

When measuring the curdlan concentrations or the bacterial cell weights (West, 2006), a 5 mL sample of culture medium was removed from each flask at selected intervals of 24 h and the medium was centrifuged at 10,000 g for 15 min at 25°C. The cell pellet was washed with 5 mL of 0.01 M HCl and again centrifuged at 10,000 g for 15 min at 25°C. To the cell pellet, 5 mL of 0.5 M NaOH was added to dissolve the curdlan capsule on the cells and the suspension was incubated at 25°C for 60 min. The suspension was centrifuged at 10,000 g for 15 min at 25°C to pellet the cells. The supernatant was collected and used in the curdlan determination while the cells were washed with 5 mL of water and centrifuged at 10,000 g for 15 min at 25°C. The cells were collected by filtration on preweighed filters. The filters were dried at 80°C to constant weight. The cell dry weight levels were expressed as g cell dry weight L^{-1}. To quantitate the concentration of curdlan in the culture medium, 5 mL of 2 M HCl was added to the previously collected supernatant and mixed rigorously. The mixture containing the precipitated curdlan was centrifuged at 10,000 g for 15 min at 4°C and the pellet containing the precipitated curdlan was collected on preweighed filters and dried to constant weight at 80°C. Curdlan levels were given as g curdlan L^{-1}. All values represent the mean of three independent determinations involving three separate cultures. The student's t-test was utilized during statistical analysis.

RESULTS

In the present study, the effect of supplementing 0.5% yeast extract into the corn syrup-containing medium was investigated. In the medium containing 50 g L^{-1} solubles, curdlan production by ATCC31749 was enhanced by more than 2-fold after 48 h of growth relative to the unsupplemented medium (Table 1). A significant difference ($p<0.01$) in curdlan production by ATCC 31749 was noted between the yeast extract supplemented medium and unsupplemented medium. Similarly, curdlan production by ATCC 31749 was significantly higher ($p<0.01$) on the yeast extract-supplemented medium than the unsupplemented medium after 72 or 96 h. After 120 h of growth,

curdlan production by ATCC 31749 was 1.5-fold higher on the supplemented medium compared to the unsupplemented medium with the difference in production being statistically significant ($p<0.01$). As can be seen in Table 1, biomass production by ATCC 31749 grown on the 50 g L^{-1} solubles was increased by more than 7-fold after 24 h when yeast extract was supplemented into the medium. A statistically significant ($p<0.01$) increase in biomass production by the strain was observed after 24, 48, 72, 96 and 120 h of growth in the yeast extract-containing medium compared to the unsupplemented medium.

When a concentration of 100 g L^{-1} condensed corn distillers solubles was present in the corn syrup containing medium, the presence of yeast extract increased polysaccharide production by ATCC 31749 about 2-fold after 48 or 72 h of growth compared to the unsupplemented medium (Table 2). No increase in curdlan production by the strain was noted after 24 h but biomass production was higher (Table 2). The difference in curdlan production by ATCC31749 between the yeast extract supplemented medium and unsupplemented medium after 48 or 72 h was determined to be statistically significant ($p<0.01$). After 96 or 120 h of growth, curdlan production by the strain was increased by 1.4-fold in the yeast extract-containing medium compared to the unsupplemented medium (Table 2) with the difference in production being statistically significant ($p<0.01$). Biomass production by ATCC 31749 cells grown on the 100 g L^{-1} solubles was increased by about 4-fold after 24, 48, 72 and 96 h of growth when yeast extract was included in the medium (Table 2). The difference in biomass production by the bacterium following growth for 24, 48, 72 and 96 h on the supplemented and unsupplemented medium was statistically significant ($p<0.01$). After 120 h of ATCC 31749 growth (Table 2), biomass production was observed to be 3.3-fold higher on the yeast extract-supplemented medium than on the unsupplemented medium with the difference in production being statistically significant ($p<0.01$).

Using the medium containing 200 g L^{-1} solubles, the addition of yeast extract increased ATCC 31749 polysaccharide production by 1.7-fold or 1.8-fold, respectively, after 48 and 72 h relative to production on the unsupplemented medium (Table 3). This contrasts the decrease in curdlan production by ATCC 31749 after 24 h in the yeast extract-supplemented medium where biomass production was increased (Table 3). Compared to the solubles medium alone, the presence of yeast

Table 1: Effect of yeast extract on curdlan and biomass production by ATCC 31749 grown on corn syrup-containing medium with 50 g L^{-1} condensed corn distillers solubles

	Curdlan (g L^{-1})		Biomass (g L^{-1})	
Growth (h)	Control	Yeast extract	Control	Yeast extract
0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
24	0.55±0.24	0.95±0.38	0.36±0.16	2.71±0.11
48	1.83±0.36	4.30±0.75	0.29±0.14	3.02±0.54
72	3.07±0.56	5.66±0.32	0.51±0.21	2.95±0.67
96	3.53±0.51	5.69±0.33	0.31±0.05	1.81±0.35
120	3.50±0.14	5.39±0.60	0.29±0.01	2.71±0.12

Values represent Mean±SD of 3 trials

Table 2: Effect of yeast extract on curdlan and biomass production by ATCC 31749 grown on corn syrup-containing medium with 100 g L^{-1} condensed corn distillers solubles

	Curdlan (g L^{-1})		Biomass (g L^{-1})	
Growth (h)	Control	Yeast extract	Control	Yeast extract
0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
24	1.54±0.05	0.58±0.25	0.68±0.09	2.39±0.26
48	2.85±0.25	5.95±0.58	0.67±0.05	2.67±0.17
72	3.67±0.34	7.28±0.09	0.61±0.12	2.78±0.40
96	5.37±0.30	7.74±0.02	0.60±0.07	2.58±0.21
120	6.14±0.36	8.53±0.23	0.87±0.15	2.84±0.15

Values represent Mean±SD of 3 trials

Table 3: Effect of yeast extract on curdlan and biomass production by ATCC 31749 grown on corn syrup-containing medium with 200 g L^{-1} condensed corn distillers solubles

	Curdlan (g L^{-1})		Biomass (g L^{-1})	
Growth (h)	Control	Yeast extract	Control	Yeast extract
0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
24	1.94±0.14	0.57±0.09	1.29±0.14	2.28±0.12
48	3.09±0.65	5.37±0.56	1.29±0.10	3.58±0.04
72	5.43±0.83	9.84±0.81	1.43±0.23	4.34±0.27
96	6.08±0.43	7.39±0.91	1.85±0.07	2.63±0.50
120	8.27±0.72	9.75±0.11	2.17±0.56	3.65±0.29

Values represent Mean±SD of 3 trials

extract significantly elevated curdlan production by the bacterium ($p<0.01$). After ATCC 31749 was grown on the yeast extract-containing medium for 96 and 120 h, its polysaccharide production was increased by 1.4-fold relative to the unsupplemented medium (Table 3). The difference in curdlan production by ATCC 31749 following growth on the supplemented medium and supplemented medium was significant after 96 h ($p<0.05$) and 120 h ($p<0.01$). Yeast extract addition to the medium containing solubles and corn syrup increased biomass production by the strain about 3-fold following 48 or 72 h of growth (Table 3). The difference in biomass production produced by ATCC 31749 following growth for 48 or 72 h on the yeast extract-containing medium compared to the unsupplemented medium was highly significant ($p<0.01$). After 96 h or 120 h of ATCC 31749 growth on the medium with yeast extract present, biomass production was elevated by 1.4-fold or 1.7-fold, respectively, relative to biomass production on the medium containing solubles and corn syrup alone (Table 3). A statistically significant difference in biomass production after ATCC 31749 growth for 96 h ($p<0.05$) and 120 h ($p<0.01$) on the yeast extract-supplemented medium relative to the unsupplemented medium was observed.

Comparing curdlan production on the three concentrations of solubles tested, yeast extract stimulated curdlan production by ATCC 31749 after 120 h to a greater degree on the 100 g L^{-1} solubles medium compared to the 50 g L^{-1} solubles medium (Table 1, 2). When the solubles concentration in the medium was raised from 100 to 200 g L^{-1}, the supplementation of yeast extract to the medium had little effect on elevating curdlan production by ATCC 31749 (Table 2, 3).

DISCUSSION

Previously, it has been shown that *Agrobacterium* sp. ATCC 31749 can produce curdlan on such corn substrates such as a high maltose corn syrup or condensed corn distillers solubles (Portilho *et al*., 2006; West and Nemmers, 2008). Using the ethanol-processing coproduct from corn dry-milling, namely condensed corn distillers solubles, as a source of carbon and nitrogen, low levels of curdlan was produced (West and Nemmers, 2008). It appeared that the addition of a carbon source would increase polysaccharide production. In this investigation, curdlan production by ATCC 31749 was found to more than double when the strain was grown for 120 h on different concentrations of solubles supplemented with 3% corn syrup as a carbon source relative to curdlan production on unsupplemented solubles-containing medium (West and Nemmers, 2008). The findings indicated that carbon source was limiting in the medium and resulted in less curdlan being formed. The quality of the curdlan produced by ATCC 31749 on the medium containing solubles was the same as produced on other mineral salts medium in that the polysaccharide was readily alkali-soluble. In addition to carbon source, polysaccharide production can also be influenced by the addition of growth supplements such as yeast extract. It has been shown in earlier studies that yeast extract supplementation of the culture medium could elevate the production of other bacterial polysaccharides (Lo *et al*., 1997; West and Fullenkamp, 2000; Bajaj *et al*., 2006).

The findings from this study indicated that the supplementation of yeast extract into the medium containing corn syrup and solubles stimulated biomass production by *Agrobacterium* sp. ATCC 31749 which subsequently resulted in an increase in bacterial curdlan production. Also, as the solubles concentration in the corn syrup-containing medium was increased, curdlan production after 120 h was also noted to increase independent of whether yeast extract was added to the medium but its production was not proportional to the solubles level in the medium. The effect of yeast extract supplementation upon bacterial polysaccharide production has been explored previously (Lo *et al*., 1997; West and Fullenkamp, 2000; Bajaj *et al*., 2006). The prior studies examined how the addition of yeast extract to the culture medium affected the production of bacterial polysaccharides. It was shown that gellan production by the bacterium *Sphingomonas paucimobilis* ATCC 31461 could be stimulated by yeast extract supplementation to a medium containing casamino acids or ammonium nitrate as a nitrogen source and either glucose or high maltose corn syrup as a carbon source (West and Fullenkamp, 2000; Bajaj *et al*., 2006). Unlike this study, the presence of yeast extract in the medium only increased gellan production but failed to elevate biomass production by the bacterial cells (West and Fullenkamp, 2000). This appeared to be a species-dependent difference. Another investigation explored the effects of yeast extract and glucose concentration on production of the bacterial polysaccharide xanthan by *Xanthomonas campestris* ATCC 13951 (Lo *et al*., 1997). Xanthan production by ATCC 13951 grown on 5% glucose in batch cultures for 72 h was found to increase when the yeast extract concentration was elevated in the medium from 0.2 to 0.3% (Lo *et al*., 1997). Similar to the findings of the present study, it was demonstrated that the production of *X. campestris* cellular biomass was influenced by the presence of yeast extract. As the level of yeast extract in the 5% glucose-containing medium was increased from 0.2 to 0.3%, an elevation of ATCC 13951 biomass production was found to occur (Lo *et al*., 1997).

Overall, yeast extract supplementation of a medium containing corn syrup and selected concentrations of condensed corn distillers solubles increased curdlan production by *Agrobacterium* sp. ATCC 31749. The highest curdlan level was produced by the strain after 120 h of growth on the medium containing corn syrup and 200 g L^{-1} solubles indicating it was the correct concentration for use during curdlan production. Biomass production by ATCC 31749 was also increased following the addition of yeast extract to the medium. It was concluded that this increase in cellular biomass production caused by yeast extract supplementation was responsible for the observed elevation in curdlan production by the strain. Future study will focus on optimization of curdlan production by ATCC 31749 on the solubles medium by studying additional fermentation experiment parameters.

ACKNOWLEDGMENTS

This study was supported by the South Dakota AES and U.S. Department of Agriculture Grants No. 2005-35504-15460 and No. 2003-35504-13622. The technical assistance of Beth Nemmers was greatly appreciated.

REFERENCES

Bajaj, I.B., P.S. Saudagar, R.S. Singhal and A. Pandey, 2006. Statistical approach to optimization of fermentative production of gellan gum from *Sphingomonas paucimobilis* ATCC 31461. J. Biosci. Bioeng., 102: 150-156.

Ham, G.A., R.A. Stock, T.J. Klopfenstein, E.M. Larson, D.H. Shain and R.P. Huffman, 1994. Wet distillers byproducts compared with dried distillers grains with solubles as a source of protein and energy for ruminants. J. Anim. Sci., 72: 3246-3257.

Harada, T., A. Misaki and H. Saito, 1968. Curdlan: A bacterial gel-forming beta-1, 3-glucan. Arch. Biochem. Biophys., 124: 292-298.

Kanke, M., K. Koda, Y. Koda and H. Katayama, 1992. Application of curdlan to controlled drug delivery. I. The preparation and evaluation of theophylline-containing curdlan tablets. Pharm. Res., 9: 414-418.

Lee, I.Y., W.T. Seo, G.J. Kim, M.K. Kim and Y.H. Park, 1997. Production of curdlan using sucrose or sugar cane molasses by two-step fed-batch cultivation of *Agrobacterium* species. J. Ind. Microbiol. Biotechnol., 18: 255-259.

Lo, Y.M., S.T. Yang and D.B. Min, 1997. Effects of yeast and glucose on xanthan production and cell growth in batch culture of *Xanthomonas campestris*. Applied Microbiol. Biotechnol., 47: 689-694.

Nakao, Y., A. Konno, T. Taguchi, T. Tawada, H. Kasai, J. Toda and M. Terasaki, 1991. Curdlan: Properties and applications to foods. J. Food Sci., 56: 769-772.

Phillips, K.R. and H.G. Lawford, 1983a. Production of curdlan-type polysaccharide by *Alcaligenes faecalis* in batch and continuous culture. Can. J. Microbiol., 29: 1331-1338.

Phillips, K.R. and H.G. Lawford, 1983b. Theoretical maximum and observed product yields associated with curdlan production by *Alcaligenes faecalis*. Can. J. Microbiol., 29: 1270-1276.

Portilho, M., G. Matioli, G.M. Zanin, F.F. de Moraes and A.R.P. Scamparini, 2006. Production of the insoluble exopolysaccharide of *Agrobacterium* sp. (ATCC 31749 and IFO 13140). Applied Biochem. Biotechnol., 129-132: 21-31.

Rausch, K.D. and R.L. Belyea, 2006. The future of coproducts from corn processing. Applied Biochem. Biotechnol., 128: 47-86.

Saudagar, P.S. and R.S. Singhal, 2004. Fermentative production of curdlan. Applied Biochem. Biotechnol., 118: 21-31.

Spicer, E.F.J., E.I. Golenthal and T. Ikeda, 1999. A toxicological assessment of curdlan. Food Chem. Toxicol., 37: 455-479.

West, T.P. and N. Fullenkamp, 2000. Ability of casamino to support gellan production by *Sphingomonas paucimobilis* ATCC 31461. Microbios, 102: 89-101.

West, T.P., 2006. Pyrimidine base supplementation effects curdlan production in *Agrobacterium* sp. ATCC 31749. J. Basic Microbiol., 46: 153-157.

West, T.P. and B. Nemmers, 2008. Curdlan production by *Agrobacterium* sp. ATCC 31749 on an ethanol-processing coproduct. J. Basic Microbiol., 48: 65-68.