Research Journal of Microbiology

ISSN 1816-4935

Academic Journals Inc.

www.academicjournals.com

Research Journal of Microbiology 7 (5): 273-279, 2012
ISSN 1816-4935 / DOI: 10.3923/jm.2012.273.279

Polysaccharide Production by an *Agrobacterium* sp. Curdlan Overproducer Mutant on a Grain Fermentation Coproduct

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

ABSTRACT

The aim of this study was to determine if a curdlan overproducer *Agrobacterium* sp. mutant strain was able to produce higher levels of the polysaccharide than its parent strain from the grain processing coproduct condensed corn distillers solubles. The curdlan overproducer mutant was grown in a medium containing 400 g L^{-1} solubles for 120 h with curdlan and biomass levels being determined daily. The results with significant value were that curdlan could be produced at a higher level by the mutant strain on condensed corn distillers solubles as a source of carbon and nitrogen alone or supplemented with 3% corn syrup in less fermentation time than its parent strain. Other results with significant value was that the highest curdlan concentration was produced by the mutant strain (5.39 g L^{-1}) or parent strain (4.97 g L^{-1}) on condensed corn distillers solubles alone at 120 h. When the solubles were supplemented with corn syrup, the highest curdlan concentration was also produced by the mutant strain (12.34 g L^{-1}) or parent strain (10.51 g L^{-1}) at 120 h. Biomass production by ATCC 31749 (2.67 g L^{-1}) was slightly higher than the mutant strain (2.50 g L^{-1}) at 120 h of growth on condensed corn distillers solubles alone. When the solubles were supplemented with corn syrup, biomass production by the parent strain (4.25 g L^{-1}) was much higher than the mutant strain (2.43 g L^{-1}) at 120 h. The conclusion of this study with beneficial value is that the commercially valuable gum curdlan could be produced from a low value coproduct corn distillers solubles.

Key words: Curdlan, mutant, fermentation coproduct, biomass, polysaccharide, solubles

INTRODUCTION

Curdlan is an exopolysaccharide produced by *Agrobacterium* sp. (Harada *et al*., 1968; Phillips and Lawford, 1983). This high molecular weight polysaccharide exists as an unbranched chain of glucosyl residues connected by β-D-(1>3) bonds (Harada *et al*., 1968). This polysaccharide exists as a complex tertiary structure with its degree of polymerization being about 135 glucose residues (Harada *et al*., 1968). Curdlan is soluble in alkali but is insoluble in acid or water (Harada *et al*., 1968). This non-toxic carbohydrate polymer has applications in the food and pharmaceutical industries (Nakao *et al*., 1991; Kanke *et al*., 1992; Franz and Alban, 1995; Spicer *et al*., 1999; McIntosh *et al*., 2005; Rinaudo, 2008; Wang *et al*., 2010; Lehtovaara and Gu, 2011; Zhan *et al*., 2012). Curdlan production has been found to begin following stationary phase of a culture limiting in nitrogen but with excess carbon present (Phillips and Lawford, 1983; Lawford and Rousseau, 1992). Polysaccharide biosynthesis involves the utilization of nucleotide sugars as substrates and can be blocked if the phospholipid composition of the membrane is altered (Kim *et al*., 1999; Karnezis *et al*., 2002; Ruffing *et al*., 2006; West, 2006). A number of carbon

sources support curdlan production by *Agrobacterium* sp. ATCC 31749 including glucose, sucrose, maltose as well as high glucose and high maltose corn syrups (Portilho *et al*., 2006; West, 2006, 2009a; West and Nemmers, 2008). Carbon dioxide is produced in addition to curdlan during polysaccharide formation by the bacterium when glucose serves as the carbon source (Phillips *et al*., 1983).

A major coproduct resulting from grain-based ethanol production using corn is condensed corn distillers solubles. Following corn fermentation to ethanol, condensed corn distillers solubles is recovered by evaporation (Rausch and Belyea, 2006). With significant volumes of corn-based ethanol being produced, a large quantity of the low value coproduct distillers solubles exists that could be used to support bacterial polysaccharide production. It has previously been shown that bacterial curdlan production can occur on condensed corn distillers solubles (West and Nemmers, 2008; West, 2009b). The objective of this study was to test both unsupplemented condensed corn distillers solubles and corn syrup-supplemented distillers solubles as substrates for curdlan and biomass production by an *Agrobacterium* sp. curdlan overproducer mutant strain (West, 2009a) relative to its parent strain to learn if the mutant strain also overproduced curdlan on the unsupplemented and corn syrup-supplemented distillers solubles.

MATERIALS AND METHODS

Strains: The curdlan-producing strains *Agrobacterium* sp. ATCC 31749 and strain ECP-1, a mutant strain capable of elevated curdlan production, were used in this study (Harada *et al*., 1966; West, 2009a). The parent strain was maintained on nutrient agar plates while the mutant strain was maintained on nutrient agar plates containing ampicillin (100 mg L^{-1}). For long term storage, the mutant and parent strains were stably maintained in lyophilized cultures.

Medium and composition of distillers solubles: The strains were grown in a modified minimal medium (pH 6.8) consisting of 0.174% KH_2PO_4, 0.049% K_2HPO_4, 0.37% $Na_2SO_4.2H_2O$, 0.025% $MgCl_2.6H_2O$, 0.0024% $FeCl_3.6H_2O$, 0.0015% $CaCl_2.2H_2O$, 0.001% $MnCl_2.4H_2O$, 0.021% sodium citrate.$2H_2O$, 3% Na_2HPO_4 and 400 g L^{-1} condensed corn distillers solubles (Harada *et al*., 1966; West and Nemmers, 2008). When the medium was supplemented with additional carbon source, 3% corn syrup (42% maltose syrup from ICN Biomedical, Inc., Aurora, OH, USA) was added to the medium. The condensed corn distillers solubles (Dakota Ethanol, LLC, Wentworth, SD, USA) contained 25% solids, 1.2% glucose and 17.9% protein (West, 2009b). This research project was conducted from January 2005 to December 2011.

Growth conditions: Each batch culture was inoculated with 2×10^7 cells from 48 h cultures of ATCC 31749 and ECP-1 grown at 200 rpm at 30°C on the same medium. Following inoculation, batch cultures (50 mL) in 250 mL Erlenmeyer flasks were shaken at 200 rpm at 30°C for 120 h.

Curdlan and biomass analysis: When the curdlan concentrations or the bacterial cell weights were measured, a sample of culture medium (5 mL) was removed from each flask at selected intervals of 24 h and centrifuged at 10,000 xg for 15 min at 25°C. The cell pellet was washed with 0.01 M HCl (5 mL) and again centrifuged. To the cell pellet, 0.5 M NaOH (5 mL) was added to dissolve the curdlan and the suspension was incubated at 25°C for 60 min (West, 2006). The suspension was centrifuged at 10,000 xg for 15 min at 25°C to pellet the cells. The supernatant was collected and used for curdlan determination while the cells were washed with water and collected

at 10,000 xg for 15 min at 25°C. Using preweighed filters, the cells were collected by filtration and dried to constant weight at 80°C. To measure the curdlan concentration in the culture medium, 2 M HCl (5 mL) was added to the previously collected supernatant to precipitate the curdlan. The precipitated curdlan mixture was centrifuged at 10,000 xg for 15 min at 4°C. After the pellet containing the precipitated curdlan was collected on preweighed filters, the filters were dried at 80°C to constant weight (West, 2006).

Statistical analysis: The results indicate the mean of three separate trials where three independent cultures were used in the experimental design. The findings were statistically analyzed utilizing the student's t-test.

RESULTS

The ability of the grain fermentation coproduct condensed corn distillers solubles to support polysaccharide production by *Agrobacterium* sp. ATCC 31749 and the mutant strain ECP-1 was compared in a medium containing 400 g L^{-1} distillers solubles alone or supplemented with 3% corn syrup. It was previously determined that the modified minimal medium containing 400 g L^{-1} distillers solubles supported the highest level of curdlan production by ATCC 31749 after 120 h of growth at 30°C (West and Nemmers, 2008). In the medium containing only the distillers solubles as a carbon or nitrogen source, the mutant strain produced 1.5-fold higher curdlan levels than its parent strain following 72 h of growth (Table 1). The curdlan level produced by strain ECP-1 was significantly higher ($p<0.01$) than the level produced by ATCC 31749 after 72 h of growth. Similarly, curdlan production by strain ECP-1 was 2.4-fold higher than ATCC 31749 on the distillers solubles-containing medium after 96 h of growth (Table 1) with the difference in curdlan levels being statistically significant ($p<0.01$). Interestingly, there was little difference in curdlan production between strain ECP-1 and ATCC 31749 after 120 h of growth (Table 1). It appeared that the available carbon had been utilized by the mutant strain by 96 h which allowed the parent strain to continue to produce curdlan for an additional 24 h (Table 1). Curdlan production by the parent strain increased by 2.3-fold after 120 h of growth compared to 96 h of growth (Table 1). Next, the effect of supplementing the distillers solubles-containing medium with additional carbon source was investigated. When the distillers solubles-containing medium was supplemented with

Table 1: Comparison of curdlan production between curdlan mutant (ECP-1) and its parent (ATCC 31749) strains after growth on distillers solubles mediums

	Curdlan concentration (g L^{-1})			
	Solubles		Solubles+3% corn syrup	
Fermentation time (h)	ATCC 31749	ECP-1	ATCC 31749	ECP-1
0	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
24	0.94±0.16[a]	0.85±0.01[a]	1.06±0.20[a]	0.87±0.08[a]
48	2.83±0.29[a]	2.05±0.14[b]	4.43±0.89[a]	2.60±0.04[b]
72	2.36±0.09[a]	3.42±0.26[b]	5.15±0.82[a]	7.42±0.31[b]
96	2.15±0.21[a]	5.07±0.30[b]	6.53±0.24[a]	10.88±0.75[b]
120	4.97±0.15[a]	5.39±0.39[a]	10.51±0.87[a]	12.34±0.76[a]

Values are Mean±SD of three independent trials, Superscripts in the same row of data that do not have a common letter are statistically different at $p<0.01$ using student's t-test

Table 2: Comparison of biomass production by *Agrobacterium* sp. mutant (ECP-1) and its parent (ATCC 31749) strain grown on corn distillers solubles mediums

	Biomass concentration (g L^{-1})			
	Solubles		Solubles+3% corn syrup	
Fermentation time (h)	ATCC 31749	ECP-1	ATCC 31749	ECP-1
0	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
24	1.75±0.06[a]	1.44±0.04[b]	1.46±0.06[a]	1.34±0.12[a]
48	2.00±0.05[a]	1.65±0.06[b]	2.18±0.30[a]	1.57±0.23[b]
72	2.04±0.21[a]	1.98±0.11[a]	2.81±0.92[a]	2.37±0.35[a]
96	3.22±0.27[a]	2.21±0.26[b]	4.47±0.68[a]	2.19±0.27[b]
120	2.67±0.23[a]	2.50±0.10[a]	4.25±0.84[a]	2.43±0.13[b]

Values are Mean±SD of three independent trials, Superscripts in the same row of data that do not have a common letter are statistically different at $p<0.01$ using student's t-test

3% corn syrup, curdlan production by both the parent and mutant strains was more than double after 120 h of growth compared to the unsupplemented medium (Table 1). In the corn syrup-supplemented medium containing 400 g L^{-1} distillers solubles (Table 1), the mutant strain produced a 1.4-fold or 1.7-fold higher curdlan level than did its parent strain after 72 h or 96 h of growth, respectively, with the differences in polysaccharide production being statistically significant ($p<0.01$). The curdlan level produced by strain ECP-1 was 1.2-fold higher than the level produced by ATCC 31749 after 120 h of growth (Table 1). The difference in curdlan production by both strains was statistically significant ($p<0.05$). The results indicate that the mutant strain ECP-1 produced higher polysaccharide levels on the medium containing the distillers solubles alone or supplemented with carbon source.

As can be seen in Table 2, biomass production by ATCC 31749 was higher than strain ECP-1 grown on the medium containing condensed corn distillers solubles alone. After 96 h of growth on the medium containing the distillers solubles (Table 2), biomass production by the parent strain was 1.5-fold higher than the mutant strain with the difference in biomass production being statistically significant ($p<0.01$). When the distillers solubles-containing medium was supplemented with corn syrup, biomass production by ATCC 31749 continued to be elevated compared to strain ECP-1 (Table 2). Biomass production by ATCC 31749 was about double the level of biomass produced by strain ECP-1 after 96 h or 120 h of growth on the corn syrup supplemented medium (Table 2). There was a significant difference ($p<0.01$) in biomass production between the strains grown on the distillers solubles-containing medium supplemented with corn syrup. The higher level of biomass production produced by ATCC 31749 compared to strain ECP-1 on the medium containing the distillers solubles alone or supplemented with corn syrup did not result in increased curdlan production by the parent strain.

DISCUSSION

The findings from this work indicated that the parent strain ATCC 31749 and the mutant strain ECP-1 utilized 400 g L^{-1} condensed corn distillers solubles as a source of carbon and nitrogen to support both curdlan and biomass production. It was previously shown that ATCC 31749 was capable of producing curdlan on various concentrations of distillers solubles but that 400 g L^{-1} distillers solubles produced the highest curdlan levels (West and Nemmers, 2008). It appeared that

curdlan production by the mutant strain ECP-1 occurred more rapidly on the distillers solubles than curdlan production by its parent strain. This was observed previously in that curdlan production by the mutant strain ECP-1 occurred over a shorter period of fermentation time more rapidly than did its parent strain when grown on 3% corn syrup maltose or glucose as a carbon source (West, 2009b). When the medium was supplemented with 3% corn syrup, curdlan levels produced by both the mutant and parent strains increased with the mutant strain continuing to produce higher curdlan levels than its parent strain. This likely indicated that the available carbon source in the distillers solubles was limiting as has been observed previously (Rausch and Belyea, 2006). In the medium containing the distillers solubles alone or supplemented with 3% corn syrup, curdlan production by the parent strain is slightly higher than the mutant strain until 48 h of growth but curdlan production by the mutant strain exceeds the parent strain following 72 h of growth. Apparently, curdlan production by the mutant strain greatly increases on the media after 48 h of growth. The data indicated that curdlan production on the distillers solubles by the mutant strain represented a novel biotechnological production method for this important food and pharmaceutical polysaccharide gum (Nakao *et al*., 1991; Franz and Alban, 1995; McIntosh *et al*., 2005; Rinaudo, 2008; Wang *et al*., 2010; Lehtovaara and Gu, 2011; Zhan *et al*., 2012).

Interestingly, biomass production by ATCC 31749 and the mutant strain ECP-1 was very similar when the strains were grown in a medium containing corn syrup, glucose or maltose as a carbon source (Portilho *et al*., 2006; West, 2009a). Biomass production by ATCC 31749 grown on sucrose was also similar to the other carbon sources tested (West, 2006). In this study, biomass production by ATCC 31749 was elevated compared to strain ECP-1 independent of whether the strains were grown on the distillers solubles alone or supplemented with corn syrup. The addition of corn syrup appeared to have a greater effect on biomass production by the parent strain than the mutant strain. It appeared that less biomass production by the mutant strain on the distillers solubles alone or supplemented with corn syrup resulted in greater curdlan production.

CONCLUSION

It was concluded that it is possible to produce elevated levels of the polysaccharide curdlan using a curdlan overproducer *Agrobacterium* sp. mutant strain compared to its parent strain on the grain fermentation coproduct condensed corn distillers solubles. It appeared that the mutant strain was capable of producing the polysaccharide more rapidly from the distillers solubles alone or supplemented with corn syrup than its parent strain did. The use of this low cost coproduct as a substrate for curdlan production by the mutant strain has potential considering the quantity of condensed corn distillers solubles being produced each year during the dry-milling of corn for ethanol production.

ACKNOWLEDGMENTS

Financial support of this work was provided by the South Dakota Agricultural Experiment Station, US Department of Agriculture Grant No. 2005-35504-15460, No. 2003-35504-13622 and NIFA Grant No. 2011-67010-20051. The expert technical assistance of Beth Nemmers was greatly appreciated.

REFERENCES

Franz, G. and S. Alban, 1995. Structure-activity relationship of antithrombotic polysaccharide derivatives. Int. J. Biol. Macromol., 17: 311-314.

Harada, T., F. Fujimori, S. Hirose and M. Masada, 1966. Growth and β-glucan 10C3K production by a mutant of *Alcaligenes faecalis* var. *myxogenes* in defined medium. Agric. Biol. Chem., 30: 764-769.

Harada, T., A. Misaki and H. Saito, 1968. Curdlan: A bacterial gel-forming β-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.

Karnezis, T., H.C. Fisher, G.M. Neumann, B.A. Stone and V.A. Stanisich, 2002. Cloning and characterization of the phosphatidylserine synthase gene of *Agrobacterium* sp. ATCC 31749 and effect of its inactivation on production of high-molecular-mass (1>3)-β-D-glucan (curdlan). J. Bacteriol., 184: 4114-4123.

Kim, M.K., I.Y. Lee, J.H. Ko, Y.H. Rhee and Y.H. Park, 1999. Higher intracellular levels of uridine monophosphate under nitrogen-limited conditions enhance metabolic flux of curdlan synthesis in *Agrobacterium* species. Biotechnol. Bioeng., 62: 317-323.

Lawford, H.G. and J.D. Rousseau, 1992. Production of β-1,3-glucan exopolysaccharide in low shear systems: The requirement for high oxygen tension. Appl. Biochem. Biotechnol., 34/35: 597-612.

Lehtovaara, B.C. and F.X. Gu, 2011. Pharmacological, structural and drug delivery properties and applications of 1,3-β-glucans. J. Agric. Food Chem., 59: 6813-6828.

McIntosh, M., B.A. Stone and V.A. Stanisich, 2005. Curdlan and other bacterial (1>3)-β-D glucans. Appl. Microbiol. Biotechnol., 68: 163-173.

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, 1983. Theoretical maximum and observed product yields associated with curdlan production by *Alcaligenes faecalis*. Can. J. Microbiol., 29: 1270-1276.

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

Portilho, M., G. Matioli, G.M. Zanin, F.F. de Moraes and A.R. Scamparini, 2006. Production of the insoluble exopolysaccharide of *Agrobacterium* sp. (ATCC 31749 and IFO 13140). Applied Biochem. Biotechnol., 131: 864-869.

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

Rinaudo, M., 2008. Main properties and current applications of some polysaccharides as biomaterials. Polym. Int., 57: 397-430.

Ruffing, A., Z. Mao and R.R. Chen, 2006. Metabolic engineering of *Agrobacterium* sp. for UDP-galactose regeneration and oligosaccharide synthesis. Metab. Eng., 8: 465-473.

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

Wang, M., C. Chen, G. Sun, W. Wang and H. Fang, 2010. Effects of curdlan on the color, syneresis, cooking qualities and textural properties of potato starch noodles. Starch/Starke, 62: 429-434.

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.

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

West, T.P., 2009a. Elevated curdlan production by a mutant of *Agrobacterium* sp. ATCC 31749. J. Basic Microbiol., 49: 589-592.

West T.P., 2009b. Effect of yeast extract supplementation on curdlan production from condensed corn distillers solubles. Res. J. Microbiology, 4: 202-207.

Zhan, X.B., C.C. Lin and H.T. Zhang, 2012. Recent advances in curdlan biosynthesis, biotechnological production and applications. Appl. Microbiol. Biotechnol., 93: 525-553.