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Utilization of fuel ethanol residues in production of the biopolymer alternan

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Abstract

Corn condensed distiller's solubles (CCDS), an abundant byproduct of fuel ethanol production from wet-milled corn, was utilized as a fermentation medium component in the production of alternan by *Leuconostoc mesenteroides*. Complex components of a conventional alternan production medium were completely replaced with CCDS at 1·5% w/v. *L. mesenteroides* strains NRRL B-1355 and NRRL B-21138 grew to higher cell densities in CCDS than in conventional medium, and produced biopolymer more rapidly. However, stationary-phase cultures of strain NRRL B-21138 lost viability in CCDS medium, suggesting that CCDS might be most suitable for batch production of alternan. © 1998 Elsevier Science Ltd

Keywords: alternan, corn condensed distiller's solubles, corn gluten feed, fuel ethanol, *Leuconostoc mesenteroides*, value-added co-products.

Introduction

The fuel ethanol industry has become an important partner of American agriculture. More than 500 million bushels of corn were used during 1995 to produce approximately 1·3 billion gallons of ethanol [1]. Nevertheless, the profitability of fuel ethanol production continues to hinge on feedstock prices and regulatory policies. Strategies to enhance the economics of ethanol production include the development of new value-added co-products [2].

The highest volume co-product of fuel ethanol production from wet-milled corn is corn gluten feed (CGF), currently marketed as a cattle feed [3]. Corn gluten feed is composed of two distinct fractions, corn fibre and corn condensed distillers' solubles (CCDS). Corn fibre includes the seed covering or pericarp, removed early in the wet-milling process. Because corn fibre is rich in hemicellulose, cellulose and adherent starch, research efforts have been directed toward utilization of this fraction for production of ethanol and other chemicals [4]. Much less work has been reported on value-added uses for CCDS.

Fuel ethanol distillation residues contain soluble protein, unfermented sugars, dead yeast cells, and chemicals such as glycerol or lactic acid [5]. This material is evaporated to approximately 50% moisture to form CCDS. Corn condensed distiller's solubles contains approximately 18% protein by weight (commercial basis), and is a rich source of nutrients and growth factors. Recent studies suggest that CCDS may be suitable as a fermentation medium for the production of valuable carotenoids or polysaccharides [6–8].

Alternan is an α-D-glucan with the unique backbone structure of alternating α-(1→6), α-(1→3) linkages, produced by rare strains of the lactic acid bacterium *Leuconostoc mesenteroides* [9–11]. Because of its unusual structure, alternan and its derivatives have properties of high solubility and low viscosity that suggest commercial applications in foods, cosmetics, etc. [12,13]. Alternan-producing strains of *Leuconostoc mesenteroides* are nutritionally demanding, and have been cultured on media containing such complex components as liver-, beef- and yeast extract, and peptones [14–18]. In commercial production, such nutrients would likely be limiting due to price and availability.

* Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

Utilization of CCDS in place of conventional complex ingredients would reduce the cost of alternan production, and provide a new value-added use for fuel ethanol residues.

Materials and methods

Strains, culture conditions

Leuconostoc mesenteroides strains NRRL B-1355 and NRRL B-21138 were obtained from the ARS Culture Collection at the National Center for Agricultural Utilization Research, Peoria, IL. Strain NRRL B-1355 is a naturally occurring isolate that produces both alternan and dextran, in approximately equal amounts [9,14]. Strain NRRL B-21138 is a highly stable mutant derived from NRRL B-1355 that produces alternan with little or no contaminating dextran [19]. The conventional medium for production of alternan contained sucrose as a carbon source, complex components and defined salts (Table 1). Solid media included 1·5% w/v agar. Liquid cultures were 10 ml in 50 ml flasks, inoculated at 1·0% v/v from 2 d preinocula and incubated at 28°C and 100 rpm. Corn condensed distiller's solubles (CCDS) was the kind gift of Pekin Energy Company, Pekin, IL.

Viable cell and polysaccharide determinations

Viable cell counts were determined by plating appropriate culture dilutions on solid medium. Polysaccharide yields were determined gravitometrically as dry weights from culture supernatants precipitated with 2 vol. ethanol. All results are reported as means and standard errors from replica cultures.

Results and discussion

Growth of L. mesenteroides on solid medium containing CCDS

Ingredients for the conventional medium used in this study are listed in Table 1. In order to test the general growth requirements of *L. mesenteroides* strains NRRL B-1355 and NRRL B-21138, solid medium plates were prepared that contained various combinations of defined and complex components (Table 2). Both strains required sucrose, basal salts and complex components for growth. Furthermore, CCDS at 2·0% w/v appeared to serve as a suitable substitute for conventional complex components. Colonies on plates containing CCDS were similar in size and mucoid appearance to colonies on conventional plates, indicating good growth and polysaccharide production.

Table 1. Components of conventional medium for production of alternan

Ingredient	Amount (g)
Carbon source	
Sucrose	20·0
Complex components	
Beef extract	1·5
Polypeptone	1·5
Yeast extract	1·5
Basal salts	
Ammonium citrate	2·0
Magnesium sulphate	0·1
Manganese sulphate	0·05
Potassium phosphate dibasic	2·0
Sodium acetate	5·0
Tween 80	1·0

Growth and polysaccharide production in liquid medium containing CCDS

L. mesenteroides strains NRRL B-1355 and NRRL B-21138 were cultured for 48 h in liquid media containing 2·0% sucrose, basal salts and from 0 to 4·0% w/v CCDS. Strain NRRL B-1355 cultured in conventional medium produced approximately $6{\cdot}8 \times 10^7$ viable cells/ml and 4·4 mg polysaccharide/ml (Fig. 1). Growth in conventional medium was accompanied by a decrease in culture pH from an initial value of pH 6·5 to a final value of pH 4·7. Liquid medium containing only sucrose and basal salts showed no culture growth ($<10^6$ viable cells/ml) or change in pH. However, approximately 1·5 mg polysaccharide/ml accumulated in these 0% CCDS cultures, presumably because inocula included low levels of glucansucrases. Cultures containing 0·25% CCDS also showed no viable cells, although some growth was implied by the final culture pH. Growth was also evidenced by the production of 5·8 mg polysaccharide/ml, significantly more than was produced in conventional medium. Cultures containing from 0·5 to 4·0% CCDS produced high yields of both viable cells and polysaccharide (Fig. 1). At the highest concentration of CCDS tested (4·0% w/v) polysaccharide yields were slightly reduced, suggesting that cell growth had depleted carbon available for polysaccharide production.

Strain NRRL B-21138, which produces alternan with little or no contaminating dextran, was similarly cultured for 48 h on media containing from 0 to 4·0% CCDS (Fig. 2). Conventional medium cultures contained nearly 10^8 viable cells/ml and produced approximately 5·3 mg polysaccharide/ml. As was the case for parental strain NRRL B-1355, cultures of NRRL B-21138 in 0% CCDS medium showed no evidence of growth and little polysaccharide accumulation. Cultures containing from 0·25 to 4·0% CCDS produced between 6·0 and 7·0 mg polysaccharide/ml. Although these cultures exhibited pH levels characteristic of growth, they contained less than 10^6 viable cells/ml.

Time courses of liquid cultures containing 0·25 or 1·5% CCDS

Cultures of strain NRRL B-1355 in conventional medium reached stationary growth phase within about

Table 2. Growth of *Leuconostoc mesenteroides* strains on solid media

	Strain NRRL B-21138			Strain NRRL B-1355		
	Complex components			Complex components		
	None	Conventional	2·0% CCDS	None	Conventional	2·0% CCDS
Defined components						
None	–	–	–	–	–	–
Basal salts	–	–	–	–	–	–
Basal salts+sucrose	–	+	+	–	+	+

12 h, although polysaccharide levels continued to increase up to approximately 24 h (Fig. 3A). Cultures grown in medium containing 1·5% CCDS exhibited higher growth rates and yields than those in conventional medium (Fig. 3B). Polysaccharides also accumulated more rapidly and to greater yields. Cultures of NRRL B-1355 grown in medium containing 0·25% CCDS showed an initial increase in viable cell counts, followed by a gradual decline (Fig. 3C). Polysaccharide continued to accumulate during this cell death phase.

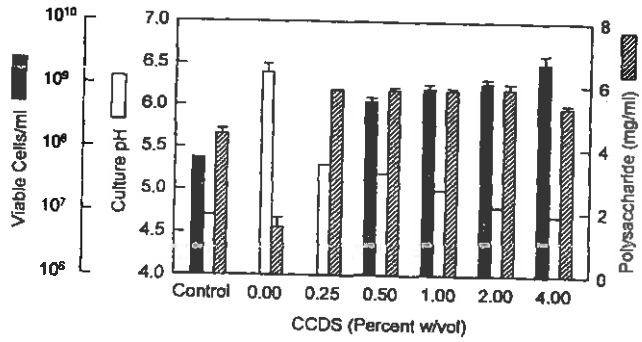

Fig. 1. Characteristics of 48-h cultures of *Leuconostoc mesenteroides* strain NRRL B-1355 grown in liquid media containing from 0 to 4·0% w/v corn condensed distiller's solubles (CCDS). Control cultures were grown on conventional medium.

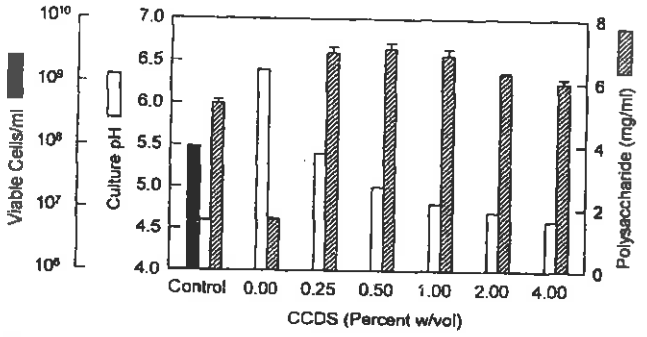

Fig. 2. Characteristics of 48-h cultures of *Leuconostoc mesenteroides* strain NRRL B-21138 grown in liquid media containing from 0 to 4·0% w/v corn condensed distiller's solubles (CCDS). Control cultures were grown on conventional medium.

These results explain the apparent contradiction between low viable counts and high polysaccharide yields observed at 48 h in 0·25% CCDS medium (Fig. 1), and suggest that stationary phase cultures require nutrients or growth factors for continued viability.

Strain NRRL B-21138 exhibited growth kinetics on conventional medium similar to those of parental strain NRRL B-1355, with somewhat greater polysaccharide yields (Fig. 4A). Also like NRRL B-1355, strain NRRL B-21138 showed enhanced rates of growth and polysaccharide production in medium containing 1·5% CCDS (Fig. 4B). However, these cultures suffered a dramatic loss in cell viability during the stationary

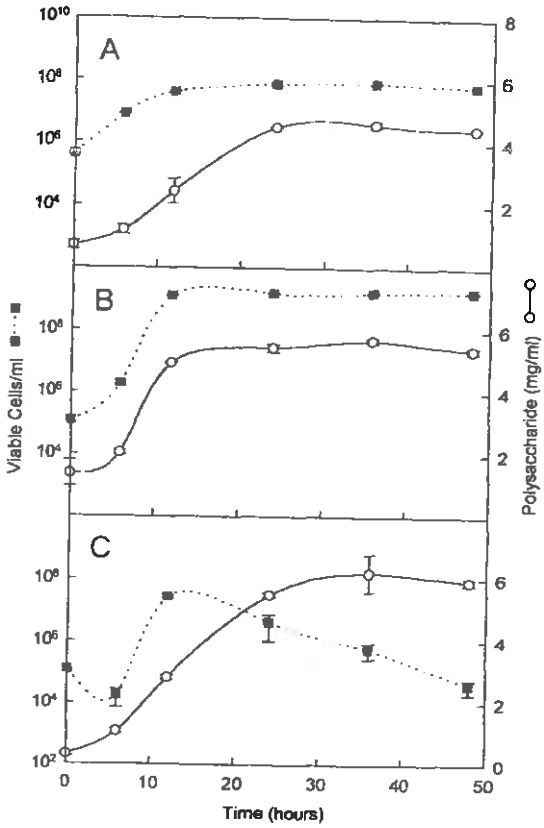

Fig. 3. Time courses of *Leuconostoc mesenteroides* strain NRRL B-1355 in liquid media containing corn condensed distiller's solubles (CCDS). Panel A, conventional medium control cultures; Panel B, medium containing 1·5% CCDS; Panel C, medium containing 0·25% CCDS.

phase. These results account for the low levels of viable cells found in 48-h cultures of NRRL B-21138 (Fig. 2), and suggest that this strain has a higher requirement for viability factors than does NRRL B-1355. It seems unlikely that the increased susceptibility of strain NRRL B-21138 to cell death is directly related to its known phenotype of enhanced alternan production. Since strain NRRL B-1355 was heavily mutagenized in the generation of NRRL B-21138 [19], the latter strain likely carries multiple mutations and may be partially auxotrophic. Cultures of strain NRRL B-21138 in medium containing 0·25% CCDS exhibited growth and death kinetics similar to NRRL B-1355 (Fig. 4C), consistent with the exhaustion of factors required for cell viability.

Conclusions

New value-added uses for fuel ethanol residues could improve the economic viability of this important industry. Corn condensed distiller's solubles (CCDS) is an abundant byproduct of fuel ethanol production from wet-milled corn, and is currently used as a component of low value animal feeds. Alternan is a unique biopolymer with numerous potential food, cosmetic and industrial uses. Results indicate that CCDS can be utilized in the production of alternan, as a substitute for the costly complex components of conventional medium. Since alternan is precipitated from culture supernatants by the addition of organic solvents, co-production of alternan could take advantage of the on-site availability of ethanol. *Leuconostoc mesenteroides* is considered a GRAS organism, and cell pastes from alternan production might be incorporated into animal feeds for at least partial recovery of the protein value of CCDS. The loss of viability of strain NRRL B-21138 in stationary phase cultures containing CCDS appears to favour batch production schemes.

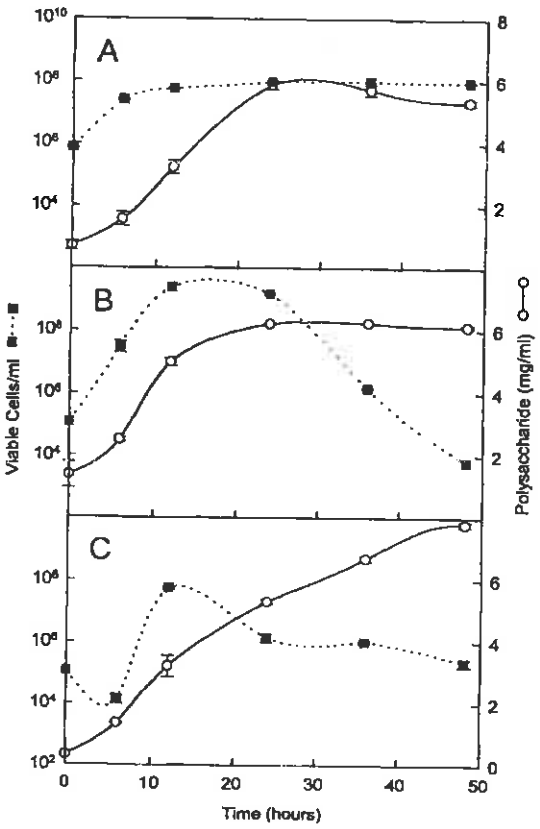

Fig. 4. Time courses of *Leuconostoc mesenteroides* strain NRRL B-21138 in liquid media containing corn condensed distiller's solubles (CCDS). Panel A, conventional medium control cultures; Panel B, medium containing 1·5% CCDS; Panel C, medium containing 0·25% CCDS.

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