BIOTECHNOLOGY LETTERS
Volume 16 No.11 (Nov.1994) p.1163-1166
Received 5th November

PRODUCTION OF PULLULAN FROM FUEL ETHANOL BYPRODUCTS BY *AUREOBASIDIUM* SP. STRAIN NRRL Y-12,974

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SUMMARY

Byproducts of fuel ethanol production from wet-milled corn, were tested as substrates for growth and pullulan production by *Aureobasidium* sp. strain NRRL Y-12,974. The strain grew well on corn fiber and corn condensed distiller's solubles (CCDS), and fermented CCDS to pullulan. CCDS compared favorably with starch as a substrate for pullulan production.

INTRODUCTION

Relatively low-value corn wet-milling byproducts and corn-based fuel ethanol byproducts are often marketed as animal feed supplements, primarily based upon their protein value (May, 1987). Nevertheless, certain of these materials often retain significant residual carbohydrate components. Microbial fermentations may have the

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corn maize
~~starch~~ → ethanol byproducts → pullulan → food → pharma → other] applic

Ethanol byp

potential to upgrade these carbohydrates into value-added products, including industrial biopolymers. Pullulan is a unique biopolymer that has numerous patented uses in manufacturing, pharmaceuticals, electronics, and food industries (Yuen, 1974). Pullulan is conventionally produced by direct fermentation of sucrose or starch by the yeast-like fungus *Aureobasidium pullulans*. (Yuen, 1974; Catley, 1979; Slodki and Cadmus, 1978). New cost-effective substrates for pullulan production could help make this biopolymer more competitive with synthetic petroleum-derived polymers.

MATERIALS AND METHODS

Growth media: A basal medium was used that contained per liter: 2.0 g $NaNO_3$, 0.5 g $MgSO_4x7H_2O$, 0.5 g NaCl, 0.01 g $FeSO_4x7H_20$, 1.0 g KH_2PO_4 and 0.4 g yeast extract (Difco). Carbon sources were added as follows: glucose, sucrose, maltose, (Sigma Chemical Co.), or soluble starch (Difco), all at 2.0% w/v; corn fiber (Pekin Energy Co.), 3.0% dry w/v; or corn condensed distiller's solubles (CCDS, Pekin Energy Co.), 10.0% wet w/v. CCDS medium was clarified by centrifugation prior to being sterilized by autoclaving, and contained 0.6% final carbohydrate as estimated by phenol-sulfuric acid analysis. Corn fiber medium contained 2.0% final carbohydrate by analysis. Clarified thin stillage (containing 2.2% carbohydrate by analysis), was directly tested as a medium without basal medium amendments.

Organism: *Aureobasidium* sp. strain NRRL Y-12,974 was isolated as a "color variant" of *Aureobasidium*, a member of a lightly pigmented subgroup genetically near *Aureobasidium pullulans* (Leathers, 1986; Leathers et al., 1988). Certain color variant isolates, including NRRL Y-12,974, have been shown to produce pullulan in good yields and without the melanin contamination often produced by typically pigmented strains (Leathers et al., 1988; Silman et al., 1990; Pollock et al., 1992).

Culture Conditions: Fifty ml cultures were prepared in triplicate in 250 ml Erlenmeyer flasks, inoculated with 0.5 ml of glucose-grown *Aureobasidium* sp. strain NRRL Y-12,974, and incubated at 28°C at 200 rpm for 9 days.

Assays: Culture growth yields were determined by direct microscopic cell counts using a hemocytometer. Total extracellular polysaccharide was determined as dry weight following precipitation with tetrahydrofuran. Pullulan content of extracellular polysaccharides was estimated by digestion with pullulanase as previously described (Leathers et al., 1988). All results are reported as mean values (with standard deviations) from triplicate cultures, and are characteristic of repeated experiments.

RESULTS AND DISCUSSION

As shown in Table 1, *Aureobasidium* sp. strain NRRL 12,974 grew equally well on all substrates tested, with the exception of clarified thin stillage. Since thin stillage lacked the basal medium amendments of CCDS medium, this result suggests that supplements are essential for growth of *Aureobasidium*.

Table 1 also summarizes total polysaccharide yields from tested substrates. Although *Aureobasidium* sp. strain NRRL 12,974 grew well on corn fiber, polysaccharide yields were low from this substrate. Corn fiber may carry residual adherent starch in addition to the hemicellulose (arabinoxylan) and cellulose of corn bran (Boyer and Shannon, 1987; May, 1987). Strain NRRL Y-12,974 is capable of producing both amylases (Leathers, 1993) and high levels of xylanase (Leathers, 1986), and can produce pullulan from the fermentation of starch but not xylan (Leathers et al., 1988). Failure to produce pullulan from corn fiber might result from either insufficient levels of starch in the corn fiber, or preferential use of starch for cell growth.

Although highest pullulan yields were obtained from cultures grown on simple sugars, both soluble starch and CCDS media supported polysaccharide production (Table 1). Pullulan purity, as judged by pullulanase sensitivity, was also similar between polysaccharides from soluble starch and CCDS cultures. Furthermore, when correction was made for both initial carbohydrate levels and pullulan purity, CCDS was converted to pullulan with an efficiency of 21%, intermediate between efficiencies for conversion of soluble starch and sugars.

Commercial production of pullulan conventionally employs starch or sucrose (Yuen, 1974). Clarified CCDS contains starch-derived oligosaccharides that have escaped complete saccharification, and consequently, fermentation by *Saccharomyces cerevisiae* to ethanol. It is possible that oligosaccharides in CCDS are shorter or otherwise more accessible than those in commercial soluble starch, resulting in a higher efficiency of CCDS conversion to pullulan.

Our study suggests that CCDS could be a promising new economical substrate for the production of pullulan. CCDS is available in large volumes as a byproduct of fuel ethanol production, and currently markets for as little as $0.01/lb. Since pullulan is recovered from culture supernatants by the addition of organic solvents, pullulan production could, in theory, take advantage of the on-site availability of ethanol at production facilities. Diluted ethanol from pullulan precipitation could then be recovered by distillation.

ACKNOWLEDGMENTS

The authors thank Stanley Janson, Chuck Sauder and Gary Welch of Pekin Energy Company for samples of fuel ethanol byproducts, as well as valuable consultations. Expert technical assistance was provided by Heidi Hefner.

Table 1. Growth and polysaccharide yields from cultures of *Aureobasidium* sp. strain NRRL Y-12,974

Substrate[1]	Growth Yield, Cells/ml	Polysacch., g/l	Pullanase Sensitivity	Bioconversion Efficiency[2]
Glucose	$3.3 \pm 0.3 \times 10^8$	10.1 ± 1.3	81 ± 5 %	41 %
Maltose	$2.9 \pm 0.3 \times 10^8$	12.5 ± 1.8	65 ± 3 %	41 %
Starch	$3.8 \pm 0.4 \times 10^8$	5.4 ± 0.8	39 ± 7 %	11 %
CF	$5.2 \pm 1.3 \times 10^8$	0.9 ± 0.1	21 ± 9 %	1 %
TS	$2.0 \pm 0.3 \times 10^5$	8.3 ± 1.7	5 ± 1 %	2 %
CCDS	$5.8 \pm 3.3 \times 10^8$	4.5 ± 0.2	28 ± 12 %	21 %

[1]Corn wet-milling/fuel ethanol byproduct substrates: CF, corn fiber; TS, thin stillage; CCDS, corn condensed distiller's solubles

[2]Bioconversion efficiency calculated as percent initial substrate converted to pullulanase-sensitive endproduct

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