DIRECT FERMENTATION OF CELLODEXTRINS TO ETHANOL BY

CANDIDA WICKERHAMII AND C. LUSITANIAE

Shelby N. Freer* and Robert W. Detroy
U.S. Department of Agriculture
Agricultural Research Service
Northern Regional Research Center
1815 N. University Street
Peoria, Illinois 61604

Summary

Production of ethanol from cellodextrins, as large as cellohexose, by Candida lusitaniae and C. wickerhamii was studied. C. lusitaniae fermented only glucose and cellobiose, whereas C. wickerhamii efficiently fermented cellodextrins. Maximum ethanol yields of 29.2 g/liter from 54 g/liter cellodextrins were achieved by C. wickerhamii in 3-4 days.

Introduction

From an initial screening of 22 yeasts, the two which fermented cellobiose most rapidly were NRRL Y-5394 Candida lusitaniae van Uden et do Carmo-Sousa and NRRL Y-2563 C. wickerhamii (Capriotti) Meyer et Yarrow (synonym Torulopsis wickerhamii Capriotti) (Yarrow and Meyer, 1978). Although both yeasts readily ferment cellobiose, striking differences between these organisms exist. For example, we expected both organisms to produce \( \beta \)-glucosidase. However, virtually no \( \beta \)-glucosidase activity was associated with the C. lusitaniae cellobiose fermentation. C. wickerhamii produced relatively high levels of \( \beta \)-glucosidase when grown anaerobically on cellobiose. In fact, this yeast appears to "over-produce" the enzyme since glucose was detected in the cellobiose fermentations at 24 hr (Freer and Detroy, submitted for publication).

Purified Trichoderma reesei Simmons \( \beta \)-glucosidase hydrolyzes not only cellobiose but also cellodextrins of DP (degree of polymerization) 3-6 (Wood, 1971). Because C. wickerhamii produces \( \beta \)-glucosidase and both yeasts ferment cellobiose, we tested these organisms for their ability to ferment cellodextrins. For comparison, similar experiments were conducted with NRRL Y-2034 Saccharomyces cerevisiae Hansen, which ferments glucose but not cellobiose or cellodextrins. This communication reports that C. lusitaniae fermented only glucose and cellobiose. C. wickerhamii, however, exhibited the unique capacity to efficiently ferment cellodextrins directly to ethanol.
MATERIALS AND METHODS

Preparation of Cellodextrins: Cellodextrins were prepared by a modified method of Miller et al. (1960) and Hsu et al. (1980). Either CF-11 cellulose or avicel was first dissolved in ice-cold fuming HCl, then incubated at 25 C to carry out the acid hydrolysis. After 2-3 hr of incubation, HCl was partially removed under vacuum and the cellodextrins were precipitated by adding 5-7 volumes of cold acetone. After 2 hr at -20 C, the precipitate was collected by centrifugation at 14,000 x g, washed three times with cold acetone, and resuspended in 200 ml of deionized H2O. The solution was neutralized by slowly adding Dowex 2-8X (100-200 mesh, Sigma Chemical Co.) that had been converted to the hydroxide form. The resin and insoluble cellodextrins were removed by centrifugation at 14,000 x g for 15 min., and the supernatant was clarified by filtration through a 0.45 µ nitrocellulose filter. The neutral, water-soluble cellodextrins were concentrated to 50 ml on a rotary evaporator at 45 C and precipitated by the addition of cold acetone. The precipitate was collected by centrifugation at 14,000 x g for 30 min. Residual acetone was removed under vacuum, and the cellodextrins were stored at -20 C. The final preparation contained glucose, cellobiose, and cellodextrins as large as cellohexose. Larger cellodextrins were probably also generated by this procedure, but we were not able to detect oligosaccharides larger than cellohexose with the HPLC analysis procedure used.

Organisms, Media, and Fermentation Conditions: The yeasts tested for their ability to ferment the cellodextrins were Candida lusitaniae NRRL Y-5394, C. wickerhamii NRRL Y-2563 and Saccharomyces cerevisiae NRRL Y-2034. The yeasts were acquired from the ARS Culture Collection, NRRL, Peoria, IL.

Inocula were prepared by transferring a loopful of cells from a fresh YM agar slant to 200 ml of basal media (5 g/liter peptone, 3 g/liter yeast extract, 3 g/liter malt extract, adjusted to pH 5.0 with HCl) containing 50 g/liter glucose. The cultures were incubated on a rotary shaker (200 rpm) at 28 C for 24 hr, and a 0.2 ml aliquot was used to inoculate 20 ml of fresh media. After 24 hr incubation, the cells were harvested by centrifugation at 7,000 x g for 5 min., washed once in sterile basal medium to remove residual glucose, and resuspended in 20 ml of sterile basal media. Fifteen milliliters of basal media containing either 45 or 90 g/liter cellodextrins in 25-ml flasks was inoculated with 0.45 ml of the cell suspension (3% v/v). Flasks were capped with serum stoppers and vented with No. 26 gauge sterile needles. The fermentations were carried out at 28 C on a rotary shaker at 200 rpm.

Ethanol and Carbohydrate Analysis: Ethanol was determined by gas-liquid chromatography with a Packard Model 402 GLC using a Porapak Q column at 150 C. Carbohydrates were analyzed by high-pressure liquid chromatography on a Waters HPLC fitted with a DuPont Zorbax-NH column. The mobile phase consisted of acetonitrile:H2O (70:30). Cellobiose was