APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Mar. 1991, p. 655–659
0099-2240/91/030655-05$02.00/0

Vol. 57, No. 3

Fermentation and Aerobic Metabolism of Cellodextrins by Yeasts

S. N. FREER

Northern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Peoria, Illinois 61604

Received 30 September 1989/Accepted 12 December 1990

The fermentation and aerobic metabolism of cellodextrins by 14 yeast species or strains was monitored. When grown aerobically, *Candida wickerhamii*, *C. guilliermondii*, and *C. molischiana* metabolized cellodextrins of degree of polymerization 3 to 6. *C. wickerhamii* and *C. molischiana* also fermented these substrates, while *C. guilliermondii* fermented only cellodextrins of degree of polymerization ≤ 3. *Debaryomyces polymorphus*, *Pichia guilliermondii*, *Clavispora lusitaniae*, and one of two strains of *Kluyveromyces lactis* metabolized glucose, cellobiose, and cellotriose when grown aerobically. These yeasts also fermented these substrates, except for *K. lactis*, which fermented only glucose and cellobiose. The remaining species/strains tested, *K. lactis*, *Brettanomyces claussenii*, *B. anomalus*, *K. dobzhanskii*, *Rhodotorula minuta*, and *Dekkera intermedia*, both fermented and aerobically metabolized glucose and cellobiose. Crude enzyme preparations from all 14 yeast species or strains were tested for ability to hydrolyze cellotriose and cellotretose. Most of the yeasts produced an enzyme(s) capable of hydrolyzing cellotriose. However, with two exceptions, *R. minuta* and *P. guilliermondii*, only the yeasts that metabolized cellodextrins of degree of polymerization >3 produced an enzyme(s) that hydrolyzed cellotretose.

Cellulose has the potential to serve as a renewable carbon or energy resource for the microbial production of fuels and chemical feedstocks; however, few industrial microorganisms utilize cellulose directly. Glucose can be produced from cellulose by either acid hydrolysis or the concerted action of the cellulase enzyme complex. Acid hydrolysis methodologies have the advantage of being relative rapid; however, potential pollutants and compounds toxic or inhibitory to microorganisms are often produced. Enzymatic saccharification of cellulose is nonpolluting. However, the reaction times are often quite long due to an accumulation of glucose that results in product inhibition (21) of the β-1,4-glucosidase (EC 3.2.1.21). This leads to an accumulation of cellobiose that results in product inhibition of the 1,4-β-glucan cellobiohydrolase (EC 3.2.1.91) and a retardation in the rate of cellulose saccharification and glucose production. When *Trichoderma reesei* Simmons cellulase preparations were supplemented with fungal culture filtrates that were rich in β-glucosidase, the amount of glucose produced was substantially increased (2, 21). Thus, β-glucosidase not only produces glucose from cellobiose, it also reduces the cellobiose inhibition, allowing the cellulolytic enzymes to function more efficiently.

An alternative approach to the production of glucose from cellulose might be to use chemical methods to produce soluble cellodextrins and then use microbial or enzymatic methods to produce value-added products. Since relatively mild chemical treatments are required to produce cellodextrins, the amount of side products and/or compounds toxic or inhibitory to microorganisms should be reduced as compared to the harsh chemical treatments required for the complete hydrolysis of cellulose.

To date, only two yeasts, *Candida wickerhamii* and *C. molischiana*, have been reported to ferment soluble cellodextrins to ethanol (7, 10). Both yeasts produce an extracytoplasmic β-glucosidase (8, 11). The *C. wickerhamii* β-glucosidase has been purified (6, 14) and shown to be a glycoprotein with an M_r of about 200,000. The β-glucosidase(s) of *C. molischiana* has not yet been purified and/or characterized.

The β-glucosidases responsible for the aerobic metabolism of cellobiose have also been purified and characterized from *Kluyveromyces marxianus* (4, 5), *K. dobzhanskii* (4, 5), *K. marxianus* × *K. dobzhanskii* (5, 12), *K. lactis* (5–7, 22), *Rhodotorula minuta* (3), *Debaryomyces polymorphus* (24, 25), and *C. guilliermondii* (19). Similarly, the enzyme responsible for the fermentative metabolism of cellobiose has been purified and characterized from *Dekkera intermedia* (1). The β-glucosidases from all of the above organisms are nonglycosylated, cytoplasmically located enzymes with M_r of about 300,000.

Since cellodextrins are not routinely used as substrates for the taxonomic identification of yeasts, the ability to ferment or assimilate cellodextrins might not be limited to *C. wickerhamii* and *C. molischiana*. I present data on the ability of the above-listed yeasts, and of several other yeasts that ferment cellobiose, to ferment cellodextrins and to metabolize them aerobically. In addition, to determine whether the yeasts tested have an enzyme(s) that hydrolyzes cellodextrins, crude enzyme preparations were tested for ability to catalyze the hydrolysis of purified cellotriose and cellotetrose.

MATERIALS AND METHODS

Source of chemicals and organisms. Yeast extract, malt extract, peptone, and glucose were purchased from Difco Laboratories, Detroit, Mich. *p*-Nitrophenyl-β-D-glucopyranoside was purchased from Sigma Chemical Co., St. Louis, Mo. A mixture of cellodextrins containing, by weight, approximately 5% glucose, 13% cellobiose, 29% cellotriose, 23% cellotetrose, 22% cellopentose, and 8% cellohexose was prepared as described previously (7, 18). Cellotriose and cellotetrose were purified from the cellodextrin mixture by charcoal-Celite chromatography as described previously (18). In addition to the above-listed yeasts from which β-glucosidases have been isolated and partially characterized, several other yeasts were included in this screening. *Pichia guilliermondii* was included in this screen because comparisons of nuclear DNA relatedness show it to be

TABLE 1. Yeasts used

NRRL designation[a]	Organism	Commonly used synonyms
Y-1118	*Kluyveromyces lactis* (Dombrowski) van der Walt	*Saccharomyces lactis* Dombrowski
Y-1140		
Y-1974	*Kluyveromyces dobzhanskii* (Shehata, Mrak & Phaff) van der Walt	*S. dobzhanskii* Shehata, Mrak & Phaff
Y-610	*Kluyveromyces marxianus* (Hansen) van der Walt	*Saccharomyces marxianus* Hansen, *K. fragilis* (Jorgensen) van der Walt
Y-1414	*Brettanomyces claussenii* Custers	
Y-1415	*Brettanomyces anomalus* Custers	
Y-1589	*Rhodotorula minuta* (Saito) Harrison	
YB-4241	*Dekkera intermedia* van der Walt	
Y-2022	*Debaromyces polymorphus* (Klocker) Price & Phaff	*Pichia polymorpha* Klocker, *Torulaspora phaffii* (Capriotti) van der Walt & Johannsen
Y-2075	*Pichia guilliermondii* Wickerham	
Y-5394	*Clavispora lusitaniae* Rodrigues de Miranda	*Candida lusitaniae* van Uden & do Carmo-Sousa
Y-324	*Candida guilliermondii* (Castelanii) Langeron & Guerra	
Y-2237	*Candida molischiana* (Zikes) Meyer & Yarrow	*Torulopsis molischiana* Zikes
Y-2563	*Candida wickerhamii* (Capriotti) Meyer & Yarrow	*Torulopsis wickerhamii* Capriotti

[a] NRRL, Northern Regional Research Laboratory.

conspecific with *C. guilliermondii* (13). *Clavispora lusitaniae*, *Brettanomyces claussenii*, and *B. anomalus* were included in this study because they ferment cellobiose efficiently (8, 11). A complete listing of the yeast species and strains used in this study in presented in Table 1. All cultures were obtained from the Agricultural Research Service Culture Collection, Northern Regional Research Center, Peoria, Ill.

Media and culture conditions. The basal medium consisted of 0.5% peptone, 0.3% yeast extract, and 0.3% malt extract. Inocula were prepared as described previously (8) by growing yeasts for 24 h in basal medium containing 20 g of glucose per liter. A 15-ml portion of basal medium containing 20 g of cellodextrins per liter in 25-ml flasks was inoculated with 0.225 ml of cell suspensions (1.5%, vol/vol). Flasks were capped with either cotton (aerobic growth) or serum stoppers and vented with 26-gauge needles (anaerobic growth). All cultures were incubated at 28°C on a rotary shaker at 300 rpm.

Carbohydrate analysis and β-glucosidase assay. The ability of yeasts to metabolize cellodextrins was determined by analyzing the culture beers for residual carbohydrates. Yeasts were grown either aerobically or fermentatively in basal medium that initially contained 20 g of the cellodextrin mixture per liter. Samples were taken at various times after inoculation, the cells were removed by centrifugation at 8,000 × *g* for 10 min, and the residual medium cellodextrins were analyzed by high-performance liquid chromatography (HPLC) on a Waters chromatograph fitted with a reverse-phase amino column (Regis, Morton Grove, Ill.). Acetonitrile-water (70:30) was used as the mobile phase. Carbohydrates were detected with a Waters R401 refractive index monitor. Only cultures that metabolized at least 20% of a particular cellodextrin after 5 days of incubation were scored as being capable of either fermenting or aerobically metabolizing it.

To determine whether the yeasts contained an enzyme(s) that hydrolyzed the larger cellodextrins, cells (1 ml) were harvested by centrifugation at 8,000 × *g* for 10 min, washed once with 25 mM sodium phosphate (pH 5.5), and resuspended in 0.5 ml of phosphate buffer. The cells were disrupted by vortexing at maximum speed for 3 min after the addition of 1.0 g of 0.25- to 0.3-mm glass beads (23). Cellular debris was removed by centrifugation at 12,000 × *g* for 15 min. The crude enzyme preparations (0.1 ml) were mixed with 0.1 ml of either 2.5 mM cellotriose or cellotetrose in 25 mM sodium phosphate (pH 5.5) and incubated for 18 h at 28°C. The reactions were terminated by heating at 100°C for 3 min, and the production of glucose and/or smaller oligomers was determined by HPLC as described above.

To determine whether the cultures produced an extracytoplasmic β-glucosidase activity, cells plus media were in-

TABLE 2. In vivo metabolism of cellodextrins and in vitro hydrolysis of cellotriose and cellotetrose by various yeasts

Organism	NRRL no.[a]	Growth condition[b]		In vitro hydrolysis[c]			
				G_3		G_4	
		$+O_2$	$-O_2$	$+O_2$	$-O_2$	$+O_2$	$-O_2$
K. lactis	Y-1118	G_2	G_1	−	−	−	−
B. claussenii	Y-1414	G_2	G_1	−	−	−	−
K. marxianus	Y-610	G_2	G_2	−	−	−	−
B. anomalus	Y-1415	G_2	G_2	−	−	−	−
R. minuta	Y-1589	G_2	G_2	+	+	+	+
K. dobzhanskii	Y-1974	G_2	G_2	−	−	−	−
D. intermedia	YB-4241	G_2	G_2	+	+	−	−
K. lactis	Y-1140	G_3	G_2	+	+	−	−
D. polymorphus	Y-2022	G_3	G_3	+	+	−	−
P. guilliermondii	Y-2075	G_3	G_3	+	+	+	−
C. lusitaniae	Y-5394	G_3	G_3	+	+	−	−
C. guilliermondii	Y-324	G_6[d]	G_3	+	+	+	−
C. molischiana	Y-2237	G_6[d]	G_6[d]	+	+	+	+
C. wickerhamii	Y-2563	G_6[d]	G_6[d]	+	+	+	+

[a] NRRL, Northern Regional Research Laboratory.

[b] Yeasts were grown either aerobically ($+O_2$) or fermentatively ($-O_2$) in medium containing 20 g of the cellodextrin mixture per liter. After 5 days of incubation, the medium was analyzed for residual cellodextrins by HPLC. The symbols represent the largest cellodextrin that was metabolized. Cellodextrins smaller than those listed for a particular yeast were also metabolized in all cases. G_1 represents glucose, G_2 represents cellobiose, G_3 represents cellotriose, and G_6 represents cellohexose.

[c] Crude enzyme preparations from 5-day cultures were incubated with either purified cellotriose (G_3) or cellotetrose (G_4). After 18 h of incubation, the carbohydrates were analyzed by HPLC. The reactions in which either glucose or cellodextrins smaller than the initial substrate were detected were rated as positive. The reactions in which the initial substrate was not hydrolyzed were rated as negative.

[d] Appeared to produced an extracytoplasmic β-glucosidase.

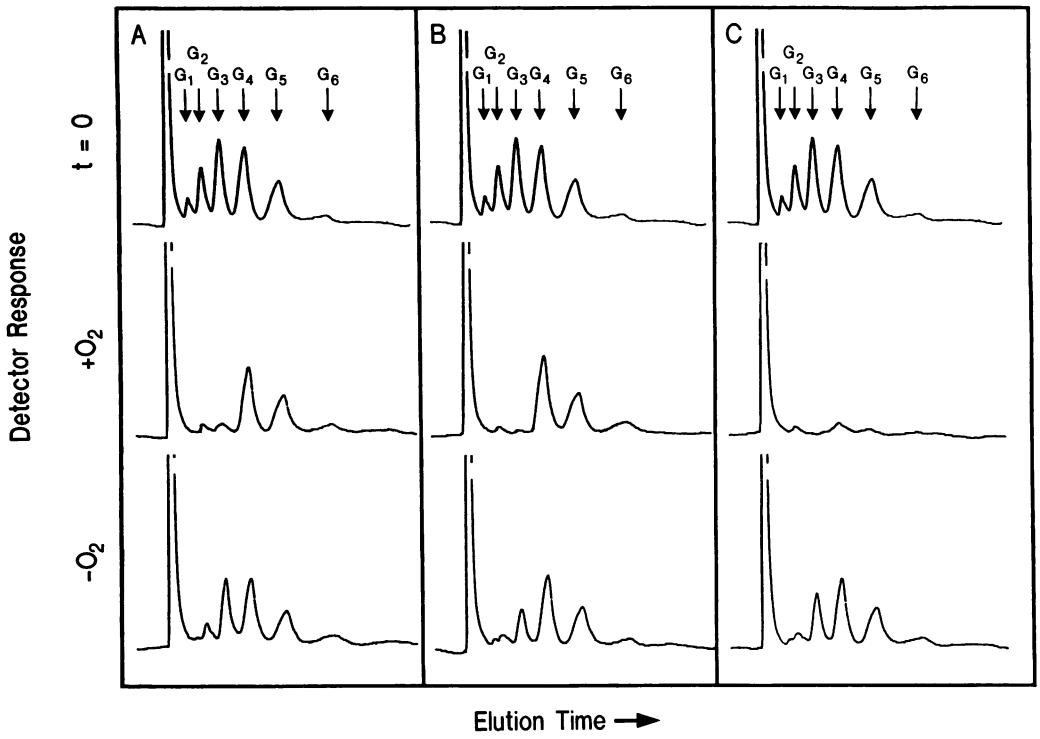

FIG. 1. Residual cellodextrins present in the culture beers after 5-day incubation of *D. polymorphus* (A), *C. lusitaniae* (B), and *C. guilliermondii* (C). The yeasts were grown either aerobically ($+O_2$) or fermentatively ($-O_2$) in basal medium initially containing 20 g of the cellodextrin mixture per liter ($t = 0$).

cubated at 28°C with 0.5 ml of 2.5 mM *p*-nitrophenyl-β-D-glucopyranoside (pNPG) in 10 mM phosphate buffer (pH 5.5). After 30 min, the reactions were stopped by the addition of 0.5 ml of 1 M glycine (pH 10.8) and scored for the production of the *p*-nitrophenol chromophore (8).

RESULTS

Aerobic metabolism of cellodextrins. The results of the experiment to determine whether the yeasts aerobically metabolized cellodextrins are presented in Table 2. The yeasts could be grouped into three classes: class 1, seven yeasts metabolized only glucose and cellobiose; class 2, four yeasts metabolized glucose, cellobiose, and cellotriose; and class 3, three yeasts metabolized cellodextrins of degree of polymerization (DP) ≤ 6. In general, the yeasts metabolized the smaller cellodextrins more extensively than they did the larger ones. For example, of the seven yeasts in class 1, all organisms completely metabolized the glucose from the medium, but only *K. dobzhanskii* and *B. claussenii* metabolized all of the cellobiose from the medium. The other five yeasts metabolized 25 to 75% of the cellobiose initially present in the medium. Of the four yeasts in class 2, all completely metabolized the glucose and cellobiose initially present in the medium. *D. polymorphus*, *P. guilliermondii*, and *C. lusitaniae* also completely metabolized the cellotriose, while *K. lactis* NRRL Y-1140 metabolized about 45% of it. Of the three yeasts that metabolized cellodextrins of DP ≤ 6 (class 3), only *C. molischiana* completely metabolized all of the carbohydrates. The other two yeasts left small amounts of cellobiose, cellotriose, and cellotetrose in the medium.

Anaerobic fermentation of cellodextrins. When grown fermentatively, the majority of the yeasts metabolized the same spectrum of cellodextrins as when grown aerobically (Table 2); however, the degree to which they fermented the larger oligomers was generally less. Three of the more striking examples of this were the degree to which *D. polymorphus* and *C. lusitaniae* fermented cellotriose and the degree to which *C. guilliermondii* fermented cellodextrins (Fig. 1). When grown aerobically, *D. polymorphus* and *C. lusitaniae* metabolized all of the cellotriose initially present in the medium. However, when grown anaerobically, these yeasts fermented only about 30 and 50% of the cellotriose, respectively. *C. guilliermondii* fermented none of the cellodextrins of DP > 3 and only about 30% of the cellotriose initially present in the medium, even though it aerobically metabolized cellodextrins of DP ≤ 6 (Fig. 1). Of the yeasts examined, *C. molischiana* and *C. wickerhamii* were the only two that both fermented and aerobically metabolized cellodextrins of DP > 3.

In vitro hydrolysis of cellotriose and cellotetrose. The inability of the majority of the yeasts tested to metabolize cellodextrins of DP > 3 might be because either their β-glucosidases do not recognize these cellodextrins as substrates or these organisms cannot transport them into the cells. To test the former possibility, crude cellular homogenates were incubated with either cellotriose or cellotetrose. The results (Table 2) showed that 10 of the 14 yeasts tested produced an enzyme(s) that hydrolyzed the same spectrum of cellodextrins as they fermented or aerobically metabolized. Whether any of these yeasts transport the larger cellodextrins is unknown; however, most do not possess a β-glucosidase(s) that catalyzes the hydrolysis of cellodextrins larger than those they metabolize. The exceptions to this were *R. minuta*, *D. intermedia*, *K. lactis*, and *P.*

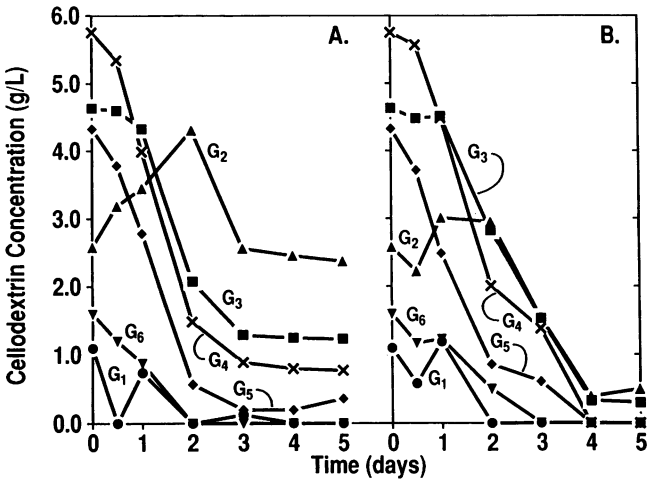

FIG. 2. Residual carbohydrates present in the culture beer when *C. wickerhamii* (A) and *C. molischiana* (B) were grown fermentatively in basal medium initially containing 20 g of the cellodextrin mixture per liter. Symbols: ●, glucose; ▲, cellobiose; ■, cellotriose; ×, cellotetrose; ◆, cellopentose; ▼, cellohexose.

guilliermondii, which produced an enzyme(s) that hydrolyzed cellodextrins larger than they metabolized in vivo. For example, *R. minuta* and *D. intermedia* fermented and aerobically metabolized only glucose and cellobiose, yet their crude cellular homogenates contained an activity(ies) that hydrolyzed cellotriose or cellotretrose or both. Similarly, crude homogenates of *K. lactis* (fermented no sugars larger than cellobiose) and *P. guilliermondii* (fermented no sugars larger than cellotriose) contained an activity(ies) that hydrolized cellotriose and cellotetrose, respectively. In these cases, it appears that the inability of these yeasts to ferment the larger cellodextrins is possibly due to an inability to transport the oligosaccharides.

Comparison of *C. molischiana* and *C. wickerhamii* fermentations. To assess the similarities or differences between *C. molischiana* and *C. wickerhamii*, the yeasts were grown both aerobically and anaerobically in basal media containing 20 g of cellodextrins per liter and the residual medium carbohydrates were analyzed at various times after inoculation. The results showed that the two yeasts appeared to metabolize the cellodextrins in a similar, but not identical, manner (Fig. 2). Both yeasts fermented the six carbohydrates concomitantly; however, *C. wickerhamii* fermented the larger cellodextrins slightly faster than did *C. molischiana*. For example, *C. wickerhamii* started fermenting the cellohexose from the cellodextrin mixture after 12 h of incubation and completely fermented it in 2 days, whereas *C. molischiana* started fermenting cellohexose after 1 day of incubation and completely fermented it in 3 days. *C. wickerhamii* also fermented cellopentose and cellotetrose slightly faster than did *C. molischiana*. The major difference in the cellodextrin fermentation patterns between the two yeasts was the greater rate at which *C. molischiana* fermented cellobiose. After 2 days of incubation, cellobiose increased in the *C. wickerhamii* fermentation broth from an initial concentration of 2.6 to 4.3 g/liter and represented about 50% of the total carbohydrate. In the *C. molischiana* fermentation broth, cellobiose increased to a concentration of 3.0 g/liter and represented about 34% of the total carbohydrate. Even though the *C. molischiana* fermentation broth contained less total carbohydrate than did the *C. wickerhamii* fermentation broth at 2 days, it contained about 30% more cellodextrins of DP 3 to 6 than did the *C. wickerhamii* fermentation broth. After 3 days of incubation, both cultures fermented most of the cellodextrins initially present in the medium; however, *C. molischiana* utilized more total carbohydrate than did *C. wickerhamii*. Similar cellodextrin metabolism patterns were found when these yeasts were grown aerobically, except that the cultures metabolized the carbohydrates more rapidly (data not shown).

DISCUSSION

Little is known about the mechanisms by which yeasts transport and metabolize cellobiose and cellodextrins. The β-glucosidases are cytoplasmically located enzymes in the majority of the yeasts from which they have been purified and partially characterized (1, 3–5, 12, 15, 16, 19, 24, 25). These yeasts metabolize only cellodextrins of DP ≤ 3, even though some contain an enzyme(s) capable of hydrolyzing larger cellodextrins. Only yeasts that appeared to produce an extracellular β-glucosidase (*C. molischiana*, *C. wickerhamii*, and *C. guilliermondii* when grown aerobically) metabolized cellodextrins of DP > 3 (Table 2) (6, 7, 10, 14). As these yeasts metabolized the larger cellodextrins, there was a concomitant increase in cellobiose (Fig. 2). This suggests that these yeasts do not transport cellodextrins directly, but, rather, first hydrolyze the cellodextrins and transport the hydrolysis products. Thus, it appears that one of the major factors limiting the metabolism of cellodextrins of DP > 3 is the inability of yeasts to transport the larger oligomers.

It should be noted that many yeast β-glucosidases are repressed by glucose and that the medium used in this study initially contained approximately 1.1 g of glucose per liter. The degree to which the enzymes were repressed in the individual yeasts studied here is unknown; however, from time course studies, glucose was not detected in any of the culture beers after 2 days, while the data reported represent samples taken after 5 days of incubation. Also, all of the crude enzyme preparations, except those from *K. lactis* and *B. claussenii* grown fermentatively, contained an activity(ies) that hydrolyzed both *p*-nitrophenyl-β-D-glucopyranoside and cellobiose (data not shown).

A comparison of the residual medium carbohydrates in the *C. wickerhamii* and *C. molischiana* fermentation broths indicated that *C. wickerhamii* preferentially metabolized the larger cellodextrins while *C. molischiana* showed a substrate preference for the smaller cellodextrins (Fig. 2). However, the gross mechanism by which both yeasts metabolized cellodextrins appears to be similar. The differences in substrate preference shown by these yeasts might be the result of enzymes that function similarly, yet have slightly different kinetic parameters. Multiple substrates of a single enzyme behave kinetically as if they are competitive inhibitors of each other (20). If the *C. molischiana* β-glucosidase had a lower K_m and/or a larger V_{max} for cellobiose than did the *C. wickerhamii* β-glucosidase, then less cellobiose would accumulate in the medium and the hydrolysis of the larger cellodextrins would be retarded, as compared with the *C. wickerhamii* culture broth. Alternatively, the variation in substrate utilization might result from differences in the ability of these yeasts to transport small oligomers. *C. wickerhamii* can transport only glucose (9). If *C. molischiana* transported cellobiose and cellotriose as well as glucose, this could also explain the apparent differential

substrate preference. Experiments are under way to investigate these possibilities.

REFERENCES

1. **Blondin, B., R. Ratomahenina, A. Arnaud, and P. Galzy.** 1983. Purification and properties of the β-glucosidase of a yeast capable of fermenting cellobiose to ethanol: *Dekkera intermedia* van der Walt. Eur. J. Microbiol. Biotechnol. **17**:1–6.
2. **Desrochers, M., L. Jurasek, and M. G. Paige.** 1981. High production of β-glucosidase in *Schizophyllum commune*: isolation of the enzyme and effects of culture filtrates on cellulose hydrolysis. Appl. Environ. Microbiol. **23**:222–228.
3. **Duerksen, J. D., and H. Halvorson.** 1958. Purification and properties of an inducible β-glucosidase of yeast. J. Biol. Chem. **233**:1113–1120.
4. **Fleming, L. W., and J. D. Duerksen.** 1967. Purification and characterization of yeast β-glucosidases. J. Bacteriol. **93**:135–141.
5. **Fleming, L. W., and J. D. Duerksen.** 1967. Evidence for multiple molecular forms of yeast β-glucosidase in a hybrid yeast. J. Bacteriol. **93**:142–150.
6. **Freer, S. N.** 1985. Purification and characterization of the extracellular β-glucosidase produced by *Candida wickerhamii*. Arch. Biochem. Biophys. **243**:515–522.
7. **Freer, S. N., and R. W. Detroy.** 1982. Direct fermentation of cellodextrins to ethanol by *Candida wickerhamii* and *C. lusitaniae*. Biotechnol. Lett. **4**:453–458.
8. **Freer, S. N., and R. W. Detroy.** 1983. Characterization of cellobiose fermentations to ethanol by yeasts. Biotechnol. Bioeng. **25**:541–557.
9. **Freer, S. N., and R. V. Greene.** 1990. Transport of glucose and cellobiose by *Candida wickerhamii* and *Clavispora lusitaniae*. J. Biol. Chem. **265**:12864–12868.
10. **Gondé, P., B. Blondin, M. LeClerc, R. Ratomahenina, A. Arnaud, and P. Galzy.** 1984. Fermentation of cellodextrins by different yeast strains. Appl. Environ. Microbiol. **48**:265–269.
11. **Gondé, P., B. Blondin, R. Ratomahenina, A. Arnaud, and P. Galzy.** 1982. Selection of yeast strains for cellobiose alcoholic fermentation. J. Ferment. Technol. **60**:579–584.
12. **Hu, A. S. L., R. Epstein, H. O. Halvorson, and R. M. Bock.** 1960. Yeast β-glucosidase: comparison of the physical-chemical properties of purified constitutive and inducible enzyme. Arch. Biochem. Biophys. **91**:210–219.
13. **Kurtzman, C. P.** Personal communication.
14. **LeClerc, M., P. Gondé, A. Arnaud, R. Ratomahenina, P. Galzy, and M. Nicolas.** 1984. The enzyme systems in a strain of *Candida wickerhamii* Meyer et Yarrow participating in the hydrolysis of cellodextrins. J. Gen. Appl. Microbiol. **30**:509–521.
15. **Marchin, G. L., and J. D. Duerksen.** 1968. Purification of β-glucosidase from *Saccharomyces lactis* strains Y-14 and Y-1057A. J. Bacteriol. **96**:1187–1190.
16. **Marchin, G. L., and J. D. Duerksen.** 1968. Purification of β-glucosidase from *Saccharomyces lactis* strain Y-123. J. Bacteriol. **96**:1181–1186.
17. **Marchin, G. L., and J. D. Duerksen.** 1969. Comparison of the catalytic and immunological properties of β-glucosidases from three strains of *Saccharomyces lactis*. J. Bacteriol. **97**:237–243.
18. **Miller, G. L., J. Dean, and R. Blum.** 1960. A study of methods for preparing oligosaccharides from cellulose. Arch. Biochem. Biophys. **91**:21–26.
19. **Roth, W. W., and V. R. Srinivasan.** 1978. Affinity chromatographic purification of β-glucosidase of *Candida guilliermondii*. Prep. Biochem. **8**:57–71.
20. **Segal, I. H.** 1975. Enzyme kinetics. Behavior and analysis of rapid equilibrium and steady-state enzyme systems, p. 113–118. Wiley-Interscience, New York.
21. **Sternberg, D., P. Vijayakumar, and E. T. Reese.** 1977. β-Glucosidase: microbial production and effect on enzymatic hydrolysis of cellulose. Can. J. Microbiol. **23**:139–147.
22. **Tingle, M., and H. O. Halvorson.** 1972. Biochemical and genetic characterization of β-glucosidase mutants in *Saccharomyces lactis*. J. Bacteriol. **110**:196–201.
23. **Van Etten, J. L., and S. N. Freer.** 1978. Simple procedure for disruption of fungal spores. Appl. Environ. Microbiol. **35**:622–623.
24. **Villa, T. G., V. Notario, and J. R. Villanueva.** 1975. β-Glucosidases of the yeast *Pichia polymorpha*. Arch. Microbiol. **104**: 201–206.
25. **Villa, T. G., V. Notario, and J. R. Villanueva.** 1979. β-Glucosidases in the yeast *Pichia polymorpha*. FEMS Microbiol. Lett. **6**:91–94.