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Thermotolerant Yeast for Simultaneous Saccharification and Fermentation of Cellulose to Ethanol

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ABSTRACT

Ten promising microbial strains were screened for glucose fermentation over the temperature range of 37–47°C, and five temperature-tolerant yeasts (*Saccharomyces cerevisiae* SERI strain (D_5A), *S. uvarum,* and *Candida* genera *acidothermophilium, brassicae,* and *lusitaniae*), were chosen for SSF evaluation on Sigmacell-50 cellulose with Genencor 150 L cellulase enzyme. *Brettanomyces clausenii* (Y-1414) was included for comparison to previous studies both by itself and in mixed culture with *S. cerevisiae* (D_5A). Good conversion rates were achieved at temperatures as high as 43°C with *C. brassicae* and *S. uvarum;* mixed cultures of either of these yeasts with the thermotolerant cellobiose fermenting yeast *C. lusitaniae* achieved higher rates and yields than any of the three yeasts alone. However, the mixed culture of *B. clausenii* and *S. cerevisiae* at 37°C achieved as high conversion rates and higher yields than any of the other yeasts tested.

Index Entries: Simultaneous saccharification and fermentation (SSF); yeast screening; thermotolerant yeasts; mixed cultures; cellulose conversions and rates.

INTRODUCTION

The rationale for researching lignocellulosic materials conversion to ethanol for fuel has been discussed in many previous publications (*1–15*).

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An important point is that lignocellulosics provide a low cost substrate for ethanol production. In addition, ethanol can be blended with gasoline as an octane enhancer and fuel extender or used as a neat fuel in internal combustion engines. This benefit is clearly illustrated by the Brazilian ethanol program. Although either acid or enzyme catalyzed hydrolysis can be used, enzymatic hydrolysis of the cellulosic substrate has at least two potential advantages; less expensive equipment may be possible at the more mild reaction conditions and higher recovery of fermentable substrates is possible, since enzymes are highly specific. However, to compete with the price of petroleum, the cost of enzyme, rate of hydrolysis, and product yield must be improved for enzymatic hydrolysis processes to prove viable.

Research on the simultaneous saccharification and fermentation (SSF) process has shown a potential for high rates and high ethanol yields from lignocellulosic materials via enzymatic hydrolysis coupled with yeast fermentation in the same vessel. This process, first studied by Takagi et al. (*1*), substantially reduces end-product inhibition of the cellulose enzyme while eliminating the need for one fermenter.

A number of yeast and several bacterial strains have been studied for cellulose, glucose, cellobiose, and/or xylose fermentations at various temperatures. Blotkamp et al. (*3*) looked at *S. cerevisiae* 4126 and 4132, *S. uvarum* (*carlsbergensis*), and *Candida brassicae* for SSF. In these studies, enzyme from *T. reesei* was used at 40 and 45°C, and the effects of different yeasts, cellulase loadings, and substrate concentrations were observed. *S. uvarum* was also used in a SSF study using *Penicillium funicolsum* cellulase (*4*). *Schizosaccharomyces pombe* (*1*), *Candida brassicae* (*1,5,7–11*), *C. lusitaniae* (*12–14*), and *C. acidothermophilium* (*15*) have all been used in various fermentation studies involving cellulose to ethanol production. In addition, a *S. cerevisiae* mutant strain (D_5A) has been used in two of our recent publications (*2,14*) for SSF research. In a recent publication (*2*), we found that a mixed culture of *Brettanomyces clausenii* and *S. cerevisiae* (D_5A) with Genencor 150L cellulase performed better at 37°C than either yeast alone or straight saccharification at 50°C. In addition to these yeast studies, the ethanol producing bacterium *Zymomonas mobilis* has been reported to produce comparable results to *S. cerevisiae* in several publications (*16–18*).

Although substantial improvements have been made in SSF, it is desirable to increase the temperature as high as possible since the rate limiting cellulose saccharification step would increase. Although several microbes look promising based on the referenced studies, all of the previous research was performed with different substrate types, enzymes, and substrate and enzyme loadings. Hence, this study was undertaken to measure the SSF performance of the most promising microorganisms identified in the literature so that we could compare them on a common basis and select the best ones possible for SSF.

MATERIALS AND METHODS

Materials

The following strains were ordered from the American Type Culture Collection (ATCC), Rockville, MD: *Candida acidothermophilium* 20831, *Candida brassicae* 32196, *Saccharomyces cerevisiae* 4126 and 4132, *Saccharomyces uvarum* 26602, and *Zymomonas mobilis* 10988 and B4490. *Schizosaccharomyces pombe* 1358, *Candida lusitaniae* 5394, and *Brettanomyces clausenii* Y1414 were obtained from the Northern Regional Research Laboratories (NRRL), USDA, Peoria, IL. SERI strain *S. cerevisiae* (D_5A) was derived by genetic improvements from commercial Red Star baker's yeast. Chemicals were purchased from the Sigma Chemical Company, as was the cellulose substrate Sigmacell-50. Other growth and fermentation media came from Difco. The cellulase enzyme employed was Genencor 150L from Genencor Inc., San Francisco, CA. This enzyme was characterized for activities and properties in our previous SSF paper (*2*). The Sigmacell-50 substrate is a relatively pure cellulose powder containing less than 3% xylose and has a crystallinity index of about 85.

Methods

The initial glucose (8.2 and 12.2%) screening fermentations (25 mL) were done at various temperatures (37, 41, 45, and 47°C) in 50 mL flasks constructed to vent CO_2 into water traps. The SSF fermentations were run in 250 mL flasks at 100 mL vol with water traps. All fermentations used 1% yeast extract and 2% peptone, and were agitated at 150 rpm. The substrate for SSFs was Sigmacell-50 at 7.5, 10, and 15% (w/v), and the enzyme was Genencor 150L cellulase at 7, 13, and 26 IU/g of substrate. In this paper, IU represents International Units of filter paper activity in micromoles of glu/min (*19*). A mixture of penicillin and streptomycin at 10 mg/L was used to inhibit contamination. A lipid mixture of ergosterol (5 mg/L) and oleic acid (30 mg/L) was also included in the media before autoclaving. Inoculations were 1:10 yeast/total vol in all cases. All components of the fermentation were added at the same time and allowed to become anaerobic on their own.

Residual glucose and cellobiose were measured in screening fermentations on a Model 27 glucose analyzer from Yellow Springs Instruments, Yellow Springs, OH. Cellobiose was measured as total sugar minus glucose by incubating the sample with 2 mg/mL almond extract (β-glucosidase) for 1 h at 37°C. Growth was also measured in these glucose fermentations with a Bausch and Lomb Spectronic 21. Ethanol was determined for both glucose and SSF fermentations via gas chromatography (Hewlett Packard 5880A, Porpack Q80/100 col) with 4% isopropanol as an internal standard. The percent equivalent conversion of cellulose reported here for all SSF experiments is determined by dividing the g/L ethanol by the

substrate concentration in g/L and multiplying by 196; this calculation accounts for the stoichiometry of ethanol production and assumes 90% conversion of sugars to ethanol by yeast, leaving 10% for cell growth. The result was used to provide an indication of cellulose utilization for ethanol production so that SSFs with different substrate concentrations could be compared to one another and to straight saccharification of cellulose.

RESULTS

Based on the referenced studies, ten strains of microorganisms were selected for this study: *Candida brassicae, C. lusitaniae, C. acidothermophilium, S. cerevisiae, S. uvarum, Schizosaccharomyces pombe,* and *Zymomonas mobilis.* To test for the thermotolerance of these microorganisms, fermentations were first run for 7 d at 37, 41, 45, and 47°C with glucose concentrations of 8.2 and 12.2% for all strains. Results for these glucose fermentations are shown in Table 1 as percent equivalent conversion efficiencies at the various temperatures. As a result of the initial glucose screening tests, three of the eight yeasts (i.e., *S. cerevisiae* 4126 and 4132 and *Schizosaccharomyces pombe* 1356) were eliminated because of poor performance at 41°C and above. Fermentations by the bacterium *Zymomona* 10988 and B4480 at 41°C and above were not promising enough to warrant consideration for experiments. The five strains left, *C. acidothermophilium* 20381, *C. brassicae* 32196, *S. uvarum* 20381, *C. lusitaniae* Y-5394, and *S. cerevisiae* (D_5A), could still ferment glucose at 45 and 47°C with yields ranging from 90–35% at 45°C and 60–17% at 47°C with the yeasts listed in order from best to least fermenters. *Brettanomyces clausenii* was also run at 37 and 41°C to confirm that it could not be employed above 37°C.

The five yeasts that passed the initial glucose temperature tolerance screening were run in 100 mL SSF cultures at substrate loadings of 7.5, 10, and 15% Sigmacell-50 cellulose and cellulase concentrations of 7, 13, and 26 IU/g of substrate, resulting in nine fermentations for each yeast at each temperature. Since previous publications on the thermotolerant yeasts reported growth up to 45°C, this temperature (45°C) was chosen as the high limit for SSFs. Furthermore, 45°C is the optimal temperature for cellulose degradation by the Genencor 150 L cellulase enzyme (Fig. 1).

C. brassicae, S. uvarum, and *C. lusitaniae* gave higher conversion rates than *S. cerevisiae* and *C. acidothermophilium* in small-scale SSFs, especially at the higher temperature of 43°C. As an example, Fig. 2 presents ethanol production data for *C. brassicae* in a small-scale SSF at an enzyme loading of 13 IU/g and the selected temperatures to reveal its increase in conversion rate with increase of temperature from 37–43°C, especially at the lower substrate concentrations. *S. uvarum* demonstrates similar perfor-

Table 1
Percent Yields of Ethanol from Glucose Fermentations for Comparison of the Ten Microbes for SSF at 37–47°C Temperatures and Substrate Loadings of 8.2 and 12.2% (w/v) Glucose

		37°C		41°C		43°C		45°C		47°C	
	Strain	8.2%	12.2%	8.2%	12.2%	8.2%	12.2%	8.2%	12.2%	8.2%	12.2%
C. acidothermophilum	20831	81.5	80.5	82.5	79.0	82.5	70.0	92.0	73.2	63.0	47.0
C. brassicae	32196	82.0	82.0	82.5	75.0	83.0	71.0	82.0	58.5	66.5	48.0
S. uvarum	26602	89.5	87.0	86.0	77.0	78.5	62.5	74.0	57.5	50.5	48.0
C. lusitaniae	5394	90.0	79.5	86.5	77.5	76.5	56.0	67.5	49.0	28.0	22.5
S. cerevisiae	(D_5A)	99.0	82.0	93.0	83.0	70.0	55.0	35.5	28.5	17.0	21.0
S. cerevisiae	4126	100.0	82.1	83.0	79.0	48.5	40.0	—	—	—	—[a]
S. cerevisiae	4132	85.0	80.5	20.0	15.0	—	—	—	—	—	—
S. pombe	1358	98.0	89.5	53.5	43.0	14.3	24.5	—	—	—	—
Z. mobilis	10988	90.0	95.0	27.5	38.5	—	—	—	—	—	—
Z. mobilis	14490	94.0	89.0	81.5	62.0	—	—	—	—	—	—

[a]—No ethanol production.

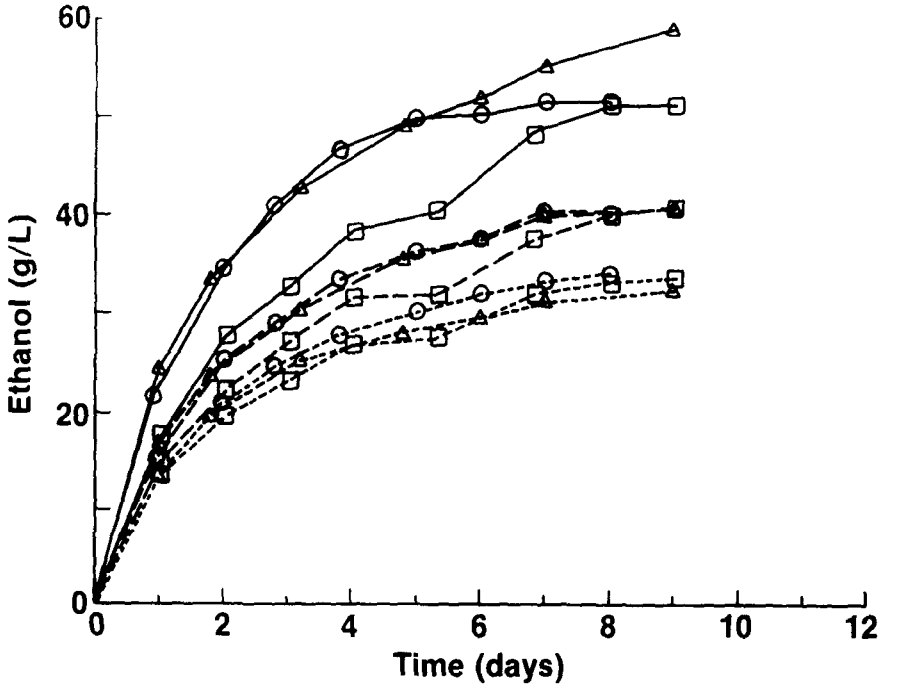

Fig. 1. Comparison of Genencor 150 L saccharification at 30 (◇), 37 (□), 45 (○), and 50°C (△) at cellulase loading of 26 IU/g with Sigmacell 50 cellulose at 10 (closed) and 15% (open) w/v.

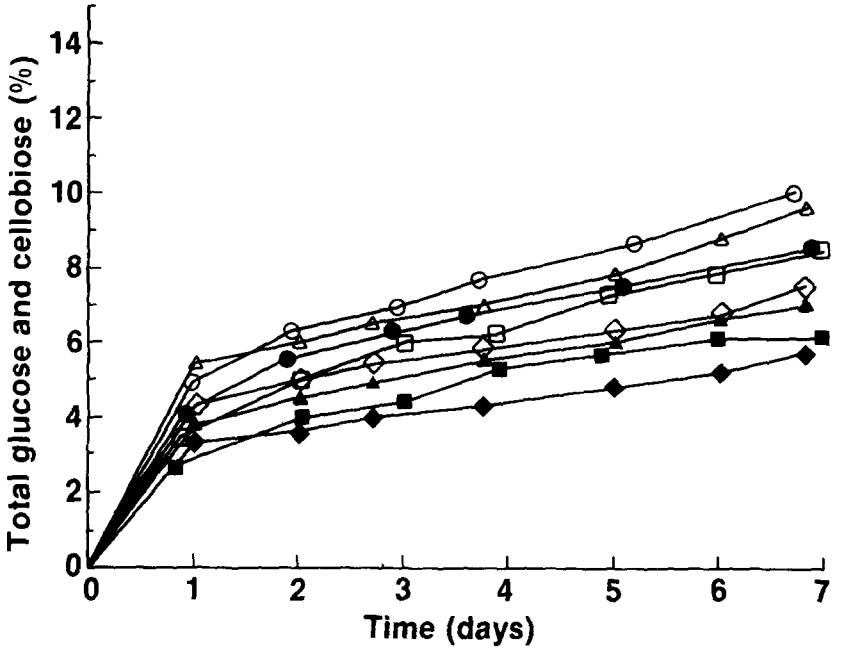

Fig. 2. Ethanol production in small scale SSF experiments for *C. brassicae* with Genencor 150 L cellulase enzyme at 37 (□), 41 (△), and 43°C (○). Enzyme loadings are 13 IU/g Sigmacell 50 cellulose substrate at 7.5 (---), 10 (--), and 15% (-) w/v.

mance to *C. brassicae,* as seen in Fig. 3. *C. lusitaniae* displays faster initial ethanol production rates in Fig. 4 and attains higher final ethanol conversions at 37°C. However, at 41 and 43°C, the final concentration is less than for *C. brassicae* or *S. uvarum.* Although not shown in the figures, *S. cerevisiae* (D_5A) and *C. acidothermophilium* perform about the same at 37 and 41°C, and the equivalent conversions of *S. cerevisiae* drop considerably at 43°C, whereas *C. acidothermophilium* stays steady at this temperature.

In Fig. 5, we have compiled results of the four most thermotolerant yeasts for comparison at selected temperatures and substrate concentrations at the completion of the fermentations. These data are given as percent equivalent cellulose conversion to facilitate comparison at various substrate levels and to straight saccharification. *S. uvarum* shows the most consistent results for 37, 41, and 43°C fermentations with the different substrates. The other three yeasts, *Candida lusitaniae, brassicae* and *acidothermophilium,* demonstrate the more common pattern of a decrease in equivalent conversion with increase in substrate and temperature. All of these yeasts have poor temperature tolerance at 45°C, with the exception of *C. lusitaniae,* which maintains comparable final cellulose conversions for 43 and 45°C temperatures with 7.5 and 10% loadings. To present a rate comparison of the four more thermotolerant yeasts, results at 10% substrate concentration and 13 IU/g enzyme loading were compiled

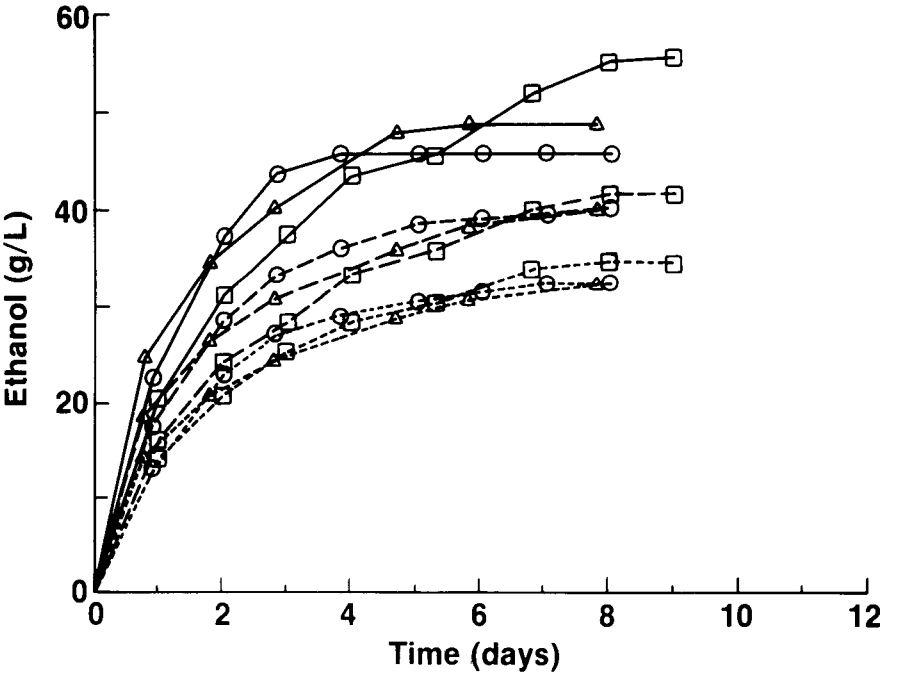

Fig. 3. Ethanol production in small scale SSF experiments for *S. uvarum* with Genencor 150 L cellulase enzyme at 37 (□), 41 (△), and 43°C (○). Enzyme loadings are 13 IU/g Sigmacell 50 cellulose substrate at 7.5 (---), 10 (--), and 15% (-) w/v.

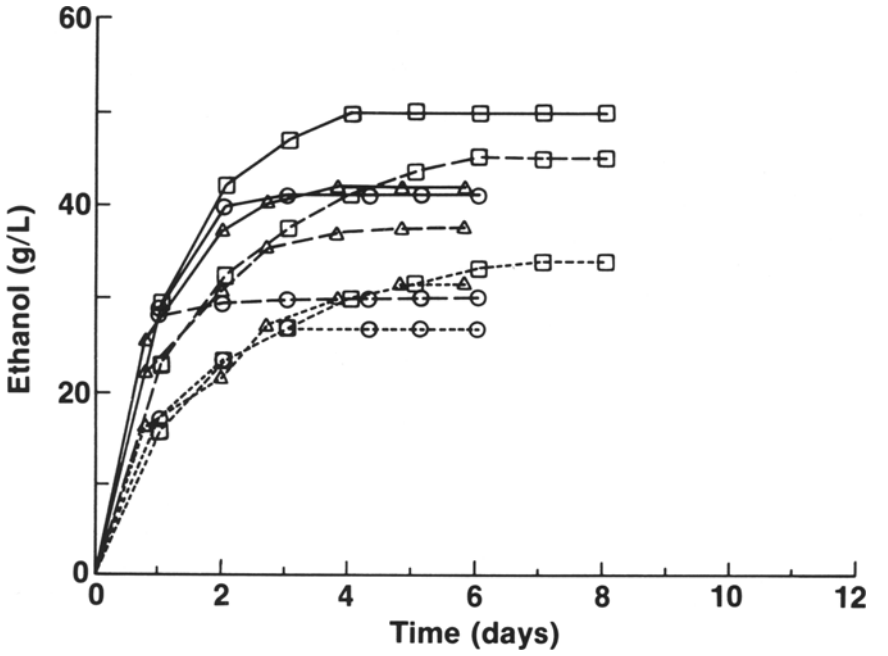

Fig. 4. Ethanol production in small scale SSF experiments for *C. lusitaniae* with Genencor 150 L cellulase enzyme at 37 (□), 41 (△), and 43°C (○). Enzyme loadings are 13 IU/g Sigmacell 50 cellulose substrate at 7.5 (---), 10 (--), and 15% (-) w/v.

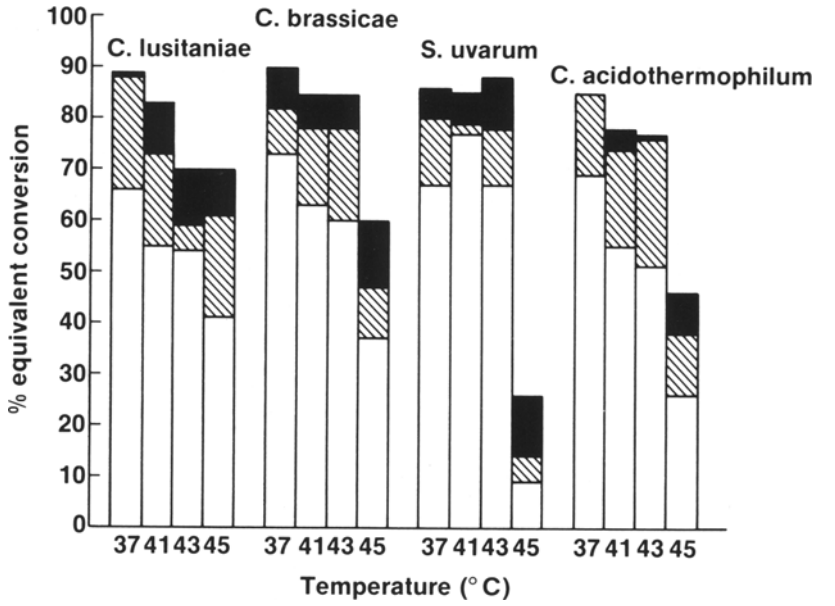

Fig. 5. Percent equivalent cellulose conversion as a function of temperature for four thermotolerant yeast at the completion of the fermentations. Genencor 150 L cellulase loading of 13 IU/g cellulose at 7.5 (■), 10 (□), and 15% (□) w/v Sigmacell 50.

in the bar graph form at different temperatures in Fig. 6. *C. lusitaniae* has the fastest 2 and 4-d fermentations at 37 and 41°C, and the other three yeasts give comparable rates of conversion.

At this point, the thermotolerant cellobiose fermenting yeast *C. lusitaniae* was evaluated in combination with the two best thermotolerant glucose fermenters *S. uvarum* (designated as mixed culture II) and *C. brassicae* (mixed culture III), as shown in Table 2. In addition, we have included *B. clausenii* and *S. cerevisiae* results from a previous paper along with the mixed culture of the two (mixed culture I).

As can be seen, mixed culture I of *B. clausenii* and *S. cerevisiae* achieved higher equivalent cellulose conversions than all other yeasts for any enzyme/substrate scenario at 37°C with the exception of the highest loadings of 26 IU for 10 and 15% substrate. At this loading, *C. brassicae* and *S. cerevisiae* were about equal to the mixed culture I 15% substrate results, and *C. lusitaniae* achieved the highest yield on 10% substrate.

If we look at the rates of ethanol production, we see another picture because cellulase performs better at higher (43–45°C) temperatures. In most cases, the rate of ethanol production was much greater when the temperature was increased from 37 to 43°C. Comparing the equivalent cellulose conversions at two days reported in Table 3, we see that mixed culture I excels at 37°C with *C. lusitaniae*, the next best. For the higher temperature runs at 41 and 43°C, the rates are quite similar, with the exception of the lower enzyme loading 7 IU/g where *C. lusitaniae* looks

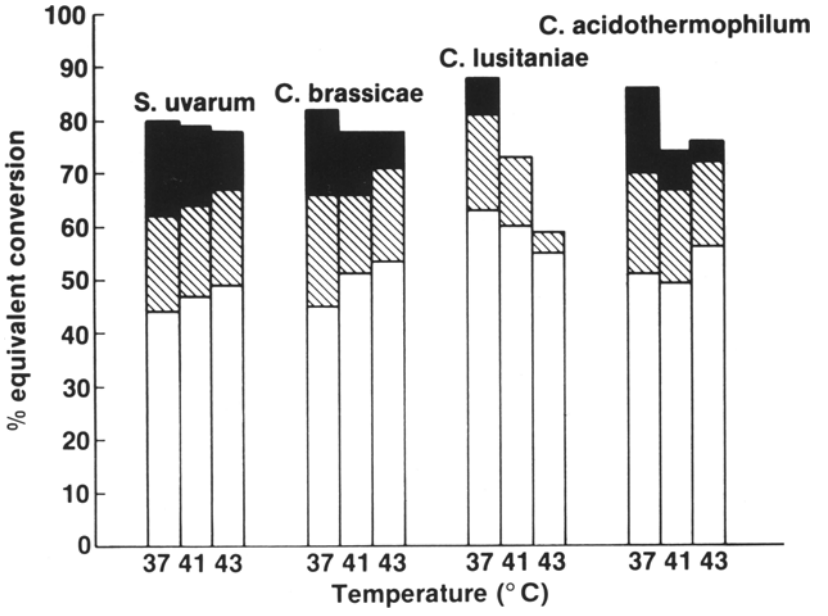

Fig. 6. Comparison of the percent equivalent cellulose conversions for four thermotolerant yeasts at selected temperatures with Genencor 150 L cellulase enzyme at a loading of 13 IU/g of 10% Sigmacell 50 cellulose substrate after 2 d (□), 4 d (□), and final (■).

Table 2
Performance of Six Yeast Strains and Their Mixed Cultures[a] Measured in Percent Equivalent Cellulose Conversions at End of Runs for Selected Temperatures, Substrate Concentrations, and Cellulase (Genencor 150 L) Loadings for Small Scale SSF Runs with Sigmacell 50 Cellulose

Substrate concentration:	7.5%					10%					15%			
Temperature:	37°C	41°C	43°C	45°C		37°C	41°C	43°C	45°C		37°C	41°C	43°C	45°C
7 IU/g														
Mixed cult. I	95	[b]	[b]	[b]	Mixed cult. I	88	[b]	[b]	[b]	Mixed cult. I	72	[b]	[b]	[b]
Mixed cult. II	84	83	78	[b]	Mixed cult. II	80	81	75	[b]	Mixed cult. II	68	67	61	[b]
Mixed cult. III	86	88	78	[b]	Mixed cult. III	83	84	76	[b]	Mixed cult. III	69	66	60	[b]
B. clausenii	86	[b]	[b]	[b]	*B. clausenii*	84	[b]	[b]	[b]	*B. clausenii*	72	[b]	[b]	[b]
C. lusitaniae	83	77	74	37	*C. lusitaniae*	80	75	60	56	*C. brassicae*	70	59	54	35
C. brassicae	83	81	84	43	*C. brassicae*	79	77	75	40	*C. lusitaniae*	65	55	48	43
S. cerevisiae	77	77	26	[b]	*S. cerevisiae*	73	77	29	[b]	*S. uvarum*	65	68	60	15
C. acidotherm.	74	76	61	42	*S. uvarum*	72	80	76	12	*S. cerevisiae*	64	66	36	[b]
S. uvarum	68	79	79	23	*S. acidotherm*	67	59	53	37	*C. acidotherm.*	55	46	45	28
13 IU/g														
Mixed cult. I	98	[b]	[b]	[b]	Mixed cult. I	89	[b]	[b]	[b]	Mixed cult. I	77	[b]	[b]	[b]
Mixed cult. II	91	85	84	[b]	Mixed cult. II	84	92	80	[b]	Mixed cult. II	74	68	64	[b]
Mixed cult. III	90	97	84	[b]	Mixed cult. III	83	85	78	[b]	Mixed cult. III	73	68	65	[b]
C. brassicae	90	84	84	60	*C. lusitaniae*	88	73	59	61	*S. cerevisiae*	76	64	37	[b]
C. lusitaniae	89	83	70	70	*C. acidotherm.*	86	74	76	38	*B. clausenii*	72	[b]	[b]	[b]
B. clausenii	89	[b]	[b]	[b]	*B. clausenii*	85	[b]	[b]	[b]	*C. brassicae*	73	63	60	37
S. uvarum	86	85	88	26	*C. brassicae*	82	78	78	47	*C. acidotherm*	69	55	51	26
S. cerevisiae	87	87	41	[b]	*S. cerevisiae*	81	76	39	[b]	*S. uvarum*	67	77	67	9
C. acidotherm.	83	78	77	46	*S. uvarum*	80	79	78	14	*C. lusitaniae*	66	55	54	41
26 IU/g														
Mixed cult. I	98	[b]	[b]	[b]	*C. lusitaniae*	96	74	64	58	*C. brassicae*	78	67	60	42
Mixed cult. II	90	90	86	[b]	Mixed cult. I	93	[b]	[b]	[b]	*S. cerevisiae*	79	67	38	[b]
Mixed cult. III	91	93	89	[b]	Mixed cult. II	82	84	80	[b]	Mixed cult. I	78	[b]	[b]	[b]
C. lusitaniae	94	88	77	67	Mixed cult. III	84	85	82	[b]	Mixed cult. II	74	67	63	[b]
C. brassicae	91	89	86	66	*C. brassicae*	93	85	78	49	Mixed cult. III	75	68	65	[b]
S. cerevisiae	91	86	55	[b]	*C. acidotherm.*	86	80	67	39	*C. uvarum*	77	78	63	27
B. clausenii	89	[b]	[b]	[b]	*S. uvarum*	86	88	80	16	*C. acidotherm.*	73	67	54	24
C. acidotherm.	89	89	88	48	*S. cerevisiae*	85	82	45	[b]	*B. clausenii*	72	[b]	[b]	[b]
S. uvarum	86	89	89	21	*B. clausenii*	83	[b]	[b]	[b]	*C. lusitaniae*	66	53	49	39

[a]Mixed Culture I: *B. clausenii* and *S. cerevisiae*; mixed Culture II: *C. lusitaniae* and *S. uvarum*, mixed Culture III: *C. lusitaniae and C. brassicae.*
[b]Not run, since yeast did not ferment glucose at this temperature.

Table 3
Percent Equivalent Cellulose Coversion after Two D for Small-Scale SSFs at Selected Temperatures, Substrate Concentrations, and Cellulase (Genecor 150 L) Loadings

	% Sigmacell 50	*B. clausenii*	Mixed cult. I[a]	Mixed cult. II[b]			Mixed cult III[b]			*S. cerevisiae* (D_5A)			*S. uvarum*			
IU/g	(w/v)	37°C	37°C	37°C	41°C	43°C	37°C	41°C	43°C	37°C	41°C	43°C	37°C	41°C	43°C	45°C
7	7.5	47	52	51	55	57	51	60	53	39	39	26	30	37	42	23
	10	51	47	49	52	50	53	49	49	38	39	30	37	43	42	12
	15	40	59	40	41	38	44	43	42	33	37	31	34	34	38	15
13	7.5	59	62	61	61	61	61	67	61	50	54	43	51	52	53	26
	10	52	53	55	62	54	58	61	53	49	32	40	44	47	49	14
	15	44	45	50	49	45	48	50	50	43	42	35	36	44	45	9
26	7.5	65	67	63	67	65	67	69	67	63	60	55	57	60	59	21
	10	53	59	59	60	60	61	70	60	56	55	44	55	55	57	16
	15	43	50	50	52	48	51	54	53	48	50	37	48	50	50	25

	% Sigmacell 50	*C. brassicae*				*C. lusitaniae*				*C. acidothermophilum*			
IU/g	(w/v)	37°C	41°C	43°C	45°C	37°C	41°C	43°C	45°C	37°C	41°C	43°C	45°C
7	7.5	43	47	51	39	50	47	61	36	33	44	45	36
	10	41	45	52	36	50	48	54	49	31	41	40	34
	15	37	38	39	34	44	43	49	34	26	38	39	25
13	7.5	54	55	60	54	61	56	63	34	44	55	60	38
	10	47	53	55	42	63	60	55	58	51	49	56	38
	15	40	46	48	32	55	49	52	41	46	46	45	25
26	7.5	60	63	67	35	70	65	67	65	60	63	65	46
	10	60	59	60	46	71	64	62	57	58	61	58	37
	15	54	52	54	42	54	49	47	39	50	52	52	25

[a] *B. clausenii* and S cerevisiae.
[b] C. lusitaniae and S uvarum.
[c] C. lusitaniae and C brassicae.

much better due to its β-glucosidase activity, especially in mixed cultures. Furthermore, *C. lusitaniae* gives an increase in ethanol yield for the higher cellulose loadings at 37°C compared to 43°C.

A 4-d comparison of the equivalent cellulose conversions in Table 4 reveals mixed culture I achieving the best results with the exception of *C. lusitaniae*, again at 37°C, which shows competitive rates at several enzyme/substrate loadings. *C. lusitaniae* also achieved about the same results as mixed culture I for a 15% substrate concentration. *C. lusitaniae* not only has cellobiose fermenting capability but has been shown to hydrolyze cellotriose as well (*13*). On the other hand, it has a low ethanol tolerance of only about 40 g/L.

If we examine mixed cultures I, II, and III, we see some close similarities. Mixed culture I has the best final equivalent cellulose conversion at 13 IU/g enzyme loading and 10% substrate concentration, with exception of the results for mixed culture II at 41°C, as illustrated in Fig. 7. The other two mixed cultures with *C. lusitaniae* show good rates initially for 41 and 43°C and tend to complete the fermentation with 24–48 h to spare, although at lower equivalent conversions than mixed culture I.

DISCUSSION

The enzymatic hydrolysis step is known to be rate limiting in the simultaneous saccharification and fermentation of cellulose to ethanol. Furthermore, since cellulase has an optimal temperature range of 45–50°C, use of yeast that will ferment at higher temperatures for SSFs should improve the rate of the SSF system. In this study, we measured the performance of several yeast and bacterial strains on a common basis, and four fermented in SSF up to temperatures of 43°C; *C. acidothermophilium, C. brassicae, C. lusitaniae, S. uvarum*. Yet, even though these strains could perform at these evaluated temperatures, the mixed culture that we previously studied still performed as well or better at 37°C than the more thermotolerant strains at any temperatures studied for an SSF system. Since sugars should be released more rapidly as the SSF temperature is increased, these results lead us to believe that the yeast does not ferment all of the sugar produced at high temperatures to ethanol. Experiments are now in progress to measure the cellulose, sugar, ethanol, yeast, and cellobiose concentrations along with cellulase activity in larger scale fermenters to determine the cause of this unexpected behavior.

Better performance was observed for mixed cultures of cellobiose fermenters with strong glucose fermenting yeast for both *B. clausenii* and *C. lusitaniae*. Thus, the ability to ferment both glucose and cellobiose readily would be very valuable. As an alternative to the mixed culture approach, it may be more desirable to genetically modify a yeast strain to achieve high ethanol tolerance, good glucose fermentation, and cellobiose utilization. Alternatively, the SSF process could be augmented with

Table 4
Percent Equivalent Cellulose Coversion after 4 D for Small Scale SSFs at Selected Temperatures, Substrate Concentrations, and Cellulase (Genencor 150 L) Loadings

	% Sigmacell 50	*B. clausenii*	Mixed cult. I	Mixed cult II[a]			Mixed cult. III[c]			*S. cerevisiae* (D_5A)			*S. uvarum*			
IU/g	(w/v)	37°C	37°C	37°C	41°C	43°C	37°C	41°C	43°C	37°C	41°C	43°C	37°C	41°C	43°C	45°C
7	7.5	67	78	68	72	71	68	75	73	55	61	26	48	58	63	23
	10	64	70	65	68	69	70	63	69	56	57	29	54	63	63	12
	15	53	56	53	54	53	57	57	54	46	52	35	49	51	54	15
13	7.5	77	84	75	75	76	75	84	81	68	75	42	70	71	72	26
	10	70	71	68	76	76	72	76	76	63	71	39	62	64	67	14
	15	54	60	64	62	60	61	63	65	57	59	37	50	60	61	9
26	7.5	78	84	76	78	82	81	84	87	78	76	55	73	77	73	21
	10	72	76	70	70	78	74	76	81	69	76	45	71	75	70	17
	15	60	66	61	63	63	63	66	64	59	63	38	63	65	63	27

	% Sigmacell 50	*C. brassicae*				*C. lusitaniae*				*C. acidothermophilum*			
IU/g	(w/v)	37°C	41°C	43°C	45°C	37°C	41°C	43°C	45°C	37°C	41°C	43°C	45°C
7	7.5	62	62	71	43	71	67	71	37	54	61	59	42
	10	60	60	69	40	70	72	61	56	49	55	49	37
	15	53	51	52	35	59	55	48	43	44	45	45	28
13	7.5	74	69	76	70	78	78	70	70	64	71	71	46
	10	66	66	71	47	81	73	59	61	70	67	72	38
	15	57	58	60	35	65	55	54	41	62	47	51	26
26	7.5	79	75	76	66	87	84	77	67	78	76	81	48
	10	78	73	76	49	86	74	64	58	75	73	63	39
	15	70	63	60	42	65	52	49	40	65	51	54	25

[a] *B clausenii* and S cerevisiae.
[b] C. lusitaniae and S uvarum.
[c] C lusitaniae and C brassicae.

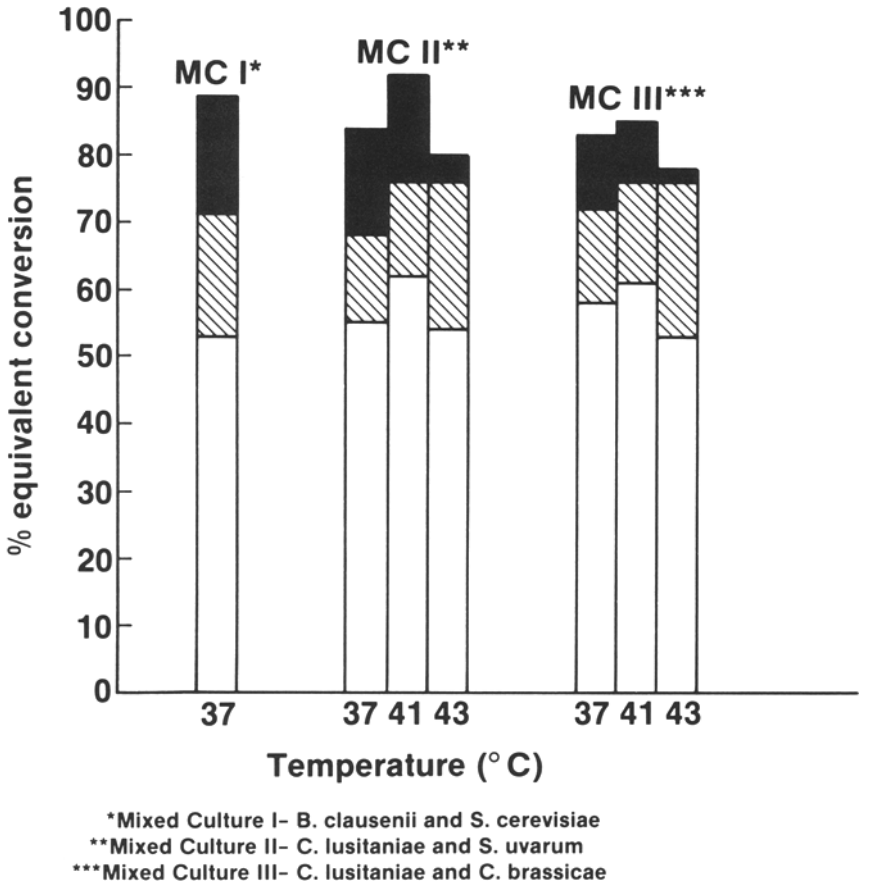

Fig. 7. Comparison of the percent equivalent cellulose conversions for mixed cultures at selected temperatures with Genencor 150 L cellulase enzyme at a loading of 13 IU/g of 10% Sigmacell 50 substrate after 2 d (□), 4 d (□), and final day (■).

extra β-glucosidase to increase the rates of fermentation, a more feasible approach in the short term.

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