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Transient-State Analysis of Metabolic Fluxes in Crabtree-Positive and Crabtree-Negative Yeasts

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In bakers' yeast, an immediate alcoholic fermentation begins when a glucose pulse is added to glucose-limited, aerobically grown cells. The mechanism of this short-term Crabtree effect was investigated via a comparative enzymic analysis of eight yeast species. It was established that the fermentation rate of the organisms upon transition from glucose limitation to glucose excess is positively correlated with the level of pyruvate decarboxylase (EC 4.1.1.1). In the Crabtree-negative yeasts, the pyruvate decarboxylase activity was low and did not increase when excess glucose was added. In contrast, in the Crabtree-positive yeasts, the activity of this enzyme was on the average sixfold higher and increased after exposure to glucose excess. In Crabtree-negative species, relatively high activities of acetaldehyde dehydrogenases (EC 1.2.1.4 and EC 1.2.1.5) and acetyl coenzyme A synthetase (EC 6.2.1.1), in addition to low pyruvate decarboxylase activities, were present. Thus, in these yeasts, acetaldehyde can be effectively oxidized via a bypass that circumvents the reduction of acetaldehyde to ethanol. Growth rates of most Crabtree-positive yeasts did not increase upon transition from glucose limitation to glucose excess. In contrast, the Crabtree-negative yeasts exhibited enhanced rates of biomass production which in most cases could be ascribed to the intracellular accumulation of reserve carbohydrates. Generally, the glucose consumption rate after a glucose pulse was higher in the Crabtree-positive yeasts than in the Crabtree-negative yeasts. However, the respiratory capacities of steady-state cultures of Crabtree-positive yeasts were not significantly different from those of Crabtree-negative yeasts. Thus, a limited respiratory capacity is not the primary cause of the Crabtree effect in yeasts. Instead, the difference between Crabtree-positive and Crabtree-negative yeasts is attributed to differences in the kinetics of glucose uptake, synthesis of reserve carbohydrates, and pyruvate metabolism.

Alcoholic fermentation during bakers' yeast production is undesirable. Ethanol formation can be prevented by growing the organism under sugar limitation. However, because bakers' yeast is produced in large fermentors, sugar concentration gradients occur. When glucose concentrations rise above 100 to 200 mg/liter, aerobic alcoholic fermentation sets in (23, 24). This fermentative response to higher sugar concentrations is known as the short-term Crabtree effect (14, 20).

The Crabtree effect has been the subject of many studies (1, 3, 4, 7, 19a, 20, 22). The main goal of these studies was to assess the role of the respiratory capacity of *Saccharomyces* species in the Crabtree effect. Käppeli (7) emphasized that *Saccharomyces* spp. possess limited respiratory capacities, which cause increased intracellular pyruvate concentrations. Only when pyruvate accumulates does the fermentative enzyme pyruvate decarboxylase become important, since its affinity for pyruvate is much lower than that of pyruvate dehydrogenase, the key enzyme of the oxidative route (6, 22). However, van Urk et al. (19a, 20) have demonstrated that the respiratory capacities of steady-state cultures of *Saccharomyces cerevisiae* CBS 8066 are not significantly lower than the respiratory capacity of *Candida utilis* CBS 621, a yeast which does not exhibit the Crabtree effect. Instead, the anabolic capacities of steady-state cultures, the ability to accumulate intracellular reserve carbohydrates, and pyruvate decarboxylase activity appear to be the important parameters in the occurrence of the short-term Crabtree effect.

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In order to ascertain the parameters of the physiological behavior of yeasts upon transition from glucose limitation to glucose excess, it was decided to perform a comparative study on a variety of yeast species.

MATERIALS AND METHODS

Strains. The following yeast strains were obtained from the Centraal Bureau voor Schimmelcultures (CBS; Delft, The Netherlands): *S. cerevisiae* CBS 8066, *Torulopsis glabrata* CBS 138, *Schizosaccharomyces pombe* CBS 356, *Brettanomyces intermedius* CBS 1943, *Candida utilis* CBS 621, *Hansenula nonfermentans* CBS 5764, *Kluyveromyces marxianus* CBS 6556, and *Pichia stipitis* CBS 5773. These strains were maintained on malt agar slopes. In addition, some experiments were carried out with a leaky pyruvate decarboxylase mutant of *S. cerevisiae* (*pdc* 2-122) (17). This strain was maintained on yeast extract-peptone agar slopes containing 2% ethanol.

Culture conditions. The organisms were grown aerobically in chemostat cultures under glucose limitation at $D = 0.1\ h^{-1}$ as described previously (20). The glucose concentration in the reservoir medium was 5 g/liter. The *pdc* mutant was grown at a dilution rate of $0.05\ h^{-1}$, since its μ_{max} was only $0.1\ h^{-1}$. The medium for the mutant was the same as that for the other yeasts, except that it also contained 40 mg of DL-leucine $liter^{-1}$. After 2 days of batch growth on ethanol, the dilution rate was set at $0.05\ h^{-1}$. After another 2 days, the ethanol-containing reservoir was exchanged for a glucose-containing reservoir, and the dilution rate was kept at $0.05\ h^{-1}$.

Glucose pulse experiments. The glucose pulse experiments

were performed in duplicate with each organism. After a steady state had been reached, the glucose feed was stopped and 50 mM glucose was added aseptically to the cultures. Samples were taken at regular intervals for the determination of metabolite concentrations, enzyme levels, and dry weight, as well as protein, glycogen, and trehalose contents of the cells. During the experiment, the oxygen consumption and CO_2 production rates were continuously monitored via analysis of the exhaust gas by a paramagnetic oxygen analyzer and an infrared carbon dioxide analyzer. The methods used for gas analysis and the determination of glucose, ethanol, acetate, and pyruvate were as described by van Urk et al. (20).

For comparative purposes, the pulse experiments were divided into three phases: the first 30 min of the experiment (phase I), a phase between 30 and 60 min after glucose addition (phase II), and hour 2 of the pulse experiment (phase III). If the glucose was consumed within 2 h (i.e., in the case of *T. glabrata*), the consumption and production rates during phase III were calculated over the period in which glucose was still present. The glucose and oxygen consumption rates and metabolite production rates (see Table 1) were calculated by linear regression of the analytical data obtained during the three respective phases.

Carbon recoveries during the steady-state situations were between 95 and 105% for all yeasts tested and between 80 and 120% during the pulse experiments for all except for *Schizosaccharomyces pombe*, which excreted other products besides pyruvate, acetate, ethanol, and carbon dioxide. The nature of these products was not investigated.

Enzyme assays. For the determination of enzyme activities, samples were taken from steady-state cultures and from cultures 30, 60, and 120 min after glucose addition during the glucose pulse experiments. Cells were washed in 10 mM potassium phosphate (pH 7.5) containing 2.5 mM EDTA and frozen at −20°C until the preparation of extracts (within 1 month). Extracts were prepared by sonication of cells in a 75 mM potassium phosphate buffer (pH 6.5) containing 5 mM $MgSO_4$, 1 mM thiamine PP_i, and 2 mM dithiothreitol with an MSE-150 W sonifier. Enzyme activities were assayed in freshly prepared extracts with a Hitachi 100-60 spectrophotometer at 340 nm. In all cases, the enzyme activity was linearly proportional to the amount of extract used. The assays of the individual enzymes were performed as described by Postma et al. (16).

Determination of glycogen and trehalose. Glycogen and trehalose contents of cells were determined as described by van Urk et al. (20).

RESULTS

Selection of strains. We selected eight yeast species for the determination of transient responses after the exposure of yeasts to glucose excess. These species had already been characterized in the literature with respect to the occurrence of the Crabtree effect. Generally, however, information on their responses to excess sugar concerned only long-term adaptation, which involves the repression of respiratory enzymes in aerobic batch cultures. It was anticipated that like *S. cerevisiae*, other Crabtree-positive yeasts would respond to a sudden exposure to glucose excess by an instantaneous, aerobic alcoholic fermentation (i.e., a short-term Crabtree effect [14, 20]). This proved to be the case in all four Crabtree-positive yeasts selected (*S. cerevisiae* CBS 8066, *T. glabrata* CBS 138, *Schizosaccharomyces pombe* CBS 356, and, to a lesser extent, *B. intermedius* CBS 1943). This phenomenon was not observed in *C. utilis* CBS 621, *H. nonfermentans* CBS 5764, *K. marxianus* CBS 6556, or *P. stipitis* CBS 5773, which are known to carry out alcoholic fermentation under oxygen-limited conditions. Despite its name, *H. nonfermentans* also belongs to this class of facultatively fermentative yeasts, although it scores negative in the classical taxonomic fermentation test (19).

Rate of biomass production. The rate of biomass production of three of the Crabtree-positive yeasts immediately after glucose addition (phase I) remained about equal to that during the steady state in the chemostat (Table 1). *T. glabrata* behaved differently. Its rate of biomass production during phase I was twice that of the steady-state culture. After adaptation to glucose excess, the growth rates of the four yeasts increased (Table 1). The protein content of the Crabtree-positive yeasts remained approximately constant during the experiments (Table 2); it may be concluded that the rate of biomass production is directly related to the growth rate. In the Crabtree-negative yeasts, the growth rate cannot be calculated directly from the increase in dry weight, since synthesis of reserve carbohydrates in these organisms was triggered by a glucose pulse (Table 2). *K. marxianus* behaved differently in this respect: its content of reserve carbohydrates remained constant (Table 2). The growth rates of the Crabtree-negative yeasts should be calculated on the basis of the protein content of the cells and the rate of biomass production (20). In all Crabtree-negative species, the protein content decreased during phase I. When the growth rate is estimated on the basis of protein, it appears that also these yeasts initially (phase I) continued to grow at the same rate as during glucose limitation. This conclusion may be drawn even in the case of *K. marxianus*, although the decrease in protein content did not coincide with an increase in glycogen or trehalose (Table 2). Possibly, other reserve materials such as lipids were accumulated in *K. marxianus*.

Respiration rate. From a comparison of the Crabtree-positive and Crabtree-negative yeasts, no clear correlation between respiratory activity (q_{O_2}) and fermentation rate ($q_{ethanol} + q_{acetate}$) (Table 1) can be drawn. For instance, the respiration rates of the Crabtree-negative yeasts *H. nonfermentans* and *P. stipitis* after transition to glucose excess were even lower than the respiration rate of *S. cerevisiae*. Moreover, a low rate of respiration did not coincide with a high rate of fermentation, as is evident from the behavior of *B. intermedius* (Table 1).

Glucose consumption rate. The Crabtree-positive yeasts generally exhibited a higher rate of glucose consumption than the Crabtree-negative species. *C. utilis* and *B. intermedius* behaved exceptionally. The rate of glucose consumption was markedly higher in *C. utilis* than in the other three Crabtree-negative yeasts. However, this organism accumulated more glycogen as a reserve carbohydrate than the rest. This accumulation of reserve carbohydrates results in a low glycolytic rate. The rate of glycolysis can be estimated, roughly, as follows: $q_{glycolysis} = q_{glucose} - q_{glycogen} - q_{trehalose}$ (in millimoles of glucose units · $gram^{-1}$ · $hour^{-1}$). It can be calculated that the $q_{glycolysis}$ in the Crabtree-negative yeasts during phase I was 1.8 to 3.5 mmol · g^{-1} · h^{-1}, whereas this parameter was 6.2 to 7.5 in the Crabtree-positive yeasts, excluding *B. intermedius*. In *B. intermedius*, $q_{glycolysis}$ during phase I was much lower (1.1 mmol · g^{-1} · h^{-1}) than in the other Crabtree-positive yeasts. This is not surprising, in view of the low rates of growth, respiration, and fermentation encountered in this organism (Table 1). Altogether, these results establish that the carbon

TABLE 1. Rates of biomass production[a]

Type and yeast	Phase	μ_{DW} (h^{-1})	q (mmol · g of cells^{-1} · h^{-1}) for:					
			O_2	CO_2	Glucose	Ethanol	Acetate	Pyruvate
Crabtree positive								
S. cerevisiae	SS	0.10	2.7	2.9	1.1	0	0	0
	I	0.09	6.0	10.3	5.4	5.6	1.3	0.01
	II	0.30	7.0	11.7	8.8	7.4	1.3	0.08
	III	0.43	6.8	18.1	10.4	10.6	0	0.09
T. glabrata	SS	0.10	3.2	3.4	1.2	0	0	0
	I	0.20	4.7	11.2	7.1	9.2	0	0.08
	II	0.17	4.9	13.0	8.1	11.1	0	0.10
	III	0.28	5.2	11.8	8.5	9.6	0.03	0.08
Schizosaccharomyces pombe[b]	SS	0.10	3.6	3.9	1.3	0	0	0.03
	I	0.08	4.2	9.8	7.1	5.3	0.3	1.20
	II	0.11	4.3	11.1	8.5	6.8	0.3	1.08
	III	0.16	4.6	12.4	7.2	6.7	0.3	0.64
B. intermedius	SS	0.10	1.9	2.0	1.0	0	0	0
	I	0.13	2.3	2.6	1.1[c]	0.1	0.2	0
	II	0.11	2.5	2.9	1.1[c]	0.5	0.4	0
	III	0.14	2.6	3.3	2.0	0.8	0.8	0.01
Crabtree negative								
C. utilis	SS	0.10	2.7	2.9	1.1	0	0	0
	I	0.59	6.6	6.3	5.0	0.05	0.05	0
	II	0.36	7.4	6.9	5.0	0.05	0.05	0.01
	III	0.34	8.1	8.0	3.4	0.05	0.05	0.10
H. nonfermentans	SS	0.10	3.7	3.9	1.3	0	0	0
	I	0.25	5.1	5.6	2.5[c]	0	0	0.23
	II	0.26	5.7	6.3	2.5[c]	0	0	0.22
	III	0.16	6.2	6.6	2.9	0	0	0.08
K. marxianus	SS	0.10	4.2	4.2	1.4	0	0	0
	I	0.21	7.1	7.1	3.5[c]	0	0	0
	II	0.26	10.5	10.1	3.5[c]	0	0	0
	III	0.45	11.0	10.5	3.9	0	0	0.03
P. stipitis	SS	0.10	3.8	4.2	1.3	0	0	0
	I	0.28	5.7	6.5	3.3[c]	0	0	0
	II	0.32	5.7	6.4	3.3[c]	0	0	0
	III	0.27	4.9	5.5	4.1	0	0	0

[a] Calculated from dry weight measurements (μ_{DW}), specific consumption rates of oxygen (q_{O_2}) and glucose ($q_{glucose}$), and specific production rates of CO_2 (q_{CO_2}), ethanol ($q_{ethanol}$), acetate ($q_{acetate}$), and pyruvate ($q_{pyruvate}$) under steady-state conditions (SS) and after a glucose pulse (phases I, II, and III; see Materials and Methods). The estimated error is ±0.03 h^{-1} for the μ_{DW} and ±0.3 mmol · g of cells^{-1} · h^{-1} for the specific oxygen consumption and CO_2 production rates. The estimated error in the other specific rates is about 25%.

[b] Carbon recovery during the pulse experiment was not 100%, because of the formation of additional products.

[c] Because of a low glucose consumption rate, a reliable value could be calculated only for the rate during phases I and II together.

flux via glycolysis is an important factor in the occurrence of the short-term Crabtree effect.

Excretion of pyruvate. The key enzyme of alcoholic fermentation, pyruvate decarboxylase, is known to have a low affinity for pyruvate compared with the enzyme needed for oxidation of pyruvate, pyruvate dehydrogenase (5, 22). Various investigators have stressed the importance of this difference in substrate affinity for explaining the Crabtree effect (5, 14). When a bottleneck in the metabolism outside the glycolytic pathway occurs, pyruvate may accumulate and fermentation may result. Indeed, a high rate of alcoholic fermentation was generally accompanied with the excretion of pyruvate (Table 1). However, the reverse does not hold. In phase III, *C. utilis* rapidly excreted pyruvate, but the rate of ethanol production was very low. *H. nonfermentans* immediately excreted pyruvate upon exposure to glucose excess, but there was no alcoholic fermentation (Table 1). Also, the *pdc* mutant of *S. cerevisiae* excreted pyruvate at a high rate, but alcoholic fermentation was low (Table 3).

Enzyme levels. As discussed above, the high fermentation rates of Crabtree-positive yeasts must result from high intracellular pyruvate concentrations and high pyruvate decarboxylase activities. Indeed, the pyruvate decarboxylase activities in these yeasts were higher than those in the Crabtree-negative yeasts (Table 4). Steady-state cultures of a *pdc* mutant of *S. cerevisiae* contained a pyruvate decarboxylase level that was lower than that encountered in the Crabtree-negative yeasts (compare Tables 4 and 5). Nevertheless, the mutant exhibited alcoholic fermentation upon a glucose pulse. These results clearly show that the level of pyruvate decarboxylase is not the only determinant for the occurrence of aerobic alcoholic fermentation after the transition of cells from glucose limitation to glucose excess.

The activities of pyruvate decarboxylase increased signif-

TABLE 2. Protein, glycogen, and trehalose contents of yeasts grown under glucose-limited conditions (SS) and during glucose pulse experiments (30 and 60 min after glucose addition to steady-state cultures)[a]

Type and yeast	Time (min)	Amt of protein (g · g [dry wt]$^{-1}$ · 100)	Amt [(g of glucose units · g of dry wt^{-1}) · 100] of:	
			Glycogen	Trehalose
Crabtree positive				
S. cerevisiae	SS	42	6	4
	30	43	2	0
	60	44	2	0
T. glabrata	SS	41	7	1
	30	40	5	2
	60	40	7	1
S. pombe	SS	42	2	5
	30	42	2	1
	60	42	3	1
B. intermedius	SS	40	6	0
	30	37	6	0
	60	40	6	0
Crabtree negative				
C. utilis	SS	53	3	0
	30	40	17	0
	60	39	14	0
H. nonfermentans	SS	39	2	1
	30	37	6	4
	60	37	8	5
K. marxianus	SS	52	1	0
	30	50	1	0
	60	48	1	0
P. stipitis	SS	48	1	0
	30	39	10	0
	60	35	16	0

[a] $D = 0.1\ h^{-1}$. The estimated error is ±3 for the protein determination and ±1 for the glycogen and trehalose determinations.

icantly in the Crabtree-positive yeasts during glucose pulse experiments (Table 4). In contrast, the activities of pyruvate decarboxylase in the Crabtree-negative yeasts remained much lower compared with those encountered in the Crabtree-positive yeasts (Table 4). The other important enzyme for fermentation, alcohol dehydrogenase, was present at high levels in the Crabtree-positive yeasts. Of the Crabtree-negative yeasts, only *P. stipitis* did not have detectable levels of this enzyme, which explains its Crabtree-negative behavior. On the other hand, all Crabtree-negative yeasts had acetaldehyde dehydrogenase activity and hence should have been able to produce acetate. The reason why the Crabtree-negative yeasts excreted little if any acetate (Table 1) may be that acetate was immediately metabolized to acetyl coenzyme A (CoA) by the enzyme acetyl-CoA synthetase (Table 4). In the Crabtree-positive yeasts, this bypass oxidation route may also be used (6, 10) (Table 4). However, because the activity of the enzyme pyruvate decarboxylase in these yeasts was higher than that of acetyl-CoA synthetase, alcoholic fermentation was the predominant process at high intracellular pyruvate concentrations.

TABLE 3. Rates of growth, substrate consumption, and product formation of *S. cerevisiae pdc* 2-122 (17)[a]

Phase	μ_{DW} (h^{-1})	q (mmol · g of cells^{-1} · h^{-1}) for:					
		O_2	CO_2	Glucose	Ethanol	Acetate	Pyruvate
SS	0.05	2.6	2.7	0.7	0	0	0
I	0	3.3	3.4	1.7[b]	0.4	0.1	0.94
II	0	3.4	4.0	1.7[b]	1.0	0.2	0.81
III	0	3.3	4.9	2.0	1.8	0.5	0.71

[a] Growth rates (μ_{DW}), specific consumption rates of oxygen (q_{O_2}) and glucose ($q_{glucose}$), and specific production rates of ethanol ($q_{ethanol}$), acetate ($q_{acetate}$) and pyruvate ($q_{pyruvate}$) under steady-state conditions (SS) and after a glucose pulse (phases I, II, and III; see Materials and Methods). The estimated error is ±0.03 h^{-1} for μ_{DW} and ±0.3 mmol · g of cells^{-1} · h^{-1} for specific oxygen consumption and CO_2 production rates. The estimated error in the other specific rates is about 25%.

[b] Because of a low glucose consumption rate, a reliable value could be calculated only for the rate during phases I and II together.

DISCUSSION

The results of our comparative study on the metabolic behavior of eight yeast species show that several parameters are decisive for the occurrence of the short-term Crabtree effect. These include the rate of glucose consumption, the capacity to accumulate reserve carbohydrates, the level of pyruvate decarboxylase in the cells during exposure to glucose excess, and the growth rate under this condition.

It is evident that a prerequisite for alcoholic fermentation is the presence of the enzymes of the fermentative route, pyruvate decarboxylase and alcohol dehydrogenase. This prerequisite was fulfilled in all cases but one (i.e., *P. stipitis*, which did not contain alcohol dehydrogenase under the culture conditions employed [Table 4]). Generally, a high rate of alcoholic fermentation correlated with high levels of pyruvate decarboxylase. This can be seen from a plot of the rates of fermentation (i.e., $q_{ethanol} + q_{acetate}$) of the various yeasts and their pyruvate decarboxylase levels (Fig. 1). A similar pattern for *pdc* mutants of *S. cerevisiae* was found by Schmitt and Zimmermann (17) under conditions in which long-term effects are relevant. The correlation in Fig. 1 is, however, not strict, indicating that other regulating factors are also involved. For example, the *pdc* mutant of *S. cerevisiae* had approximately the same level of pyruvate decarboxylase as the Crabtree-negative yeasts but nevertheless carried out alcoholic fermentation, albeit at a low rate. An important factor in this respect is the branching point at the level of acetaldehyde, at which fermentation and respiration compete for the same substrate. In the Crabtree-negative yeasts, the rate of respiration of acetaldehyde is probably high enough to prevent even a low rate of ethanol production.

An enhanced flux through the glycolytic pathway is the basis of the short-term Crabtree effect (see above). High rates of alcoholic fermentation were typically encountered in the species that exhibited high rates of glucose consumption (Fig. 2). However, the physiological interpretation of the glucose consumption rate should be made carefully. This parameter is not identical to the rate of glycolysis. In *C. utilis*, for example, a high rate of glucose consumption was observed, but this was largely due to the accumulation of glycogen. In addition, not only the rate of catabolism (fermentation and respiration) but also the rate of anabolism contributes to the rate of glycolysis. This is because the synthetic pathways of many cell constituents originate at or beyond the level of pyruvate.

TABLE 4. Enzyme activities (units · milligram of protein^{-1}) under steady-state conditions (SS) and after a glucose pulse (phases I, II, and III)

Type and yeast	Phase	Pyruvate decarboxylase	Alcohol dehydrogenase (NAD^+)	Acetaldehyde dehydrogenase (NAD^+)	Acetaldehyde dehydrogenase ($NADP^+$)	Acetyl-CoA synthetase
Crabtree positive						
S. cerevisiae	SS	0.58	7.00	0.71	0.23	0.05
	I	0.71	6.56	0.77	0.23	0.05
	II	0.85	5.74	0.79	0.22	0.03
	III	1.12	6.39	0.78	0.24	0.02
T. glabrata	SS	0.73	3.73	0.07	0.04	0.21
	I	0.79	3.60	0.08	0.03	0.21
	II	0.94	3.60	0.05	0.03	0.18
	III	1.05	3.98	0.03	0.04	0.15
Schizosaccharomyces pombe	SS	0.29	0.97	0.17	0.05	0.07
	I	0.31	1.21	0.17	0.04	0.06
	II	0.41	1.45	0.15	0.04	0.04
	III	0.61	1.48	0.05	0.02	0.04
B. intermedius	SS	0.24	3.85	0.44	0.18	0.16
	I	0.26	3.90	0.41	0.18	0.18
	II	0.32	3.76	0.37	0.17	0.19
	III	0.37	3.64	0.36	0.17	0.17
Crabtree negative						
C. utilis	SS	0.08	2.91	0.13	0.04	0.50
	I	0.09	2.86	0.13	0.03	0.54
	II	0.11	2.22	0.13	0.02	0.44
	III	0.11	1.61	0.10	0.02	0.44
H. nonfermentans	SS	0.08	0.40	0.19	0.03	0.11
	I	0.08	0.30	0.19	0.03	0.10
	II	0.08	0.25	0.18	0.03	0.10
	III	0.09	0.25	0.17	0.22	0.10
K. marxianus	SS	0.05	1.04	0.64	0.10	0.36
	I	0.05	1.12	0.54	0.09	0.37
	II	0.05	1.23	0.54	0.09	0.37
	III	0.06	1.17	0.49	0.07	0.39
P. stipitis	SS	0.09	0.00	0.10	0.00	0.09
	I	0.11	0.00	0.10	0.00	0.09
	II	0.13	0.00	0.10	0.00	0.05
	III	0.12	0.00	0.09	0.00	0.05

The primary event that causes an enhanced rate of glycolysis is the increased uptake of glucose from the environment. Different mechanisms of glucose uptake exist in *Saccharomyces* spp. and *Candida* spp. In *Saccharomyces* spp., glucose is taken up by facilitated diffusion (8, 9), and in *Candida* spp., glucose is taken up by proton symport (11, 13, 15, 18). Recent work in our laboratory on glucose uptake has shown that this difference between *Saccharomyces* spp. and *Candida* spp. holds for Crabtree-positive and Crabtree-negative yeasts in general (21), i.e., all the Crabtree-negative yeasts tested exhibited glucose uptake by active transport (21).

TABLE 5. Enzyme activities (units · milligram of protein^{-1}) under steady-state conditions (SS) and after a glucose pulse (phases I, II, and III) of *S. cerevisiae pdc* 2-122 (17)

Phase	Pyruvate decarboxylase	Alcohol dehydrogenase (NAD^+)	Acetaldehyde dehydrogenase (NAD^+)	Acetaldehyde dehydrogenase ($NADP^+$)	Acetyl-CoA synthetase
SS	0.03	2.13	0.23	0.16	0.04
I	0.05	2.75	0.26	0.15	0.05
II	0.09	2.70	0.26	0.13	0.07
III	0.11	2.50	0.24	0.13	0.05

The short-term Crabtree effect in *Saccharomyces uvarum* H2055 (14) has been explained in terms of a limited respiratory capacity. Although this may apply to this particular strain, it cannot be a general explanation for this effect. Alcoholic fermentation in the Crabtree-positive yeasts upon transition from glucose limitation to glucose excess was not associated with low respiratory activity (Table 1). Moreover, compared with the steady-state respiratory activities, all yeasts increased their specific oxygen consumption rates upon transition to glucose excess. Apparently, they possessed a catabolic overcapacity but, in most cases, no anabolic overcapacity. In other words, the protein synthesis rates before and immediately after glucose addition were equal in most of the yeasts tested. As discussed previously (20), under glucose excess, this will lead to an uncoupling of catabolic and anabolic reactions. Therefore, overflow reactions will occur in the Crabtree-positive and Crabtree-nega-

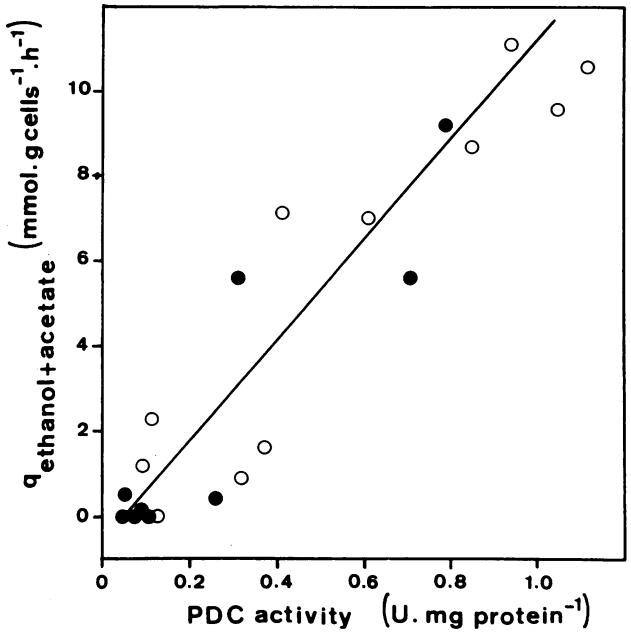

FIG. 1. Relationship between the rates of ethanol and acetate excretion and pyruvate decarboxylase activities during glucose pulse experiments. Symbols: ●, phase I; ○, phase II and phase III. Data from Tables 1 and 3–5.

tive yeasts. Similar results were obtained by Neijssel and Tempest (12) with aerobic bacterial chemostats of *Klebsiella aerogenes* which were pulsed with excess glucose.

We conclude that the different behaviors of Crabtree-positive and Crabtree-negative yeasts must be explained in terms of the kinetics of glucose uptake, the rate of glycolysis, anabolic limitations, and the levels of pyruvate decarboxylase, alcohol dehydrogenase, acetaldehyde dehydrogenases, and acetyl-CoA synthetase.

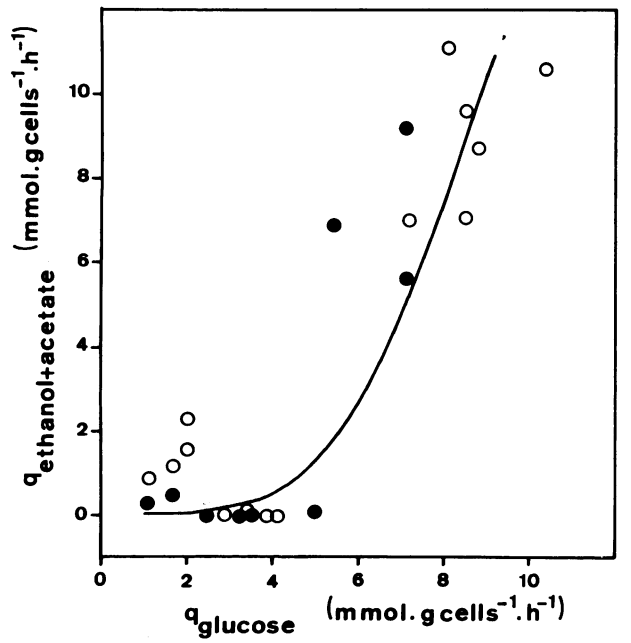

FIG. 2. Relationship between the rates of ethanol and acetate excretion and glucose consumption rates during glucose pulse experiments. Symbols: ●, phase I; ○, phase II and phase III. Data from Tables 1 and 3.

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LITERATURE CITED

1. **Barford, J. P., P. M. Jeffery, and R. J. Hall.** 1981. The Crabtree effect in *Saccharomyces cerevisiae*—primary control mechanism or transient? p. 255–260. *In* M. Moo-Young, C. W. Robinson, and C. Vezina (ed.), Advances in biotechnology, vol. 1. Pergamon Press, Inc., Toronto.
2. **Bisson, L. F., and D. G. Fraenkel.** 1983. Involvement of kinases in glucose and fructose uptake by *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **80**:1730–1734.
3. **De Deken, R. H.** 1966. The Crabtree effect: a regulatory system in yeast. J. Gen. Microbiol. **44**:149–156.
4. **Fiechter, A., G. F. Fuhrmann, and O. Käppeli.** 1981. Regulation of glucose metabolism in growing yeast cells. Adv. Microb. Physiol. **22**:123–183.
5. **Holzer, H.** 1961. Regulation of carbohydrate metabolism by enzyme competition. Cold Spring Harbor Symp. Quant. Biol. **26**:277–288.
6. **Holzer, H., and H. W. Goedde.** 1957. Zwei Wege von Pyruvat zu Acetyl-Coenzym A in Hefe. Biochem. Z. **329**:175–191.
7. **Käppeli, O.** 1986. Regulation of carbon metabolism in *Saccharomyces cerevisiae* and related yeasts. Adv. Microb. Physiol. **28**:181–209.
8. **Kotyk, A., and D. Michaljanicová.** 1978. Transport kinetics of 6-deoxy-D-glucose in *Candida parapsilosis*. Folia Microbiol. **23**:18–26.
9. **Lang, J. M., and V. P. Cirillo.** 1987. Glucose transport in a kinaseless *Saccharomyces cerevisiae* mutant. J. Bacteriol. **169**: 2932–2937.
10. **Llorente, N., and I. Núñez de Castro.** 1977. Physiological role of yeasts NAD(P)$^+$ and NADP$^+$-linked aldehyde dehydrogenases. Rev. Esp. Fisiol. **33**:135–142.
11. **Loureiro-Dias, M. C.** 1987. Glucose and polyol transport systems in *Candida intermedia* and their regulation. J. Gen. Microbiol. **133**:2737–2742.
12. **Neijssel, O. M., and D. W. Tempest.** 1976. The role of energy-spilling reactions in the growth of *Klebsiella aerogenes* NCTC 418 in aerobic chemostat culture. Arch. Microbiol. **110**:305–311.
13. **Ongjoco, R., K. Szkutnicka, and V. P. Cirillo.** 1987. Glucose transport in vesicles reconstituted from *Saccharomyces cerevisiae* membranes and liposomes. J. Bacteriol. **169**:2926–2931.
14. **Petrik, M., O. Käppeli, and A. Fiechter.** 1983. An expanded concept for the glucose effect in the yeast *Saccharomyces uvarum*: involvement of short- and long-term regulation. J. Gen. Microbiol. **129**:43–49.
15. **Postma, E., W. A. Scheffers, and J. P. van Dijken.** 1988. Adaptation of the kinetics of glucose transport to environmental conditions in the yeast *Candida utilis* CBS 621: a continuous-culture study. J. Gen. Microbiol. **134**:1109–1116.
16. **Postma, E., C. Verduyn, W. A. Scheffers, and J. P. van Dijken.** 1989. Enzymic analysis of the Crabtree effect in glucose-limited chemostat cultures of *Saccharomyces cerevisiae*. Appl. Environ. Microbiol. **55**:468–477.
17. **Schmitt, H. D., and F. K. Zimmermann.** 1982. Genetic analysis of the pyruvate decarboxylase reaction in yeast glycolysis. J. Bacteriol. **151**:1146–1152.
18. **Spencer-Martins, I., and N. van Uden.** 1985. Catabolite interconversion of glucose transport systems in the yeast *Candida wickerhamii*. Biochim. Biophys. Acta **812**:168–172.
19. **van Dijken, J. P., E. van den Bosch, J. J. Hermans, L. Rodrigues de Miranda, and W. A. Scheffers.** 1986. Alcoholic fermentation by 'non-fermentative' yeasts. Yeast **2**:123–127.

19a. **van Urk, H., P. M. Bruinenberg, M. Veenhuis, W. A. Scheffers, and J. P. van Dijken.** 1989. Respiratory capacities of mitochondria of *Saccharomyces cerevisiae* CBS 8066 and *Candida utilis* CBS 621 grown under glucose limitation. Antonie van Leeuwenhoek **56**:211–220.

20. **van Urk, H., P. R. Mak, W. A. Scheffers, and J. P. van Dijken.** 1988. Metabolic responses of *Saccharomyces cerevisiae* CBS 8066 and *Candida utilis* CBS 621 upon transition from glucose limitation to glucose excess. Yeast **4**:283–291.

21. **van Urk, H., E. Postma, W. A. Scheffers, and J. P. van Dijken.** 1989. Glucose transport in Crabtree-positive and Crabtree-negative yeasts. J. Gen. Microbiol. **135**:2399–2406.

22. **van Urk, H., D. Schipper, G. J. Breedveld, P. R. Mak, W. A. Scheffers, and J. P. van Dijken.** 1989. Localization and kinetics of pyruvate-metabolizing enzymes in *Saccharomyces cerevisiae* CBS 8066 and *Candida utilis* CBS 621. Biochim. Biophys. Acta **992**:78–86.

23. **Verduyn, C., T. P. L. Zomerdijk, J. P. van Dijken, and W. A. Scheffers.** 1984. Continuous measurement of ethanol production by aerobic yeast suspensions with an enzyme electrode. Appl. Microbiol. Biotechnol. **19**:181–185.

24. **Woehrer, W., and M. Roehr.** 1981. Regulatory aspects of bakers' yeast metabolism in aerobic fed-batch cultures. Biotechnol. Bioeng. **23**:567–581.