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Abstract

To select a *Saccharomyces cerevisiae* reference strain amenable to experimental techniques used in (molecular) genetic, physiological and biochemical engineering research, a variety of properties were studied in four diploid, prototrophic laboratory strains. The following parameters were investigated: 1) maximum specific growth rate in shake-flask cultures; 2) biomass yields on glucose during growth on defined media in batch cultures and steady-state chemostat cultures under controlled conditions with respect to pH and dissolved oxygen concentration; 3) the critical specific growth rate above which aerobic fermentation becomes apparent in glucose-limited accelerostat cultures; 4) sporulation and mating efficiency; and 5) transformation efficiency via the lithium-acetate, bicine, and electroporation methods. On the basis of physiological as well as genetic properties, strains from the CEN.PK family were selected as a platform for cell-factory research on the stoichiometry and kinetics of growth and product formation.

Keywords: Yeast; Strain choice; Physiology; Genetics; Engineering

1. Introduction

Optimization of the yeast cell factory for industrial applications requires a multidisciplinary effort of (molecular) geneticists, physiologists, and biochemical engineers. Each of these disciplines imposes specific requirements on the yeast strains used for experimental work. Required properties, that often seem to be noncompatible in a single strain, include high transformation efficiency, fast growth in defined mineral media under controlled conditions, high rates of respiratory growth in sugar-limited chemostat cultures, etc. (Table 1).

The conflicts of interest that may arise in selecting a suitable strain platform for multidisciplinary research are exemplified by *Saccharomyces cerevisiae* CBS 8066. This prototrophic, diploid wild-type strain has been extensively characterized with respect to its physiology [1–5]. However, strain CBS 8066 is heterozygous for a number of auxotrophic markers and poorly transformable, which is a major complication in integrated genetic/physiological studies.

As a result of their different immediate research goals, geneticists, physiologists and biochemical engineers use a

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Table 1
Summary of desired properties of a reference strain for quantitative studies on the *Saccharomyces cerevisiae* cell factory

- Fast growth in defined mineral media without supplements other than vitamins
- Wide range of carbon and nitrogen sources for growth
- High biomass yield on carbon source
- Fast aerobic, respiratory growth in glucose-limited chemostat cultures
- Growth in defined media under strictly anaerobic conditions
- High sporulation efficiency, spore viability, and mating efficiency
- High transformation efficiency
- Genetically stable
- Good production of heterologous proteins, both intra- and extracellularly

variety of *S. cerevisiae* strains. Even within each single discipline, a multitude of strains are studied because, for obvious reasons, a particular research group tends to adhere to the "house strain" with which previous research has been carried out. It is evident that a common reference strain, and isogenic mutants derived from it, is of great value for studies on quantitative aspects of growth and metabolism. Such studies require the use of prototrophic strains and defined mineral media. Cultivation of strains carrying auxotrophic markers requires the addition of amino acids, purine or pyrimidine bases to the medium, which complicates physiological studies [6] and in some cases obscures changes in physiological properties caused by genetic modification. For example, *S. cerevisiae* mutants lacking the E1α subunit of the mitochondrial pyruvate-dehydrogenase complex exhibit a partial leucine requirement [7] and mutants lacking glucose-6-phosphate dehydrogenase require methionine [8]. These interesting effects would remain unnoticed in leucine/methionine auxotrophs or in complex media.

In view of the lack of consensus on the choice of a common reference strain for genetic, physiological, and engineering studies, we decided to characterize a number of laboratory strains that are currently in use in our laboratories. Emphasis was given to standard transformation tests and cultivation under well-defined conditions. The results obtained show that strains from the CEN.PK family offer an acceptable compromise between the criteria set by different research disciplines.

2. Materials and methods

2.1. Strains

Four diploid, prototrophic laboratory strains were used in this study:

1. *Saccharomyces cerevisiae* CBS8066. This strain was obtained from the Centraal Bureau voor Schimmelcultures (CBS, Delft, The Netherlands) and included because it has been extensively characterized with respect to its physiological properties [1–5]. A drawback of this diploid strain for genetic studies is that it is heterozygous for the auxotrophic recessive alleles *his4*, *leu1*, and *met2* (H.Y. Steensma and J. Bauer, unpublished).
2. *S. cerevisiae* BAY.17. This strain was developed by J. Bauer and P. Niederberger as a a homozygous prototrophic diploid derived from CBS8066, with the aim to combine the good growth performance of CBS8066 with 1) a diploid, highly isogenic background for good genetic performance (high spore viability, good chromosome segregation); 2) homozygous prototrophy; and 3) heterothallic behavior. The strain development was the result of spore dissection of asci obtained from strain CBS8066 followed by three cycles of genetic crossing of segregants. Two segregants of CBS8066 of opposite mating type were crossed, the resulting diploid was sporulated and the tetrads dissected. Two of the resulting segregants (R8066-2A and R8066-9A) were crossed and again sporulation was followed by tetrad dissection. One of the segregants was back-crossed with one of the haploid parent strains (R8066-2A), resulting in the diploid strain BAY.17. For each generation a total of 20 segregants were characterized. The criteria for the selection of the segregants for further crossing steps were the existence of a mating type (heterothallic behavior), the absence of sporulation, prototrophy, and high specific growth rates in shake-flask cultures on a mineral medium containing 0.7% (v/v) ethanol and 0.3% (v/v) acetic acid as carbon sources.
3. *S. cerevisiae* X2180 was obtained from the Yeast Genetic Stock Center (University of California at Berkeley, USA). The haploid test strains X2180-1A (*MATa*) and X2180-1B (*MAT*α) were obtained from Dr. J. Bauer. X2180 is a popular laboratory strain for molecular genetic and cell biology studies on *S. cerevisiae*. Most literature data on this strain have been obtained with cells grown in shake-flask cultures [9–11].
4. *S. cerevisiae* CEN.PK122 as well as the prototrophic haploids derived from this strain were obtained from Dr. P. Kötter (J.W. Goethe Universität, Frankfurt, Germany). The CEN.PK strain family was constructed as part of an interdisciplinary German research project ("Stofflüsse in Mikroorganismen" supported by the Volkswagen Stiftung) by the groups of the late professor Michael Ciriacy and of professor K.-D. Entian, with the express aim of meeting the requirements of physiologists, geneticists, and engineers. The construction of the CEN.PK strains started with two laboratory strains and involved a series of crosses and backcrosses. Construction of completely isogenic strains involved introduction of a plasmid-borne *HO* gene in a haploid strain. After loss of the plasmid, sporulation of the resulting diploid strain

yielded completely isogenic haploid strains of opposite mating type. In addition to the isogenic, protrophic haploid (*MATa* and *MATα*), and diploid strains, the CEN.PK family contains isogenic strains with all possible combinations of the auxotrophic markers *ura3*, *his3*, *leu2*, and *trp1* (Dr. P. Kötter, personal communication).

2.2. Maintenance of yeast strains

Shake-flask cultures of all strains were grown on YPD medium (Difco yeast extract, 10 g/l, Difco peptone, 10 g/l; glucose, 20 g/l). Sterile glycerol was added to overnight cultures to give a final concentration of 15% (v/v). One milliliter stock vials were stored at −80°C and used to inoculate precultures for growth experiments.

2.3. Shake-flask cultivation

Growth studies in shake-flask cultures were performed in 500-ml shake flasks containing 100 ml Difco Yeast Nitrogen Base (YNB) without amino acids, prepared, and filter-sterilized according to the manufacturers' instructions. Carbon sources were added to an initial concentration of 10 g/l, with two exceptions. Sodium acetate and L-lactate were added at 5 g/l. Moreover, for these two carbon sources the pH of the complete medium was adjusted to pH 5.0 before filter sterilization. Cultures were incubated at 30°C at 200 rpm. Inocula (1% v/v) were prepared by pregrowing cells on the same carbon source and under the same conditions to mid-exponential phase. Where this was not possible due to absence of growth, cultures were inoculated from stationary-phase glucose-grown shake-flask cultures. Specific growth rates were calculated from optical density measurements at 660 nm [12].

2.4. Fermentation and gas analysis equipment

Batch and chemostat cultivation was performed in laboratory fermenters with working volumes of 0.3–2.0 l. The various laboratories involved in this study use commercial equipment (Applikon, The Netherlands; Braun, Germany; MBR, Switzerland; Setric, France) and, in some cases, equipment manufactured in house. The minimum stirring speed and gas flow rate (air or nitrogen) were 800 rpm and 0.5 vvm (volume gas per volume culture per minute), respectively. Exhaust gas from the fermenters passed through condensers, which were cooled at 2°C to minimize evaporation of volatile metabolites. The extent of ethanol evaporation from steady-state cultures was estimated from blank experiments, in which reservoir vessels containing known concentrations of ethanol were coupled to sterile fermenters running under the same conditions (stirrer speed, gas flow rate, temperature). In every steady state chemostat culture, biomass dry weights were determined in samples taken directly from the cultures and from the effluent line, to confirm proper sampling [13]. The dry weights of culture and effluent samples differed by less than 2%. Gas analysis was performed with paramagnetic or magneto-acoustic oxygen analyzers and photograph-acoustic or infrared carbon dioxide analyzers. Procedures for gas analysis have been described in detail elsewhere [14,15].

2.5. Batch cultivation in fermenters

For batch cultivation in laboratory fermenters, a defined medium with vitamins [3] was used. For aerobic cultivation (dissolved oxygen concentration above 40% of air saturation throughout batch growth), the initial glucose concentration was 15 g/l. For anaerobic cultivation the initial glucose concentration was 25 g/l and the medium was supplemented with 10 mg/l ergosterol and 420 mg/l Tween 80 [2]. Cultures were grown at 30°C and at pH 5.0. 2 M KOH was used as a titrant. For anaerobic cultivation, fermenters were equipped with Norprene tubing and flushed with high-purity nitrogen or argon gas. Inocula for batch cultivation were pregrown in shake-flask cultures on mineral medium with 10 g/l glucose as the sole carbon source.

2.6. Glucose-limited aerobic chemostat cultivation

A defined medium with vitamins [3] was used, supplemented with 7.5 g/l glucose. Cultures were grown at 30°C and kept at pH 5.0 by automatic titration with 2 M KOH or NaOH. The dissolved oxygen concentration was kept above 40% of air saturation. Unless specified otherwise, the dilution rate was 0.10/h. Data reported for chemostat cultures refer to steady-state situations of nonsynchronous growth. Chemostat cultures were assumed to be in steady state when at least five volume changes had passed since the last change in growth conditions and when data from gas analysis and from the analysis of biomass, ethanol, and glycerol had been constant (<2% change) for at least two volume changes.

2.7. Glucose-limited anaerobic chemostat cultivation

Strains were grown at a dilution rate of 0.10/h on 25 g/l glucose as the carbon and energy source on mineral medium with vitamins [3], supplemented with 10 mg/l ergosterol and 420 mg/l Tween 80. All tubing consisted of Norprene. Not only the fermenter, but also the reservoir medium was sparged with high-purity nitrogen gas. Cultures were grown at pH 5.0 and at 30°C.

2.8. Accelerostat cultivation

For accelerostat cultivation [16], strains were first grown to steady state at a dilution rate of 0.05/h in aerobic, glucose-limited chemostat cultures (see above). The dilution rate was then continuously increased with an acceleration factor (a) of 0.01/h, using a computer-controlled medium

supply pump. The critical dilution rate at which aerobic fermentation set in was assessed by measurement of the ethanol concentration in culture supernatants and by measurements of the respiratory quotient (RQ; ratio of the specific rate of carbon dioxide production and the specific rate of oxygen consumption).

2.9. Analytical procedures

Culture dry weights were determined by filtering culture samples over 0.45 μm membrane filters. The filters were washed with demineralized water and dried to constant weight in a microwave oven [1]. Use of a microwave oven enables fast (15 min) drying of biomass without decomposition. To minimize effects of variations in laboratory air humidity, filters were predried and cooled in a dessicator prior to weighing. Concentrations of glucose, ethanol, glycerol, succinate, acetate, pyruvate, and lactate were determined both by high-pressure liquid chromatography (HPLC) and with specific enzymic methods [2–4].

2.10. Sporulation efficiency

Five milliliters of an overnight batch culture, grown at 30°C on YPD medium to a density of ca. 1×10^8 cells/ml, were harvested by centrifugation (2000 $\times$ *g* room temperature). After washing twice with 5 ml sterile distilled water, cells were resuspended in 200 μl sterile distilled water. Ten microliters of the resulting suspension was spotted onto a sporulation plate (potassium acetate, 10 g/l; Difco Bacto agar, 20 g/l). After a 3- and 5-day incubation at 30°C, one loop-full of sporulated material was resuspended in 1 ml sterile distilled water. Ten microliters of the resulting suspension was pipetted onto a micro slide or, alternatively, an appropriate volume was placed into a counting chamber. Degrees of sporulation were assessed by counting cells and asci under a microscope, discriminating between two-, three- and four-spore asci.

2.11. Spore viability

A loop-full of sporulated material (see above) was resuspended in 50 μl of sterile distilled water. After addition of 1 μl β-glucuronidase/arylsulfatase (Snail enzyme; Boehringer Mannheim), the suspension was incubated for 5–10 min for adequate digestion. Digestion was arrested by dilution in 1 ml sterile distilled water and centrifugation (2000 $\times$ *g* 10 min). After carefully discarding the supernatant, the pellet was used for spore dissection. At least 25 asci (or 100 spores) were dissected with a micromanipulator. Spore viability was scored after 3 days of incubation on YPD agar at 30°C and expressed as a percentage (colonies formed/spores dissected).

2.12. Mating efficiency

The haploid test strains X2180-1A and X2180-1B, as well as four segregants from the diploid strains to be tested, were grown overnight at 30°C on YPD medium to a density of ca. 1×10^8 cells/ml. Five microliters of each test strain was mixed with an equal volume of the haploid culture. Two to five microliters of the mixture was spotted on a YPD agar plate and incubated for 4 h at 30°C. One loopful of the mating mixture was then resuspended in 100 μl sterile distilled water. Ten microliters of the resulting suspension were pipetted onto a micro slide or, alternatively, an appropriate volume was placed into a counting chamber. The efficiency of mating after 4 h was quantified by counting two times 50 cells and zygotes under the microscope. For verification of diploidy, three zygotes from each segregant were isolated by micromanipulation and checked for sporulation and lack of mating.

2.13. Transformation efficiency

The four diploid strains were tested for transformation efficiency by performing three commonly used methods. For each method, transformation efficiency was determined with and without the addition of 20 μg carrier DNA (calf thymus DNA, Boehringer; dissolved in sterile distilled water to a final concentration of 4 mg/ml and sheared to an average fragment size of 5–10 kb by sonication). Transformation was performed according to the following published procedures: 1) lithium acetate method [17]; 2) Bicine method [18]; and 3) electroporation [19]. 1 and 10 μg DNA of the plasmid pCEN-ILV2^{SMR} was used in each transformation protocol. Transformants were selected for on SM agar (Difco YNB without amino acids, 6.7 g/l; glucose, 20 g/l; Sulfomethuron Methyl, 30 μg/l; and Difco Bacto agar, 20 g/l). Plates were incubated for 3 days at 30°C before transformants were scored.

2.14. Duplication of experiments

Experiments in this study were performed in at least two laboratories. The data presented are the average from these independent replicates. Only growth rates on glucose were determined by all 10 laboratories (see Results).

3. Results

3.1. Physiological tests

The maximum specific growth rate in vitamin-supplemented mineral media was determined in shake-flask cultures via optical-density measurements. To obtain an impression of the reproducibility of these assays, the specific growth rate on glucose was estimated in all our 10 laboratories. A SD of ca. 20% was obtained for data collected in

Table 2
Specific growth rates of *S. cerevisiae* strains on various carbon sources in shake-flask cultures

Carbon source	Specific growth rate (h^{-1})			
	CBS8066	BAY.17	X2180	CEN.PK122
Glucose	0.44	0.42	0.34	0.41
Sucrose	0.42	0.42	0.34	0.38
Maltose	0.41	0.39	NG	0.40
Galactose	0.32	0.29	NG	0.28
Ethanol	0.12	0.08	0.13	0.12
Acetate	0.20	0.11	0.14	0.17

The mineral medium used was Difco Yeast Nitrogen Base without amino acids. Data are the average of at least four experiments performed in at least two independent laboratories. NG, no growth observed.

the various laboratories. However, duplicate experiments performed within one laboratory generally differed by less than 5%. From the results of these shake-flask experiments, summarized in Table 2, it is clear that strain X2180 displays a number of negative traits. It does not grow on maltose or galactose and grows relatively slow on glucose. Strain CBS8066 stands out as the fastest growing strain on the range of substrates tested (Table 2). Growth on L-lactate or glycerol as the sole carbon source was either extremely slow ($\mu < 0.05\ h^{-1}$) or absent in all four strains (data not shown).

Biomass yields of the four strains were similar under the cultivation conditions that were employed in the fermenter experiments (Table 2). During batch cultivation on excess sugar, the biomass yields under aerobic conditions were slightly higher than under anaerobic conditions. The major products in both cases were carbon dioxide, ethanol, and glycerol. In addition, small amounts of succinate, acetate, pyruvate, and lactate were produced. In aerobic batch cultures, all these metabolites were reconsumed after glucose was exhausted and, mainly as a result of ethanol consumption, a second growth phase was observed for all strains.

A striking difference between aerobic glucose-limited growth in chemostat cultures and the other growth conditions was the high biomass yield of approximately 0.5 g biomass (g/glucose) in the aerobic chemostats (Table 3). During aerobic, glucose-limited growth, biomass and carbon dioxide were the major products. In none of these cultures, grown at a dilution rate of 0.10/h, could ethanol be detected. Glycerol and acetate concentrations were less than 0.1 mM.

In anaerobic glucose-limited chemostat cultures the biomass yields on glucose were equal or slightly higher than during anaerobic growth in batch cultures (Table 3). In anaerobic chemostat cultures ethanol [1.5 mol/mol of glucose] and glycerol [0.15 mol/mol of glucose)] were the main organic fermentation products. As observed in the anaerobic batch cultures, minor amounts of succinate, acetate, pyruvate, and lactate were formed. Yields of these products were in the range of 0.5–10 mmol/mol of glucose). For all steady-state chemostat cultures, a closing carbon balance (100 $\pm$ 3% carbon recovery) was obtained.

Table 3
Biomass yields on glucose of four *S. cerevisiae* strains during cultivation under defined conditions in batch and chemostat cultures (30°C, pH 5.0)

Cultivation method		Biomass yield (g biomass/g glucose)			
		CBS8066	BAY.17	X2180	CEN.PK122
Batch	Aerobic	0.12	0.11	0.12	0.12
Batch	Anaerobic	0.09	0.09	0.10	0.09
Chemostat	Aerobic	0.50	0.50	0.50	0.51
Chemostat	Anaerobic	0.10	0.11	0.10	0.10

The yield in aerobic batch cultures refers to the phase of glucose use. Chemostat cultures were growth under glucose limitation at a dilution rate of 0.10/h. The data presented are the average of at least two experiments carried out in different laboratories. the SD in replicate experiments was less than 5%, irrespective of whether they were performed in the same or in different laboratories.

All *Saccharomyces* strains that have so far been investigated exhibit a mixed respiro-fermentative metabolism in glucose-limited chemostat cultures at high specific growth rates [1,20,21]. Aerobic fermentation sets in above a so-called critical dilution rate (D_{crit}), the value of which is a strain-dependent property. Because steady-state analysis of growth in chemostat cultures is a very labor-intensive enterprise, a different method was used to compare the D_{crit} values of the four strains, namely accelerostat cultivation [16]. Cultures were initially grown as steady-state glucose-limited chemostat cultures at a dilution rate of 0.05/h, after which a linearly increasing specific growth rate (a = 0.01/h) was applied. For each strain, this resulted in a reproducible dilution rate at which aerobic fermentation set in, as judged from an increase in the respiratory quotient and the appearance of ethanol in the culture. In practice, the latter parameter appeared the most sensitive indicator for the onset of respirofermentative metabolism. It should be stressed that the value of D_{crit} measured in this dynamic system, although a useful measure to compare different strains, differs from the value determined in steady-state experiments (P. Duboc and J.T. Pronk, unpublished). Using this the accelerostat method, *S. cerevisiae* CBS8066 exhibited the highest D_{crit}, whereas the lowest D_{crit}, found with strain X2180, was about 35% lower (Table 4).

Table 4
Critical dilution rates (h^{-1}) of four *S. cerevisiae* strains determined in aerobic, glucose-limited accelerostat experiments (initial dilution rate 0.05/h, a = 0.01/h)

Strain	Critical dilution rate (h^{-1})
CBS 8066	0.34
BAY.17	0.32
X2180	0.22
CEN.PK122	0.27

In all experiments, the dissolved oxygen concentration remained above 30% of air saturation. The data presented are the average of two experiments. The SD among duplicates were less than 5% whether carried out in the same or different laboratories.

3.2. Genetic tests

Because all four strains tested are diploids, sporulation was tested directly as described in the Section 2. Strains CBS8066, BAY.17 and CEN.PK122 sporulated well, with percentages of asci ranging from 30 to 48% after 5 days at 30°C. After being subjected to the same sporulation protocol, sporulation of strain X2180 varied between 3% and 18%. After three days of incubation, sporulation was almost as efficient as after 5 days. Slight improvements of the efficiency could be obtained by harvesting the cells in the stationary phase or by incubating at 25°C (data not shown).

The viability of spores was determined after dissection of asci. With the exception of one experiment, the viability was at least 90% for all four strains, the exception being a single experiment in which a 70% spore viability was found for CEN.PK122. An independent duplicate experiment gave with this strain gave 100% spore viability.

The haploid cultures derived from tetrads of each of the four strains were further tested for their mating efficiency as described in Section 2. The number of schmoo's observed in the mating mixes after 4 h varied considerably, with a variance of 50% in some cases. However, the average values were between 20 and 30% for all four strains, provided that the heterozygous *HO/ho* genotype of CBS8066 and BAY.17 is taken into account. As a consequence of this, half of their spore cultures do not mate as they are already diploid. There was no significant difference between the efficiency of the two test strains, which mated to each other with an efficiency of ca. 35%. Thus, with the exception of the poor sporulation of strain X2180, there are no significant differences between the four strains for their accessibility to "classical" genetics.

Large differences among the four strains were found when their transformation efficiencies were compared (Table 5). Strain CBS8066 and especially its homozygous derivation BAY.17 were poorly transformable by the lithium acetate method. Better results were obtained with the Bicine and electroporation methods. With all three methods tested, strain X2180 exhibited the highest transformation efficiencies, whereas CEN.PK122 gave intermediate efficiencies. The effect of carrier-DNA addition was not clear cut, as it varied both with the method and the yeast strain (Table 5).

Table 5
Transformation efficiencies of four *S. cerevisiae* strains with three different methods (see Materials and methods for references)

Strain	Transformed DNA (μg)		Transformation efficiency (cfu/μg plasmid DNA)		
	Plasmid	Carrier	Li-acetate method	Bicine method	Electro-poration
CBS8066	1	20	7	131	64
	10	20	7	393	408
	1	0	5	101	30
	10	0	9	409	292
BAY.17	1	20	0.3	18	58
	10	20	0	9	60
	1	0	1	14	0
	10	0	0.3	15	52
X2180	1	20	220	1864	>2000
	10	20	1070	>2000	1040
	1	0	327	736	186
	10	0	999	>2000	ca. 700
CEN.PK122	1	20	18	585	929
	10	20	46	696	1356
	1	0	43	1057	317
	10	0	35	783	833

4. Discussion

4.1. Physiological and genetic characteristics

In comparison with many other yeasts, the range of carbon substrates that support growth of *S. cerevisiae* is rather narrow. Even so, not all relevant substrates have been tested in this study. For example, oleate [22] was not included, as growth on this substrate is difficult to quantify. Furthermore, from the many nitrogen sources supporting growth of *S. cerevisiae* strains [23], only ammonia was tested. Nevertheless, the data presented in Table 2 show that strain X2180 is inferior compared to the other strains with respect to its growth kinetics and range of carbon substrates. None of the four strains grew well when glycerol or L-lactate was provided as sole carbon source in a defined mineral medium supplemented with vitamins. Nevertheless, all strains metabolized these compounds after they had been produced in the first phase of aerobic batch cultivation on glucose. Further work showed that both glycerol and lactate are readily consumed when provided together with ethanol in defined media (J.T. Pronk et al., unpublished).

Biomass yield on sugar is a key parameter in the industrial cultivation of *S. cerevisiae* as bakers' yeast or as a host for heterologous-protein production [24,25]. Occurrence of alcoholic fermentation should be avoided in such processes as it lowers the biomass and product yields. The results presented in Table 3 illustrate this point: only during aerobic, glucose-limited growth at a low dilution rate, high biomass yields on glucose were obtained. During aerobic growth with sugar excess, the biomass yields were only slightly higher as compared to anaerobic conditions. This can be explained from the small contribution of respiratory ATP production in the presence of excess glucose, because of glucose repression of respiratory enzymes [21]. In aerobic, glucose-limited chemostat cultures grown at a fixed dilution rate of 0.10/h, the biomass yields on glucose of the four strains were comparable (Table 3). However, significant differences were observed in the critical dilution rate at which aerobic fermentation set in in accelerostat cultures (Table 4). Also in this test, strain X2180 did not perform as well as the other strains. The high D_{crit} (0.34/h) of strain CBS8066 observed in the accelerostat system is in good agreement with the value of 0.38/h found in steady-state

chemostat cultures [1]. These values are the highest reported for any *S. cerevisiae* strain in the literature.

A unique property of *S. cerevisiae* in comparison with other yeasts is its ability to grow rapidly under anaerobic conditions, provided that sterols and unsaturated fatty acids are available [4,26–28]. To enable studies on anaerobic metabolism and ethanol production, any reference strain of *S. cerevisiae* should grow well under strictly anaerobic conditions. All strains could be grown without problems in anaerobic steady-state chemostat cultures at a dilution rate of 0.10/h (Table 3), which is not possible with other facultatively fermentative yeasts such as *Candida utilis* and *Kluyveromyces lactis* [28,29].

Genetic modification of metabolic networks has become indispensable for quantitative studies on *S. cerevisiae*, both in applied and in fundamental research (see e.g. [6]). Therefore, good accessibility to 'classical' and molecular genetic techniques is an absolute requirement for a reference *S. cerevisiae* strain. Amenability to classical genetic procedures (sporulation, mating) did not present an insurmountable barrier for any of the four strains. However, irrespective of the method applied, significant differences were observed with respect to transformation efficiency (Table 5). Strains CBS8066 and BAY.17, which performed well in the physiological tests, were poorly transformable. Conversely, strain X2180, which displayed poor physiological properties, scored highest in the transformation tests. As in the physiological tests, *S. cerevisiae* CEN.PK122 occupied an intermediate position.

4.2. CEN.PK strains, an acceptable reference for quantitative yeast research?

The use of different laboratory strains of *S. cerevisiae* is not always a problem. In some cases, strain differences may even be an interesting research topic [30]. However, especially in multidisciplinary research involving different research groups, strain differences are more often a source of confusion and delay than of new insights. Researchers working with other microorganisms have recognized this. For example, the suggestion to use *K. lactis* CBS2359 as a reference for physiological and genetic studies [31] is now widely followed.

The present study illustrates that, in all likelihood, "the ideal *S. cerevisiae* strain" does not exist. Nevertheless, based on its growth characteristics in shake-flask cultures (Table 2), range of specific growth rates that support respiratory growth (Table 4) and transformation efficiency (Table 5), CEN.PK122 offered a good compromise between physiological properties and genetic accessibility. After a decision by our groups to use the CEN.PK strain family as a common reference, many experimental data have been gathered using these strains. Most of these observations support our choice:

1. The prototrophic haploid strain CEN.PK113–7D (*MAT*a) is very similar in its physiological characteristics to the diploid strain CEN.PK122. For example, activities of all glycolytic enzymes, measured in cell extracts of aerobic, glucose-limited chemostat cultures, differed by less than 20% between the two strains. (J.T. Pronk et al., unpublished results). Even after prolonged cultivation in chemostat cultures, haploid CEN.PK strains did not exhibit mating type reversal.
2. Many gene-disruption and overexpression mutants have been constructed in CEN.PK strains (P. Kötter, J. Bauer, et al. unpublished). In EUROFAN, the European research program aimed at functional analysis of the yeast genome, CEN.PK strains are used as a second reference strain, in addition to the S288C strain used for the sequencing of the genome. This has yielded a large number of additional gene-replacement mutants. Two types of markers have been successfully applied for genetic modifications in CEN.PK strains: dominant markers such as the *kanMX* cassette [32] in prototrophic strains and, secondly, auxotrophic marker genes to complement auxotrophic strains back to prototrophy. Both types of constructs have been stably maintained for many generations in chemostat cultures (J.T. Pronk et al., unpublished).
3. CEN.PK strains are excellent hosts for heterologous protein production. Specific productivities of up to 2 mg/(g biomass · h) have been realized in CEN.PK strains using an integrated *GAL7*-based cassette expressing a *Fusarium* cutinase gene (M. Giuseppin, C.T. Verrips, unpublished). These strains have been successfully grown in high-cell-density fermentations (130 g dry biomass/l), leading to extracellular cutinase concentrations exceeding 2 g/l (P. Duboc et al., unpublished).
4. Although not isogenic to the S288C strain used in the genome-sequencing project, transcription of over 90% of the open reading frames in the genome of the haploid strain CEN.PK113–7D was detectable in genome-wide transcription studies with commercially available DNA microarrays (J.T. Pronk et al., unpublished).

Close scrutiny of any reference strain is bound to reveal traits that are less desirable for certain lines of research and CEN.PK strains are no exception in this respect. In particular, signal-transduction studies have revealed that, upon exposure to excess glucose, these strains lack the characteristic transient accumulation of c-AMP found with other strains (J.M. Francois, C. Gancedo, unpublished). Although this does not seem to affect growth and extracellular product formation under the conditions so far examined, it is clear that this phenomenon requires further research. Furthermore, in certain applied studies, CEN.PK and other labora-

tory strains may be too genetically remote from industrial (e.g. baker's and brewer's) *S. cerevisiae* strains to directly translate results to the industrial practice [30]. Clearly, we do not advise our colleagues to turn a blind eye to such possible drawbacks. However, based on our own positive experiences with CEN.PK strains, we anticipate that a more widespread implementation of these strains as a common reference will facilitate cooperation and exchange of data among the global community of yeast researchers.

Requests for CEN.PK Strains

Requests for CEN.PK strains [33] should not be addressed to the authors of this paper, but to Dr. P. Kötter, Institute of Microbiology, J.W. Goethe Universität, Marie-Curie-Strasse 9, D-60439 Frankfurt, Germany (e-mail koetter@em.uni-frankfurt.de).

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