PHYSIOLOGICAL CHARACTERIZATION OF ADAPTIVE CLONES IN EVOLVING POPULATIONS OF THE YEAST, *SACCHAROMYCES CEREVISIAE*

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ABSTRACT

Populations of a diploid strain of *S. cerevisiae* were grown in glucose-limited continuous culture for more than 260 generations. A series of seven sequential adaptive changes were identified by monitoring the frequency of cycloheximide resistance in these populations. Samples were taken from the continuous cultures following each adaptive shift and characterized physiologically to determine (1) the range of phenotypes that can be selected in a precisely defined constant environment and (2) the order and predictability of the occurrence of the adaptive mutations in evolving populations. The clones were characterized with respect to the growth parameters, maximum growth rate, saturation coefficient and yield, as well as for changes in cell size and geometry and rate of glucose uptake. The maximum growth rates of the seven adaptive clones were very similar, but in contrast the saturation coefficients differed substantially. Surprisingly, not all clones showed reductions in the saturation coefficients, in comparison to the immediately preceding clones, as would be predicted from classical continuous culture kinetics. In addition, yield estimates first increased and then decreased for later isolated adaptive clones. In general, the results suggest epistatic interactions between the adaptive clones, consistent with earlier published results. The rate of glucose uptake, as measured by ^{14}C-xylose uptake, increased dramatically after the selection and fixation of seven adaptive clones. Progressive decreases in cell volume and changes in cell geometry, resulting in increased surface area to volume ratios, were also observed in the adaptive clones, but these changes were not always seen in other haploid and diploid yeast populations evolving under the same conditions. Such changes may be easily explainable in terms of the characteristics of the glucose-limited environment. The significance of the results to the evolution of microorganisms under nutrient-limiting conditions is discussed.

ADAPTATION in evolving asexual populations occurs by the sequential replacement of one clone by another. We have previously shown (PAQUIN

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and ADAMS 1983a) that such replacements may occur rapidly in yeast populations exposed to a glucose-limited environment, approximately once every 40 generations, in diploid cell populations of size $4–5 \times 10^9$. We have also observed a high rate of replacement of clones exposed to phosphate-limited environments (L. W. LEE and J. ADAMS, unpublished results). Furthermore, the adaptive mutations isolated show a high degree of epistatic interaction, such that the mean population fitness can decrease when compared to the initial population (PAQUIN and ADAMS 1983b).

Although glucose-limited continuous culture represents an extremely simple environment, certainly compared to any natural one, our previous results concerning the frequency of adaptive mutations and the epistatic mutations between them (PAQUIN and ADAMS 1983a,b) strongly suggest that many loci determine fitness in this environment. This is not unreasonable when it is considered that fitness is a relative measure and a function both of the growth rate of the population as well as of the ability of a genotype to alter the numbers of offspring left by its neighbors (HARPER 1977) and that each of these components may depend on many different loci.

In this paper we analyze the phenotypes of adaptive clones occurring sequentially in evolving populations of *Saccharomyces cerevisiae*, with a view to understanding the factors affecting the evolution of microorganisms in nutrient-limiting environments. In particular, we determine (1) the range of phenotypes that may be selected in a precisely defined constant environment, in which growth is determined by the concentration of the carbon source, glucose, and (2) the order and predictability of the occurrence of the adaptive clones in the evolving populations.

MATERIALS AND METHODS

Strains, media, growth and sampling: Most measurements were perfromed on the *a/a* diploid strain CP1AB-1AA, although for comparison purposes, size and shape measurements were taken on other haploid and diploid strains, isogenic to CP1AB-1AA except for the mating-type locus (PAQUIN and ADAMS 1982).

Batch cultures were grown in minimal medium at 30° in a gyratory shaker at 150–200 gyrations/min. Glucose was added as a carbon source at a concentration of either 0.08 or 2% (w/v). Continuous cultures were operated as chemostats and maintained in minimal medium at 30° in culture vessels of various sizes ranging from 150 to 200 ml. Glucose was added as the carbon source at a concentration of 0.08% (w/v). At this concentration glucose is the substrate limiting growth (PAQUIN and ADAMS 1983a). Dilution rates in the continuous cultures were approximately 0.20 hr^{-1}. Cell number was estimated using an electronic particle counter (Celloscope 111: Particle Data Inc., Elmhurst, Illinois) with a 60-μm orifice, after mild sonication with a Braunsonic 1510 sonicator with a needle probe to separate clumped cells. Dry weights of the cells were estimated by harvesting an aliquot of cells by filtration through a preweighed 0.45-μm cellulose nitrate filter, drying the cells overnight at 85° and reweighing. The values were corrected for the water lost by the untreated filters. To detect the occurrence and selection of adaptive clones, cycloheximide resistance was monitored in the diploid CP1AB-1AA population every 12–24 hr. For each sample, an aliquot of cells was frozen in 15% glycerol at −70° for later analysis. The seven adaptive clones were identified from the fluctuations in the frequency of cycloheximide resistance and isolated from the samples shown by the open, numbered circles in Figure 1. Details of these procedures and of the defined media used have been described previously (ADAMS and HANSCHE 1974; WEISS, KUKORA and ADAMS 1975; PAQUIN and ADAMS 1983a).

Estimation of growth parameters: Maximum specific reproductive rates (μ_{max}) were estimated for

each clone separately by growth in minimal medium in batch culture at 30°, with glucose added as a carbon source at a concentration of 2% (w/v). Glucose concentration (0.08 or 2%) did not affect the maximum reproductive rates of any of the clones.

Saturation coefficients (K_s) were estimated by growing each clone separately in a chemostat at 30°, estimating the equilibrium concentration of glucose in the culture vessel and, using estimates of μ_{max} obtained earlier, solving equation (1) below for K_s. Cells were harvested after ten to 15 generations of growth in the chemostat. This time is sufficient for a physiological equilibrium to be reached (DEAN 1969) but not enough to allow selection of new adaptive clones. Cell suspensions were collected directly from the chemostat and the cells removed by filtration through a 0.45-μm cellulose nitrate filter as for the estimation of dry weights. The samples so obtained were either stored at −70° for later analysis or assayed immediately for glucose. Glucose concentration was estimated using the glucose oxidase-peroxidase (Sigma) coupled reaction (RAABO and TERKILDSEN 1960).

Yield estimation: Yields were estimated both for continuous cultures and for batch cultures. Yields in continuous cultures were estimated as either the dry weight of cells or cell number divided by the weight of glucose utilized, defined as the difference between the glucose concentration in the incoming medium and the equilibrium glucose concentration in the chemostat. The same cultures were used for the estimation of both the saturation coefficients and the yield parameters.

Yields in batch cultures were defined as either the dry weight of cells or to the cell number divided by the amount of glucose added to the medium (0.08%).

Estimation of glucose uptake kinetics: A modification of a procedure described by CIRILLO (1968) was used to detect differences in the rate of glucose uptake. Cells were grown for approximately 72 hr in glucose-limited chemostats, then harvested by centrifugation and washed twice with distilled water. Incubations of the cells with labeled D-xylose were carried out at 30° in a final volume of 1 ml. D-Xylose is a nonmetabolizable analog of glucose which is taken up by the same facilitated diffusion system as glucose (HEREDIA, SOLS and DE LA FUENTE 1968). The reaction mixture contained 20 mM ^{14}C-labeled D-xylose (Amersham; specific activity, 50 μCi/mol of xylose) and approximately 9×10^6 cells. Approximately every 20 sec 0.1-ml samples of the reaction mixture were removed and added to 10 ml of ice-cold water. Samples were mixed and then filtered through glass fiber filters (Whatman GF/C). Filters were washed twice with 10 ml of ice-cold water, dried and counted in a scintillation counter. The channel ratio was used to determine the efficiency of counting. Measurements of the size and shape of the cells used in the assay were carried out similarly to the procedure described below, except that both parent and budded portions of the cells were measured.

Cell size and shape estimation: Cell size and shape were estimated both for cells grown in batch culture with 0.08% (w/v) glucose, as well as for cells grown in glucose-limited chemostats. For batch cultures, cells were harvested at a density of approximately 5×10^6 cells/ml while the cells were still in the unlimited (exponential) growth phase. For the cells grown in chemostats, cells were harvested after ten to 15 generations of growth under glucose limitation. Cells were harvested and photographed immediately, using an Olympus Vanox microscope with an Olympus SPLAN 40 PL objective. The sizes of the cells were calibrated by photographing a micrometer scale at the same magnification. The parent portions only, of an average of 100 budding cells, were measured. The major and minor axes of each cell were measured directly from the photographic negatives, and cell volumes and surface areas were then calculated on the assumption that the shape of the cells approximated a prolate spheroid. In accordance with geometric convention, the shape of the cells was described by their eccentricity. This variable, which has the range (0,1) is defined as the ratio of the distance between the two foci of the elliptic section, divided by the distance between the two vertices.

RESULTS

Isolation of adaptive clones: Figure 1 shows the changes in frequency of cycloheximide resistance in an *a/a* diploid population grown in glucose-limited chemostat culture over 264 generations. Each fluctuation in frequency signals the occurrence and selection of an adaptive clone. Initially, the frequency of

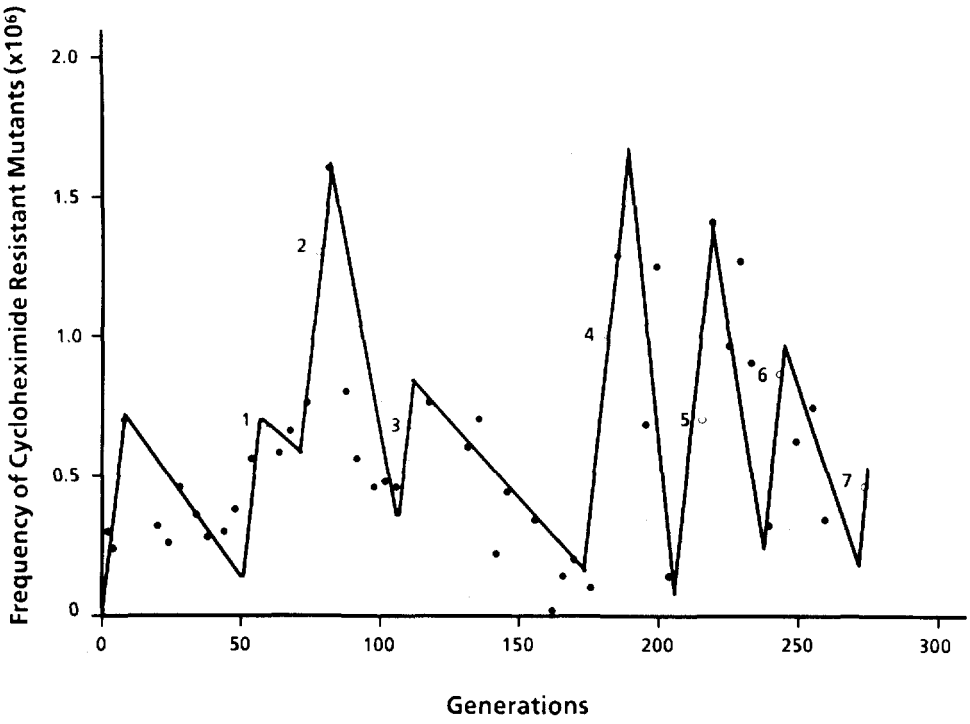

FIGURE 1.—Fluctuations in the frequency of cycloheximide resistance in a diploid *a*/*a* population of the strain CP1AB-1AA. The line indicates fluctuations in the frequency of cycloheximide resistance expected assuming the occurrence and fixation of seven adaptive mutations. The seven adaptive clones were isolated from the samples designated by the open, numbered circles.

cycloheximide resistance increases at the mutation rate until such time that an adaptive mutation occurs in the population. As the frequency of cycloheximide resistance will still be extremely low, the adaptive mutation will occur in a cycloheximide-sensitive cell with a probability of virtually 1. The original clone will be selected against, and the new clone carrying the adaptive mutation will rapidly increase in frequency. Since reproduction is exclusively asexual under our conditions, and there is no possibility of recombination between the resistance locus and the locus of the adaptive mutation, the frequency of cycloheximide resistance will, therefore, decrease. As the frequency of the selected clone becomes substantial, recurrent mutation to cycloheximide resistance within that clone will again result in an increase in cycloheximide frequency. Thus, each fluctuation in cycloheximide resistance signals the occurrence and selection of an adaptive clone in the population. In this way seven sequentially occurring adaptive clones were isolated. Further details of the rationale and justification for this procedure are given by PAQUIN and ADAMS (1983a).

Estimation of components of fitness of the adaptive clones: The rate of growth of cells in chemostat culture is generally accepted to be approximately proportional to the substrate concentration when this is low but reaches a maximal value at nonlimiting substrate concentrations. Although the precise form of

TABLE 1

Components of fitness for the original strain and the seven adaptive clones isolated

Isolated at generation	No. of adaptive clones	μ_{max} (hr^{-1} ± SEM)[a]	Doubling time (min)	K_s (μg/ml ± SEM)[a]
0 (CP1AB-1AA)	0	0.3631 ± 0.0056	115	52.0 ± 4.3
58	1	0.3602 ± 0.0045	115	53.1 ± 2.1
77	2	0.3600 ± 0.0019	116	23.1 ± 3.2
112	3	0.3484 ± 0.0106	119	34.0 ± 0.6
181	4	0.3591 ± 0.0027	116	41.3 ± 4.3
215	5	0.3465 ± 0.0021	120	22.2 ± 0.5
244	6	0.3463 ± 0.0016	120	13.8 ± 2.4
264	7	0.3528 ± 0.0021	118	23.6 ± 4.3

[a] Estimates are reported together with the standard error of the mean. Standard errors were calculated using standard linear regression procedures and, therefore, assume that the individual errors for each measurement are uncorrelated and identically distributed with mean zero and variance σ^2. Although this assumption may not be strictly correct for the growth equation, the standard errors may be considered a good first approximation to the true standard errors.

this relationship is open to discussion (*e.g.*, POWELL 1967; BECK and VON MEYENBURG 1968), the earliest and simplest form is due to MONOD (1942), who suggested that the kinetics of cell growth in chemostat culture were analogous to first order enzyme kinetics, namely,

$$\mu = \mu_{max}\left(\frac{s}{s + K_s}\right), \tag{1}$$

where μ is the reproductive rate of the cells within the chemostat, μ_{max} is the maximum specific reproductive rate when all components of the environment are in excess; K_s is the saturation coefficient (analogous to the Michaelis-Menten coefficient, K_m), the substrate concentration resulting in a reproductive rate of $\mu_{max}/2$; and s is the equilibrium substrate concentration in the chemostat. Whatever the precise form of the kinetics of growth in the chemostat, it is generally true that reproductive rate is basically determined by two parameters, μ_{max} and K_s. Therefore, if there is no interaction between the adaptive clones, apart from competition for the limiting nutrient (in our case glucose), their relative fitnesses may be described by, and partitioned into, their respective values of μ_{max} and K_s.

It is clear from a consideration of (1) that an increase in fitness may be achieved either by an increase in the μ_{max} or by a decrease in the K_s or by both. It is, of course, possible that an adaptive mutation may result in an increase in μ_{max} and K_s or a decrease in both of these parameters. Under these conditions the clone selected may also depend on the growth rate (dilution rate) of the culture (POWELL 1958).

Table 1 describes the two components of fitness μ_{max} and K_s estimated from pure cultures of each of the seven adaptive clones isolated from the *a/a* diploid population described above. The most striking aspect of these results is that there is no monotonic improvement in either component of fitness. The μ_{max}

of the seven adaptive clones and the original strain vary remarkably little. The doubling times for all of the clones occur within a range of 5 min, and there is no suggestion of an improvement in μ_{max}. In fact, μ_{max} decreases for the later isolated clones, specifically 5 and 6, although all other differences are nonsignificant. On the other hand, the K_s values are significantly reduced over the course of fixation of seven adaptive clones. However, it is not always true that the K_s for one adaptive clone is lower than the immediately preceding clone. In particular, the estimates of K_s for the third and fourth adaptive clones show significant increases. These increases are particularly surprising because, in the absence of substantial increases in μ_{max}, they would not be predicted from the classical model of chemostat kinetics described above (MONOD 1942; POWELL 1958).

Correlated physiological changes in adaptive clones

Yield: The classical model of chemostat kinetics also postulates a simple relationship between the relative growth rates in a chemostat environment and the rate of utilization of the limiting substrate, as illustrated by the following equation,

$$\frac{dx}{dt} = \mu x = -Y\frac{ds}{dt} \tag{2}$$

where x is a measure of cell biomass, and Y is the yield constant, as measured by the amount of biomass produced divided by the amount of substrate used (MONOD 1942). Thus, this variable measures the efficiency of conversion of the limiting substrate to biomass. It is generally accepted that the relationship shown in (2) is frequently an oversimplification. For example, in many microorganisms and environments, yield is dependent on the growth rate of the culture (*e.g.*, POWELL 1967; BECK and VON MEYENBERG 1968; NEIJSSEL and TEMPEST 1976). However, for our purposes it is valid to use the yield constant, so calculated, to describe differences in the adaptive clones, providing all estimates are made under the same chemostat growth conditions, in particular, at the same dilution rate.

Table 2 shows estimates of yield for the original strain and the seven adaptive clones described earlier, grown in pure culture under the same chemostat growth conditions. The yield constant was calculated using two different measures of biomass, dry weight (Y_1) and cell density (Y_2). In addition, a second series of yield estimates (Y_3, Y_4) were determined from batch cultures. These represent simply the stationary dry weight of cells (Y_3) and the stationary cell density (Y_4) in batch culture for a given initial glucose concentration [0.08% (w/v)]. Although these estimates may, strictly speaking, have no relevance to growth in chemostats, they are further measures of differences in the physiological phenotypes related to cell growth and, therefore, fitness of the clones.

All four measures of yield show a similar pattern of change, namely, an increase for the second adaptive clone followed by a progressive decrease for the remaining clones. These results are also unexpected, as *a priori* growth in

TABLE 2

Yield Estimates

Strain		Continuous culture		Batch culture	
Isolated at generation	No. of adaptive clones	Y_1 dry weight (μg dry weight cells/ μg glucose $\times$ 10^{-1} $\pm$ SEM)[a]	Y_2 cell no. (cell no./ μg glucose $\times$ 10^{-4} $\pm$ SEM)[a]	Y_3 dry weight (μg/ ml $\times$ 10^{-4} $\pm$ SEM)[a]	Y_4 (cell no. $\times$ 10^6 $\pm$ SEM)[a]
0 (CP1AB-1AA)	0	1.67 ± 0.09	1.50 ± 0.11	1.35 ± 0.11	9.71 ± 0.23
58	1	1.51 ± 0.19	1.33 ± 0.03	1.39 ± 0.13	9.63 ± 0.15
77	2	2.44 ± 0.23	2.02 ± 0.21	2.09 ± 0.20	11.01 ± 0.35
112	3	1.91 ± 0.16	1.50 ± 0.02	1.53 ± 0.19	8.94 ± 0.51
181	4	1.82 ± 0.06	1.39 ± 0.02	1.50 ± 0.17	9.81 ± 0.51
215	5	1.20 ± 0.07	1.05 ± 0.09	1.39 ± 0.07	9.06 ± 0.31
244	6	1.26 ± 0.11	1.12 ± 0.14	1.45 ± 0.00	9.14 ± 0.32
264	7	1.24 ± 0.11	1.07 ± 0.09	1.38 ± 0.04	8.75 ± 0.08

[a] Estimates are reported together with the standard error of the mean.

substrate-limited continuous culture would not be expected to select for, or against, efficiency of biomass production.

Glucose transport: As reproductive rate in chemostat environments is limited by the concentration of an essential nutrient, mutations that increase the ability of the organism to scavenge the extremely low quantities of the nutrient, in our case glucose, from the environment would be expected to possess a selective advantage. Certainly in natural environments, in which nutrient depletion is a common occurrence, the extremely low affinity constants for a variety of substrates and organisms suggest that such selection has already occurred during microbial evolution (TEMPEST and NEIJSSEL 1978). Therefore, the kinetics of glucose uptake were estimated in cells before and after growth in glucose-limited chemostat culture to assay for such mutations. Figure 2 shows the rate of uptake of ^{14}C-xylose, a nonmetabolizable analog of glucose, in the original strain and in the seventh adaptive clone isolated after 264 generations of growth under glucose limitation. The difference in the kinetics of uptake between the two strains is striking; the rate of glucose uptake is approximately 3.5 times higher in the seventh adaptive clone than in the original cells. In addition, the saturation pool size of xylose is approximately two-fold higher in the seventh adaptive clone.

Cell size and geometry: Given that glucose transport is the step limiting the reproductive rate of *S. cerevisiae* cells under glucose limitation, two main categories of mutations should confer increased fitness. Clearly, any mutations modifying the glucose transport system, either in activity or in the number of those moieties inserted into the cell membrane per unit area, which result in increased rates of glucose uptake, will be selected for. However, any change in the geometry of the cell that results in an increased surface area to volume ratio should also be adaptive, providing there is space limitation for insertion in the cell membrane of the glucose transport moieties. Previous results indi-

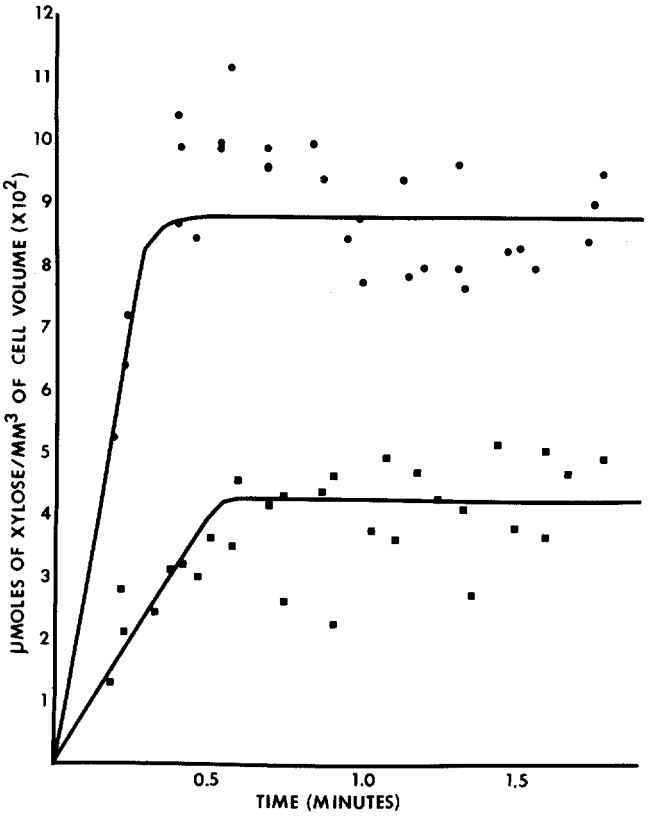

FIGURE 2.—Rate of uptake of ^{14}C-labeled xylose, a nonmetabolizable analog of glucose. ■, Strain CP1AB-1AA *a/a* diploid. ●, Seventh adaptive clone, isolated after 264 generations of growth in glucose-limited continuous culture.

cate that such space limitation exists for other enzymes located in the cell membrane (HENNAUT, HILGER and GRENSON 1970; WEISS, KUKORA and ADAMS 1975). Morphological changes could involve an increase in the elliptical shape of the yeast cell and/or a reduction in the average volume, which would also result in an increased surface area to volume ratio. Accordingly, the size and the shape of the original strain and the seven adaptive clones were measured. The results shown in Table 3 document a distinct increase in the surface area to volume ratio over the course of 264 generations. When the original strain and the seventh adaptive clone were compared, this ratio increased 28%. Photomicrographs of these two strains are shown in Figure 3 to illustrate these changes further. The data suggest that two main changes in cell size and shape were selected for. The major differences are between the first and second adaptive clones and the fifth and sixth. The increases in surface area to volume ratio are achieved not only by increases in the shape of the cells as measured by their eccentricity but also by reductions in cell volume. When the original strain and the seventh adaptive clone were compared, the contribution of the increased eccentricity to the increase in surface area to volume ratio was ap-

TABLE 3

Cell size and shape estimates

Isolated at generation	No. of adaptive clones	Cell volume (μm^3 ± SEM)[a]	Cell surface area (μm^2 ± SEM)[a]	Eccentricity ± SEM[a]	Surface area/ volume ratio ± SEM[a]
0 (CP1AB-1AA)	0	83.3 ± 1.3	93.6 ± 1.0	0.669 ± 0.009	1.13 ± 0.01
58	1	83.1 ± 1.9	92.2 ± 1.4	0.547 ± 0.001	1.13 ± 0.01
77	2	85.6 ± 1.3	96.9 ± 1.0	0.734 ± 0.010	1.14 ± 0.01
112	3	78.4 ± 1.5	91.0 ± 1.1	0.733 ± 0.009	1.17 ± 0.01
181	4	72.5 ± 1.7	87.2 ± 1.2	0.777 ± 0.008	1.22 ± 0.01
215	5	67.4 ± 1.3	83.6 ± 1.1	0.788 ± 0.008	1.25 ± 0.01
244	6	45.3 ± 1.4	67.0 ± 1.1	0.869 ± 0.008	1.52 ± 0.02
264	7	63.8 ± 1.6	91.2 ± 1.7	0.927 ± 0.006	1.45 ± 0.01

[a] Estimates are reported together with the standard error of the mean.

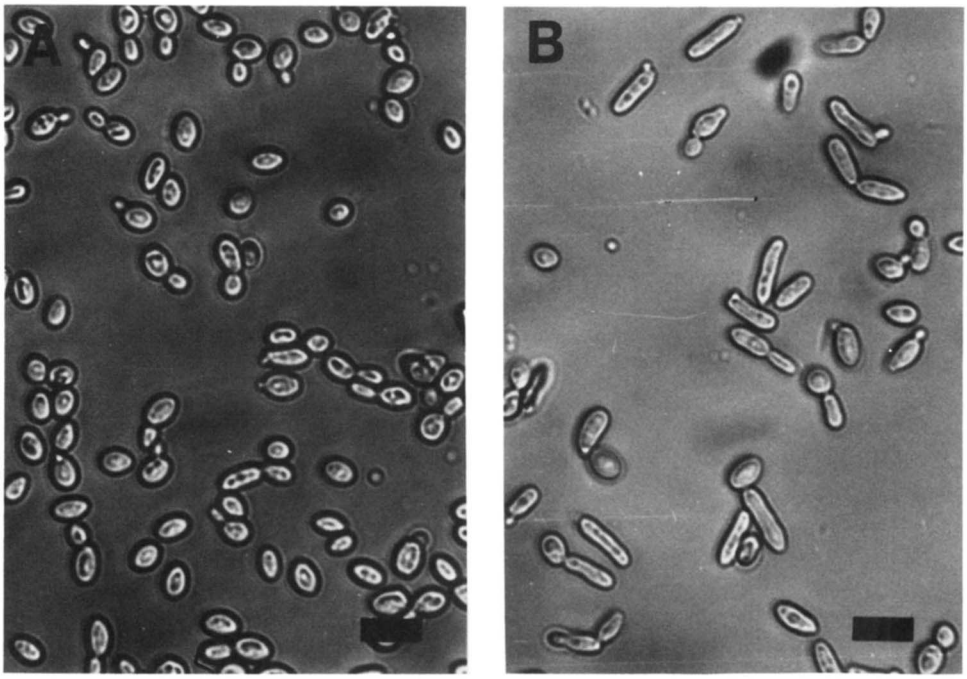

FIGURE 3.—Photomicrograph of cells before and after selection in glucose-limited continuous culture. A, Strain CP1AB-1AA. B, Strain isolated after 264 generations of growth in glucose-limited continuous culture. Bars = 10 μm.

proximately twice that from a reduction in cell volume. An increase in the eccentricity without a concomitant change in cell volume would result in an increase of this ratio of 12%, whereas a reduction in cell volume, without a concomitant change in eccentricity, would result in an increase of this ratio of 7%. Surprisingly, the cell size and shape changes of the adaptive clones are only expressed under conditions of glucose limitation. The same clones grown

in batch culture, under conditions in which glucose is nonlimiting, show little or no change in either cell size or cell shape (data not shown). For example, the surface area to volume ratio of the original strain in batch culture is 1.09, whereas this value for the seventh adaptive clone under the same conditions is 1.03.

Although cells isolated from this population clearly show changes in cell size and shape, data from other populations (PAQUIN 1982) indicate that such changes are not always a feature of genetic adaptation to glucose limitation.

DISCUSSION

The results described in this paper document the evolution of a unicellular, asexual organism, *S. cerevisiae*, in a simple defined laboratory environment. The results indicate that the nature and range of evolutionary responses can be far more complex than might be expected for such an organism in such an environment. Four aspects of these results deserve futher comment.

1. The two classical components determining fitness during substrate-limited growth in continuous culture, μ_{max} and the K_s, showed markedly different patterns of changes among the seven adaptive clones. Whereas the μ_{max} varied little, the K_s decreased for the first adaptive clones but then increased significantly for the third and fourth. According to the classical model of competition in chemostat culture (MONOD 1942; POWELL 1958), such clones would be expected to have a selective disadvantage and not increase in frequency in the chemostat. It must be concluded, therefore, that one or other of the assumptions of this model is violated. The most reasonable explanation for this apparent paradox is that some of the later adaptive clones selected interact with the immediately preceding clones beyond a simple competition for the limiting nutrient. The precise forms of these interactions remain obscure. We have previously presented evidence for epistasis between the adaptive mutations carried by the clones which can also result in decreases in fitness, relative to the fitness of the original strain (PAQUIN and ADAMS 1983b). In those experiments, fitness was measured by competition between clones not occurring immediately adjacently. Such epistasis resulting in fitness decreases may at least in part be explained by the interactions suggested by the results presented here.

In general, major changes in the K_s, but not in the maximum growth rate, for the adaptive clones are not surprising. Growth in chemostats is limited by the concentration of an essential substrate and, thus, the ability to grow at low substrate concentrations is much more likely to be selected for than growth (μ_{max}) under nonlimiting conditions. Similar results have been obtained by DYKHUIZEN and HARTL (1981) for *E. coli* evolving in long-term glucose-limited continuous culture.

2. The yield estimates increased significantly during the initial period of adaptation to glucose limitation but then showed progressive decreases. The increases in the yield parameters for the second adaptive clone indicate that the efficiency of glucose utilization in the original strain is not optimal. This

is perhaps surprising for such a substrate as glucose because it is likely that extensive selection for its utilization may have occurred in the evolutionary history of *S. cerevisiae.* Since chemostat-type continuous cultures do not necessarily select for increased efficiency, it suggests that further increases in the efficiency of glucose utilization may be possible. The decreases in the yield parameters for the last five adaptive clones indicate that reductions in efficiency may be selected for during evolution in a substrate-limited environment. Such yield changes have important implications for large scale fermentor design and practice in industry. Genetic changes to reduced yield may significantly compromise the economic properties of industrial microorganisms. Some evidence already exists for maladaptive genetic changes in long-term industrial cultures of the brewery yeast *Saccharomyces carlsbergensis* (THORNE 1968; THORNE 1970).

This pattern of results, although unexpected, is completely consistent with the classical model of chemostat kinetics. The relative fitnesses of two competing strains in substrate-limited continuous culture depends only on their respective values of μ_{max} and K_s and will be completely independent of the yields (*e.g.*, WALTMAN, HUBBELL and HSU 1980). In fact, it is not unreasonable to expect that mutations that either increase the μ_{max}, or increase the ability of an organism to scavenge limiting concentrations of an essential substrate from the medium, do so at the expense of reducing the efficiency of glucose utilization. However, it is interesting to note that in the a/α diploid yeast population described by PAQUIN and ADAMS (1983a) decreases in cell yield for the adaptive clones occurred at points where fitness decreases, as measured by competition with the original strain, were observed (PAQUIN 1982).

3. It is clear from the rate of uptake of glucose between the original strain and the seventh adaptive clone that glucose transport must be the rate-limiting step in glucose-limited continuous cultures of *S. cerevisiae*, at least during part of the period of adaptation to glucose limitation shown in Figure 1. This is consistent with the earlier results of VAN UDEN (1967), although NEIJSSEL and TEMPEST (1976) and NEIJSSEL, HEUTING and TEMPEST (1977) suggest that glucose transport is not the rate-limiting step in glucose-limited chemostat cultures of the prokaryotes *Klebsiella aerogenes* and *E. coli*, which have not undergone adaptive genetic changes to the chemostat environment. This difference between *S. cerevisiae* and the two prokaryotes may simply be explained by their evolutionary history. Extensive periods of adaptation to glucose limitation may have resulted in a highly efficient glucose uptake system such that some other step in glucose metabolism becomes rate limiting.

4. The changes in cell size and shape over the course of adaptation to a glucose-limited environment are easily understandable in terms of selection for an increase in surface area to volume ratio leading to an increase in the rate of glucose uptake. However, these changes are not always observed in other long-term populations of haploid and diploid strains of *S. cerevisiae* grown in the same environment. In addition, decreases in cell yield in these populations did not always occur after the same number of adaptive shifts (PAQUIN 1982). The emerging picture of adaptation in such populations, therefore, is that a

number of different cell phenotypes may exhibit increased fitness and that the selection of any one of them is unpredictable, depending on the random nature of the mutational events involved. Certainly in more complex natural environments the patterns of adaptation may be expected to be still more varied. In particular, interactions between the adaptive clones of the type described here and by PAQUIN and ADAMS (1983b) may be much more frequent. Probably the most important implication of such interactions is that a single optimal phenotype may not exist even for simple constant laboratory environments. Consequently, these interactions may serve to maintain genetic variation within populations of microorganisms in nature.

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