Isolation and Characterization of Yeast Strains Carrying Mutations in the Glyceraldehyde-3-phosphate Dehydrogenase Genes*

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Mutant yeast strains were constructed which carry insertion mutations in each of the glyceraldehyde-3-phosphate dehydrogenase structural genes which have been designated TDH1, TDH2, and TDH3. Haploid strains carrying autogynations in TDH1 and TDH2 as well as TDH1 and TDH3 were isolated from crosses between strains carrying the appropriate single mutations. The three single mutants as well as the two double mutants grow at wild type rates when ethanol is used as carbon source. Mutant strains lacking only a functional TDH2 allele or a TDH3 allele grow at 50 and 75% of the rate observed for wild type cells, respectively, when glucose is used as carbon source. No growth phenotype was observed for strains lacking only a functional TDH1 allele when either fermentable or nonfermentable carbon sources were used. Evidence is presented that strains lacking functional TDH2 and TDH3 alleles are not viable. These data demonstrate that the presence of a functional TDH2 or TDH3 allele is required for cell growth.

There are three glyceraldehyde-3-phosphate dehydrogenase structural genes per haploid yeast genome (1–5). The coding regions of these homologous genes are highly conserved and each contains an open reading frame capable of encoding a 331-amino acid polypeptide (2, 4, 5). The 5' termini of glyceraldehyde-3-phosphate dehydrogenase mRNAs have been mapped to each of the three genes by S1 nuclease mapping analysis using specific probes for each gene (5). These latter data demonstrate that all three genes are expressed during vegetative cellular growth.

Multiple forms of yeast glyceraldehyde-3-phosphate dehydrogenase have been identified using a number of fractionation procedures (6–8). It has not been possible, however, to determine if these different forms of the enzyme reflect true isozymes, physiologically relevant posttranslational modifications of the enzyme, or artifacts of isolation such as proteolysis or deamidation. Since glyceraldehyde-3-phosphate dehydrogenase is a tetramer, it is possible to generate a large number of isozymes if the polypeptide products of the three structural genes randomly assort into active tetramers. Alternatively, the polypeptides may assort nonrandomly to generate a more limited distribution of isozymes.

One approach to establishing the existence and distribution of putative glyceraldehyde-3-phosphate dehydrogenase isozymes is to isolate mutant strains which carry nonfunctional alleles of one or more of the glyceraldehyde-3-phosphate dehydrogenase structural genes. Utilizing these mutant strains, it should be possible to determine the level of expression of each structural gene under defined physiological conditions and the role of each structural gene in cell growth on fermentable and nonfermentable carbon sources. Using these mutant strains, it should also be possible to develop suitable assays for the polypeptide products of the three genes which are essential for the identification of specific isozymes.

In this report, we describe the isolation of yeast strains containing insertion mutations in each of the three glyceraldehyde-3-phosphate dehydrogenase structural genes which we have designated TDH1, TDH2, and TDH3 (where TDH is triose-phosphate dehydrogenase). A characterization of glyceraldehyde-3-phosphate dehydrogenase expression in these mutant strains is presented in the accompanying paper (19).

Experimental Procedures

Materials—Enzymes were purchased from the following suppliers: HindIII, EcoRI, HpaI, and BamHI from New England Biolabs; Sall and DNA polymerase I from Bethesda Research Laboratories; S1 nuclease from Boehringer Mannheim; DNase I and zymolyase 5000 from Miles Laboratories; and T, DNA ligase from P-L Biochemicals. [α-32P]dCTP (specific activity, 2000–3000 Ci/mmol) was obtained from Amersham. Yeast extract, bactopeptone, and yeast nitrogen base without amino acids were purchased from Difco.

Strains and Cell Culture Conditions—Saccharomyces cerevisiae strain S173-6B (a, leu2-3-112, his3-D1, ura3-52, trp1-289), provided by Dr. Fred Sherman, University of Rochester, was used as the recipient for the construction of insertion mutations within each of the glyceraldehyde-3-phosphate dehydrogenase genes. Strain S173-6B was crossed with an isogenic strain, designated S173-28A (a, leu2-3-112, his3-D1, ade1-101, trp1-289). A haploid strain (a, leu2-3-112, his3-D1, ade1-101, ura3-52, trp1-289) recovered from this cross was transformed with a 1.7-kb BamHI fragment containing the yeast HIS3 gene as described below to generate strain SB (a, leu2-3-112, ade1-101, ura3-52, trp1-289). Strain SB was used in crosses with strains carrying insertion mutations in each of the glyceraldehyde-3-phosphate dehydrogenase structural genes. Haploid strains recovered from these latter crosses which were used in subsequent crosses for the isolation of double glyceraldehyde-3-phosphate dehydrogenase mutants are described below.

Yeast strains were grown at 30°C in YP medium (1% yeast extract, 2% bactopeptone) or in a defined minimal medium containing: 0.67% yeast nitrogen base (without amino acids) supplemented with 20 μg/ml each of adenine, uracil, leucine, tryptophan, or histidine as required. Carbon sources were 2% glucose, 2% glycerol plus 2% lactate, or 2% ethanol. Growth rates were measured by following turbidity at 600 nm with log phase cultures grown in YP medium supplemented with 20 μg/ml each of adenine, uracil, leucine, histidine, and tryptophan; 2.5 μg/ml vitamin-free casamino acids; and 5 μg/ml vitamin B1. Growth conditions for Escherichia coli containing hybrid plasmids were as previously described (1). Hybrid plasmids containing yeast glyceraldehyde-3-phosphate dehydrogenase genes included ppc11

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1 The abbreviation used is: kb, kilobase pair.

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(5) which contains yeast genomic sequences corresponding to the
glyceraldehyde-3-phosphate dehydrogenase structural gene
designated TDH1; pgap63 (4) which contains yeast genomic DNA corre-
sponding to the TDH2 locus; and pgap49 (1) and pgap49 (2) which
contain the glyceraldehyde-3-phosphate dehydrogenase corre-
sponding to the TDH3 locus. Other plasmids included pBR322 contain-
ing a yeast HIS3 structural gene on a 1.7-kb BamHI fragment (provided
by Dr. G. Fink, Massachusetts Institute of Technology) and pBR322 contain-
ing a yeast URA3 structural gene on a 1.1-kb HindIII fragment (provided
by Dr. D. Botstein, Massachusetts Institute of Technology). Plasmid DNA isolation and E. coli transforma-
tions were carried out as previously described (1).

Construction of Plasmids Containing URA3 Insertion Mutations
within the Coding Sequences of the Three Yeast Glyceraldehyde-3-
phosphate Dehydrogenase Genes—A 1.1-kb HindIII fragment of yeast DNA containing a functional URA3 structural gene was purified by
prepaparative agarose gel electrophoresis. The termini of the fragment
were made blunt by treatment with S1 nuclease as previously de-
scribed (1). The URA3 fragment was blunt-end ligated (9) into the
HpaI site located between codons 332 and 133 within the glyceralde-
yde-3-phosphate dehydrogenase genes in pgap11 (TDH1) and
pgap63 (TDH2). The URA3 gene was also blunt-end ligated into
pgap49 containing TDH3; pgap63 (4) which contains yeast genomic
DNA corresponding to the TDH3 locus. Other plasmids included pBR322 contain-
ing a yeast HIS3 structural gene on a 1.7-kb BamHI fragment (provided
by Dr. D. Botstein, Massachusetts Institute of Technology) and pBR322 contain-
ing a yeast URA3 structural gene on a 1.1-kb HindIII fragment (provided
by Dr. G. Fink, Massachusetts Institute of Technology). Plasmid DNA isolation and E. coli transforma-
tions were carried out as previously described (1).

For isolation of URA3 insertion mutations in the TDH1 locus, a
1.7-kb BamHI fragment containing a functional yeast HIS3 structural
was ligated into the BamHI site within the bacterial plasmid
vector portion of pgap11 carrying a URA3 insert within the TDH1
coding sequences.

Construction of Yeast Glyceraldehyde-3-phosphate Dehydrogenase
Mutants—Mutant yeast strains containing URA3 insertions within
the coding regions of each glyceraldehyde-3-phosphate dehydrogenase
structural gene were obtained after transformation of yeast strain
S173-6B. Yeast transformations were carried out according to the
procedure described by Hinnen et al. (12).

For the construction of a tdh1 mutant strain, strain S173-6B was
transformed with 10 μg of a supercoiled pgap11 plasmid containing a
URA3 insertion within the TDH1 coding sequences and a yeast HIS3
structural gene in the bacterial vector portion of the plasmid. The
tdh1 mutant strain R101 was identified as previously described (1). The
URA3 insertions were confirmed by colony hybridization (11) using a [32P]dCTP-labeled
probe prepared by nick translation of the 1.1-kb HindIII fragment containing the URA3 structural gene. Restriction endonuclease clavage
maps of the URA3 insertion mutants in the yeast DNA segments corresponding to the three glyceraldehyde-3-phosphate dehydrogenase
loci are illustrated in Fig. 1.

For isolation of URA3 insertion mutations in the TDH1 locus, a
1.7-kb BamHI fragment containing a functional yeast HIS3 structural
gene was ligated into the BamHI site within the bacterial plasmid
vector portion of pgap11 carrying a URA3 insert within the TDH1
coding sequences.

Results and Discussion

Insertion of a Yeast URA3 Structural Gene into the Coding
Sequences of the Three Cloned Yeast Glyceraldehyde-3-
phosphate Dehydrogenase Genes—The three yeast glyceraldehye-
dehydrogenase genes were isolated on bacterial plasmids as previously described (1, 3–5). Based upon the
primary structures of the genes, each contains a highly conserved
coding region capable of directing the synthesis of a 331-
apptide which cross-hybridizes with the coding sequences of TDH1 and
TDH2. The second filter was hybridized with a probe obtained by
nick translation of the 1.1-kb HindIII fragment which contains the URA3 structural gene.
corresponding to the three glyceraldehyde-3-phosphate dehydrogenase loci are illustrated in Fig. 1.

Isolation of a tdh1 Mutant—Plasmid pgap11 contains the TDH1 structural gene on a 4.3-kb EcoRI fragment of yeast genomic DNA in the bacterial plasmid vector pSF2124 (5). A plasmid containing a URA3 insertion in the TDH1 coding sequences of pgap11 (Fig. 1) transforms a ura3 recipient yeast strain to prototrophy at high frequency. When these transformants are plated on a medium lacking uracil, approximately 80% of the cells have a ura⁻ phenotype, indicating that they lack the URA3 structural gene. Southern blotting analyses on genomic DNA isolated from the ura⁻ transformants demonstrated the presence of an intact autonomously replicating plasmid corresponding in size to the DNA used to transform the cells (data not shown). Since the plasmid vector contains no yeast replicative sequences, we conclude that the 4.3-kb segment of yeast DNA contains an ars sequence (where ars is autonomous replication sequence).

To replace the wild type TDH1 allele with the allele containing the URA3 insertion mutation, the recipient yeast strain (ura⁻, his⁻) was transformed with a plasmid containing a URA3 insertion in the TDH1 gene and a functional yeast HIS3 gene in the plasmid vector (Fig. 2). Transformants containing the poorly segregating autonomously replicating plasmid were grown continuously in medium lacking uracil. Stable ura⁻, his⁻ cells were isolated after 40 generations. To confirm that a gene conversion had occurred at the TDH1 locus in the cells, genomic DNA was isolated from ura⁻, his⁻ transformants and subjected to Southern blotting analysis. As illustrated in Fig. 3A, DNA isolated from wild type cells contains three HindIII cleavage fragments which hybridize with a probe containing sequences complementary to the glyceraldehyde-3-phosphate dehydrogenase coding region. The 7.7-kb fragment corresponds to the TDH1 locus and the 5.6- and 2.1-kb fragments correspond to the TDH2 and TDH3 loci, respectively. Genomic DNA isolated from the ura⁻, his⁻ transformants lacks the 7.7-kb fragment but contains a new 8.8-kb fragment. To confirm that this 8.8-kb fragment contains the inserted URA3 gene, the HindIII digests of genomic DNA were analyzed with a probe complementary to the URA3 gene sequences (Fig. 3B). This probe hybridizes strongly to a 4.7- and a 2.1-kb fragment and weakly to an 8.6-kb fragment derived from wild type cellular DNA. We assume that the presence of these latter HindIII fragments in the DNA from strain 6B reflects polymorphic differences in the HindIII cleavage pattern of the URA3 gene in strain 6B versus the strain from which the 1.1-kb HindIII fragment containing URA3 was isolated. In the case of the DNA isolated from the ura⁻, his⁻ transformants, the probe also hybridizes to an 8.8-kb fragment. These data confirm that the wild type TDH1 allele was replaced by the plasmid-derived allele containing the URA3 insertion. The phenotype of the isolated tdh1 mutant will be described below.

Isolation of tdh2 and tdh3 Mutants—Plasmids pgap63 and pgap49 containing URA3 insertions within the coding sequences of TDH2 and TDH3, respectively, transform a ura3 recipient yeast strain to prototrophy at moderately high frequency. The ura⁻ phenotype of these transformants is stable under nonselective growth conditions, and Southern blotting analysis demonstrated that in each case the transformants contained the entire plasmid integrated in tandem with the homologous triose-phosphate dehydrogenase allele. Attempts to isolate ura⁻ recombinants from these transformants in

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**FIG. 1.** Restriction endonuclease cleavage maps of segments of yeast genomic DNA containing a yeast URA3 gene insertion within the coding regions of the three cloned glyceraldehyde-3-phosphate dehydrogenase genes. The single line indicates the location of pSF2124 vector sequences. The double lines indicate yeast genomic DNA sequences corresponding to the three cloned glyceraldehyde-3-phosphate dehydrogenase genes designated TDH1, TDH2, and TDH3. The shaded region indicates the location of the glyceraldehyde-3-phosphate dehydrogenase coding regions. A 1.1-kb fragment of yeast DNA containing a functional URA3 gene (indicated by the hatched region) was inserted at HpaI sites within the TDH1 and TDH2 coding region and at a SalI site within the coding region of TDH3 as described under “Experimental Procedures.”

**FIG. 2.** Construction of a yeast strain containing a URA3 insertion mutation at the TDH1 locus. a, a 1.7-kb BamHI fragment containing a functional yeast HIS3 gene was ligated into the BamHI site within the pSF2124 vector sequences of a plasmid carrying a segment of yeast genomic DNA corresponding to the TDH1 locus. The TDH1 coding region was previously interrupted with a segment of DNA containing a functional yeast URA3 gene (Fig. 1). b, intact supercoiled plasmid DNA was used to transform yeast strain S173-6B, and his⁺, ura⁺ transformants were selected. Initially, these transformants contained an autonomously replicating plasmid corresponding in size to the DNA used to transform the yeast recipient. After continuous growth (approximately 40 cell generations), stable his⁺, ura⁺ yeast cells were isolated from the cultures and subsequently shown to contain the tdh1 insertion mutation (Fig. 3).
The wild type TDH allele was replaced with the allele containing the URA3 insertion mutation. The three mutant strains designated tdh1, tdh2, and tdh3.

To isolate the tdh2 and tdh3 mutants, we used an alternative method described by Rothstein (13) for direct gene conversion after transformation with a restriction fragment of genomic DNA containing a selectable marker gene insertion. The strategy for isolating the tdh2 and tdh3 mutants using this method is outlined in Fig. 4. To isolate the tdh2 mutant, a recipient yeast strain carrying a nonrevertible deletion mutation within the chromosomal URA3 structural gene was transformed with a 4.5-kb SalI fragment which contains a URA3 gene insertion within the coding sequences of TDH2 (Fig. 1). To isolate the tdh3 mutant, this recipient strain was transformed with a 3.2-kb HindIII fragment containing a URA3 insertion within the coding sequences of TDH3 (Fig. 1). Ura+ transformants were isolated after transformation with each fragment. Ura+ transformants were isolated after each transformation and their genomic DNA was subjected to Southern blotting analysis (Fig. 3). These data confirm the isolation of the tdh2 and tdh3 insertion mutants.

**Isolation of the tdh2, tdh2, and tdh3 Double Mutants**—Since there are three glyceraldehyde-3-phosphate dehydrogenase loci per haploid yeast genome, it should be possible to isolate three different mutant strains carrying mutations in two of the glyceraldehyde-3-phosphate dehydrogenase genes, provided these mutants are viable. These mutants would be valuable for analyzing the properties of the glyceraldehyde-3-phosphate dehydrogenase encoded by each gene since each mutant would carry only one functional gene. Previous studies using Southern blotting analysis showed that the glyceraldehyde-3-phosphate dehydrogenase structural genes are not closely linked (1, 3-5). Based on these physical mapping studies, we chose to isolate the double mutants from genetic crosses between haploid strains carrying mutations in each respective gene.

To obtain the tdh1, tdh2 mutant, a cross was made between a haploid strain carrying a tdh1 mutation and a haploid strain carrying a tdh2 mutation (α, tdh1, leu2-3-112, ura3-52, trp1-289, ade1-101 × a, tdh2, leu2-3-112, his3Δ1, ura3-52, trp1-289). The diploids obtained from this cross are homozygous for a deletion mutation at the URA3 locus and heterozygous for URA3 insertion mutations in the TDH1 and TDH2 loci. Meiotic segregation patterns for the URA3 insertion mutations in the TDH1 and TDH2 loci were determined by Southern blotting analysis of genomic DNA isolated from each of the four spores obtained after tetrad dissection. The results for five complete tetrads were: one parental ditype (PD, tdh1; tdh1; tdh2; tdh1), zero nonparental ditype (NPD, tdh1; tdh1; tdh2; tdh1), one zero nonparental ditype (NPD, tdh1; tdh2; tdh1; tdh2; WT), and four tetratype (T, tdh1; tdh2; tdh1; tdh2; WT). As expected, the segregation pattern for the ura+/? phenotype
was: PD, 4 ura"+0 ura" and T, 3 ura"+1 ura". The results of a Southern blotting analysis carried out with HindIII-digested genomic DNA isolated from the four spores of a tetrad showing the tetrate type segregation pattern are illustrated in Fig. 5. Cells containing the 8.8- and/or 6.7-kb HindIII fragments corresponding to URA3 insertions within the TDH1 and TDH2 structural genes, respectively, were identified using probes complementary to glyceraldehyde-3-phosphate dehydrogenase coding sequences (Fig. 5A) and URA3 coding sequences (Fig. 5B). As expected, one of the haploid cells derived from the tetrad contained mutations within both glyceraldehyde-3-phosphate dehydrogenase genes.

The tdh1, tdh3 double mutant was obtained from a cross between haploid cells containing URA3 insertions within the TDH1 and TDH3 structural genes, respectively (a, tdh1, lev2-3-112, ura3-52, trp1-289, ade1-101 × a, tdh3, lev2-3-112, his3Δ1, ura3-52, trp1-289). As described for the isolation of the tdh1, tdh2 double mutant, the diploids are homozygous for a deletion mutation at the URA3 locus and heterozygous for URA3 insertion mutations at the TDH1 and TDH3 loci. The meiotic segregation patterns for the URA3 insertion mutations in the TDH1 and TDH3 loci were determined by Southern blotting analysis following tetrad dissection. The results for eight complete tetrads were: two PD (tdh1, tdh3; WT; WT), two NPD (tdh1, tdh3; tdh2; WT; WT), and four T (tdh1; tdh2; tdh3; WT; WT)

Fig. 6. Identification of a mutant strain carrying URA3 insertion mutations in the TDH1 and TDH3 loci. A diploid strain which is homozygous for a deletion at the URA3 locus and heterozygous for URA3 insertion mutations at the TDH1 and TDH3 loci was isolated as described under "Experimental Procedures." After sporulation of this diploid strain, tetrads were dissected and individual spores were tested for their ability to grow in the absence of exogenously added uracil. Genomic DNA was isolated from the four spores derived from a single tetrad exhibiting a 3ura+:1ura" meiotic segregation pattern. The DNA was digested with HindIII, electrophoresed on duplicate 0.8% agarose gels, and transferred to nitrocellulose filters. Panel A, an autoradiogram of a filter after hybridization with a nick-translated probe which is complementary to glyceraldehyde-3-phosphate dehydrogenase coding sequences. Panel B, an autoradiogram of a duplicate filter after hybridization with a nick-translated probe which is complementary to the URA3 structural gene. The 8.8- and 3.2-kb HindIII fragments which hybridize with both probes correspond to URA3 insertions into the coding region of TDH1 and TDH3, respectively. The wild type spore (WT) does not contain a mutant TDH allele and is ura". The remaining spores are ura" and contain a tdh1 mutation, a tdh3 mutation, and a tdh1, tdh3 double mutation, respectively.

Table I

<table>
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<th>Number of tetrads</th>
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<td>1</td>
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Fig. 5. Identification of a mutant strain carrying URA3 insertion mutations in the TDH1 and TDH2 loci. A diploid strain which is homozygous for a deletion at the URA3 locus and heterozygous for URA3 insertion mutations at the TDH1 and TDH2 loci was isolated as described under "Experimental Procedures." After sporulation of this diploid strain, tetrads were dissected and individual spores were tested for their ability to grow in the absence of exogenously added uracil. Genomic DNA isolated from these haploid cells was further analyzed for the presence of URA3 insertion mutations within the TDH2 and TDH3 loci as described under "Experimental Procedures."
YT DH3 loci, respectively. As expected, DNA from one of the spores contains URA3 insertions in both glyceraldehyde-3-phosphate dehydrogenase structural genes.

The tdh2, tdh3 Double Mutant and the tdh1, tdh2, tdh3 Triple Mutant Are Not Viable—Isolation of a tdh2, tdh3 double mutant was initially attempted employing crosses between haploid cells carrying tdh2 and tdh3 mutations, respectively. Diploids derived from these crosses were shown to be heterozygous for URA3 insertion mutations within the TDH2 and TDH3 loci by Southern blotting analysis. After sporulation, we observed a high proportion of ascis (approximately 70%) containing three spores. This result is in contrast to the previously described crosses between tdh1 haploids and either tdh2 or tdh3 haploids where the majority of ascis contained four spores. A number of the ascis containing four spores were dissected and the genomic DNA of haploid cells derived from the spores was analyzed for the presence of URA3 insertions within the TDH2 and TDH3 loci by Southern blotting. The results of these analyses are summarized in Table I. Based on an analysis of 15 tetrads, only two showed a parental ditype segregation pattern. In 12 of the tetrads, either the tdh2 or the tdh3 mutation was lost, suggesting that a premeiotic gene conversion had occurred. In one case, two tdh2 mutants and one tdh3 mutant were recovered, suggesting that the second tdh2 mutation had been lost by postmeiotic gene conversion. In no case was a viable tdh2, tdh3 double mutant recovered.

A second approach to isolating a tdh2, tdh3 double mutant involved transformation of a haploid ura- , tdh2- mutant with the 3.2-kb HindIII fragment containing a URA3 insertion within the TDH3 coding sequences. The relative efficiency of transformation was extremely low and only two ura+ transformants were recovered. Southern blotting analysis showed that both transformants contained the tdh3 mutation but had lost the tdh2 mutation as a result of gene conversion. Based on these data and those described above, we conclude that the tdh2, tdh3 double mutant is not viable.

It is possible that our failure to recover a viable tdh2, tdh3 double mutant is due to the presence of a functional TDH1 structural gene in the mutant. If this is the case, it might be possible to recover a viable tdh1, tdh2, tdh3 triple mutant. To test this possibility, diploids were constructed which were heterozygous for URA3 insertions within all three glyceraldehyde-3-phosphate dehydrogenase genes. After sporulation and tetrad dissection, genomic DNA was isolated from haploid cells derived from individual spores and analyzed by Southern blotting. The results were similar to those described in Table I. All three single mutants as well as the tdh1, tdh2 and tdh1, tdh3 double mutants could be recovered. Neither the tdh2, tdh3 double mutant nor the tdh1, tdh2, tdh3 triple mutant was recovered from these crosses, however. These data strongly suggest that the tdh2, tdh3 and tdh1, tdh2, tdh3 mutants are not viable.

**Growth Rates of Glyceraldehyde-3-Phosphate Dehydrogenase Mutants in Glycolytic and Gluconeogenic Carbon Sources**—The growth rates for each of the single glyceraldehyde-3-phosphate dehydrogenase mutants and the two double mutants were determined in a complex medium supplemented with casamino acids and all of the auxotrophic requirements for the strains. The generation time for each mutant strain was determined using either glucose or ethanol as carbon source. As shown in Table II, the generation times for all of the mutants are similar to the wild type parent strain when 3% ethanol is used as carbon source. In contrast, the tdh2 and tdh3 mutants grow at approximately 50 and 75% of the rate of the wild type parent, respectively, when the cells are grown in the presence of glucose as carbon source. The generation time for the tdh1, tdh2 double mutant is very similar to the tdh2 single mutant whereas the tdh1, tdh3 double mutant grows more slowly than the tdh3 mutant when the strains are grown on glucose as carbon source. These data suggest a growth phenotype for the tdh1 mutation when the strain also carries a tdh3 mutation.

The observation that each of the glyceraldehyde-3-phosphate dehydrogenase single mutants as well as two of the double mutants are viable explains the inability to isolate yeast glyceraldehyde-3-phosphate dehydrogenase mutants using classical genetic approaches (18). The mutant strains described in this report have been used to identify the polypeptide products of the three glyceraldehyde-3-phosphate dehydrogenase genes and to study the expression of the genes in cells grown on fermentable and nonfermentable carbon sources. These results are presented in the accompanying report (19).

**REFERENCES**