Rapid Method for Isolation and Screening of Cytochrome c Oxidase-Deficient Mutants of Saccharomyces cerevisiae

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We describe here a new method for the specific isolation of cytochrome c oxidase-deficient mutants of Saccharomyces cerevisiae. One unique feature of the method is the use of tetramethyl-p-phenylenediamine as a cytochrome c oxidase activity stain for yeast colonies. The staining of yeast colonies by tetramethyl-p-phenylenediamine is dependent upon a functional cytochrome c oxidase and is unaffected by other lesions in respiration. Since the tetramethyl-p-phenylenediamine colony staining reaction is rapid and simple, it greatly facilitates both the identification and characterization of cytochrome c oxidase-deficient mutants. Another feature of the method, which is made possible by the tetramethyl-p-phenylenediamine colony stain, is the use of an op1 parent strain for the isolation of nuclear pet or mitochondrial mit mutants in specific protein-coding genes. A parent strain that carries this marker selects against rho0 or rho− classes of pleiotropic respiratory-deficient mutants, since these are lethal in op1 strains. We have used this method to isolate 123 independently derived cytochrome c oxidase-deficient pet mutants and 300 independently derived mit mutants.

Cytochrome c oxidase is the terminal member of the mitochondrial electron transport chain, where it transfers electrons from reduced cytochrome c to molecular oxygen. In addition, it catalyzes the redox-linked pumping of protons out of mitochondria and is therefore one of the three sites in the electron transport chain that are responsible for coupling ATP synthesis to electron transport. The Saccharomyces cerevisiae enzyme is composed of nine nonidentical protein subunits (21), two heme a and two copper prosthetic groups, and one molecule of cardiolipin (R. Walenga and R. O. Poyton, Fed. Proc. 37:2326, 1978). The three largest subunits (I, II, and III) are encoded by mitochondrial genes, and the six smallest subunits (IV, V, VI, VII, VIIa, and VIII) are encoded by nuclear genes (14, 22). Because of these features, cytochrome c oxidase has become an important model in studies of four distinct questions. (i) What is the mechanism of electron transport and redox-linked proton pumping? (ii) How are nucleus-encoded mitochondrial proteins targeted to mitochondria and imported to the correct intra-mitochondrial compartment? (iii) How is a heterooligomeric protein assembled within a membrane? (iv) Is there coordination between nuclear and mitochondrial gene expression; if so, how is this achieved?

Mutants that lack cytochrome c oxidase activity are very useful for studying the above questions. Mutants altered in the structural genes for subunits of cytochrome c oxidase enable a direct assessment of the role of each subunit in the catalytic activities of cytochrome c oxidase and, in addition, can be used to identify domains within the polypeptides that are required for targeting them to mitochondria and assembling them into a holoenzyme. So far, mit mutations in the mitochondrial genes oxi3, oxi1, and oxi2 for subunits I, II and III, respectively, and pet mutations in the nuclear gene COX5 for subunit V have been identified (2, 4, 5, 15, 30). Other mutants of interest are those that are altered in trans-acting regulatory genes required for synthesis of nucleus or mitochondrion-encoded cytochrome c oxidase subunits. These mutants can be used to study nucleus-mitochondrion coordination. Several nuclear mutants that are defective in synthesis of one or more of the mitochondrion-encoded cytochrome c oxidase subunits have been described (3, 6, 7, 16) and are beginning to be characterized in molecular detail (19, 29). Finally, mutants defective in the import or processing of subunit precursors are also of interest, since they can provide useful information concerning protein targeting and holoenzyme assembly. A temperature-sensitive nuclear mutant that accumulates the higher-molecular-weight precursor to one of the mitochondrion-encoded subunits, II, has been identified recently (23). Apparently, this mutant is defective in a nucleus-encoded protease required for processing of the subunit II precursor.

To identify additional nuclear subunit structural genes as well as genes required for the synthesis, processing, or assembly of cytochrome c oxidase, we have developed a new method for the isolation of cytochrome c oxidase-deficient mutants. This method is rapid, simple, and specific for cytochrome c oxidase deficiency.

MATERIALS AND METHODS

Yeast strains and genetic analysis. Saccharomyces cerevisiae D273-10B (ATCC 24657) is MATa. The pet mutant strains listed in Table 1 were isolated from D273-10B (6, 33) and were the generous gift of E. Ebner or A. Tzagoloff. Because D273-10B grows well, has high respiratory activity, and has already been used extensively in both genetic and biochemical analyses of respiration, we decided to isolate mutants of a strain that carries the op1 mutation in the D273-10B genetic background. This strain, JM22 (MATa his4 op1 [rho+]), was constructed as follows. First, a MATa ade1 his4 op1 [rho−] haploid was isolated from the meiotic progeny of a cross between the essentially isogenic strains 777-3A (MATa ade1 op1 [rho+]) and ABI-4A (MATa his4 [rho+]) (gifts of P. Slonimski). This derivative was then crossed to a rho0 derivative of D273-10B (D273-10Bp0). The resulting diploid was sporulated, and a haploid with the genotype MATa ade1 his4 op1 [rho−] was identified and backcrossed to D273-10Bp0. This last procedure was repeated five times. JM22 is there-
TABLE 1. Correlation of cytochrome c oxidase activity and degree of TMPD staining

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
<th>Cytochrome c oxidase activity</th>
<th>Spectrophotometric assay (mean ± standard error) (%)</th>
<th>TMPD colony assay (mean ± standard error)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D273-10B</td>
<td>+</td>
<td>+</td>
<td>100 ± 4.7</td>
<td>++</td>
</tr>
<tr>
<td>JM22</td>
<td>op1</td>
<td>+</td>
<td>63 ± 3.2</td>
<td>++</td>
</tr>
<tr>
<td>G2494</td>
<td>op1 box2-1</td>
<td>+</td>
<td>72 ± 5.5</td>
<td>++</td>
</tr>
<tr>
<td>am10-150-4D</td>
<td>+</td>
<td>+</td>
<td>2 ± 0.5</td>
<td>++</td>
</tr>
<tr>
<td>M13-249</td>
<td>+</td>
<td>+</td>
<td>8 ± 0.4</td>
<td>++</td>
</tr>
<tr>
<td>am9-3-6C</td>
<td>+</td>
<td>+</td>
<td>6 ± 0.3</td>
<td>++</td>
</tr>
<tr>
<td>E2-116</td>
<td>+</td>
<td>+</td>
<td>0 ± 0.1</td>
<td>++</td>
</tr>
<tr>
<td>E4-218</td>
<td>+</td>
<td>+</td>
<td>14 ± 1.7</td>
<td>++</td>
</tr>
<tr>
<td>E11</td>
<td>+</td>
<td>+</td>
<td>0 ± 0.1</td>
<td>++</td>
</tr>
<tr>
<td>E2-163</td>
<td>+</td>
<td>+</td>
<td>0 ± 0.1</td>
<td>++</td>
</tr>
<tr>
<td>E2-215</td>
<td>+</td>
<td>+</td>
<td>8 ± 0.4</td>
<td>++</td>
</tr>
<tr>
<td>E2-27</td>
<td>+</td>
<td>+</td>
<td>0 ± 0.1</td>
<td>++</td>
</tr>
<tr>
<td>N8-128</td>
<td>+</td>
<td>+</td>
<td>0 ± 0.1</td>
<td>++</td>
</tr>
<tr>
<td>E4-238</td>
<td>+</td>
<td>+</td>
<td>3 ± 0.2</td>
<td>++</td>
</tr>
<tr>
<td>N9-8</td>
<td>+</td>
<td>+</td>
<td>18 ± 1.2</td>
<td>++</td>
</tr>
</tbody>
</table>

* cos5-1 (5) and ms51-1 (7) have been defined previously. The other loci are designated as pet− because they are so far defined only by complementation analysis.

1. The box2-1 mutation lies in the coding sequence of the apocytochrome b gene. The oxi3, oxi4, and oxi2 mutations are in the genes for cytochrome c oxidase subunits I, II, and III, respectively.

2. The activity of D273-10B, expressed as the first-order rate constant K (minutes per mg of mitochondrial protein) was 22 ± 3.7 (mean ± standard error, n = 7). All other cytochrome c oxidase specific activities were calculated from the mean activity on two or more mitochondrial preparations. ND, Not done.

3. The degree of staining in the TMPD colony assay is indicated as + for wild-type activity, + for a faint staining reaction, and − for no staining reaction (Fig. 1).

Therefore essentially isonuclear with D273-10B and isomitochondrial with 777-3A.

To identify the class of mutation (dominant, recessive nuclear, or mitochondrial) responsible for the cytochrome c oxidase deficiency of newly isolated mutants, complementation tests with JM25 (MATa ade1 [rho−]) and JM8 (MATa ade1 [rho3]) were performed. The complementation patterns were as follows: the respiratory deficiency of dominant mutants was not complemented by either JM25 or JM8; that of recessive nuclear mutants was complemented by both JM25 and JM8; and that of mitochondrial mutants was complemented by JM25 and not by JM8. All genetic procedures were performed by standard methods (28).

A medium containing 1% yeast extract, 2% Bacto-peptone (Difco Laboratories, Detroit, Mich.), 2% dextrose (YPD medium) was used for nonselective growth of yeast. Proteotrophic selection was performed on 0.67% Difco yeast nitrogen base without amino acids–2% dextrose. Respiration proficiency was tested on a nonfermentable medium, either YPEG (1% yeast extract–2% Bacto-peptone–2% (vol/vol) ethanol–5% (vol/vol) glycerol) or SEG (0.67% Difco yeast nitrogen base without amino acids–2% (vol/vol) ethanol–5% (vol/vol) glycerol). The yeast nitrogen base-dextrose and yeast nitrogen base-ethanol-glycerol media contained adenine or histidine (or both) at 40 μg/ml, as required for growth of auxotrophic strains. All solid media contained 2% agar. In experiments requiring isolation of mitochondria, yeasts were cultured in semisynthetic galactose medium that contained the following (per liter): 3 g of yeast extract, 10 g of galactose, 0.8 g of (NH4)2SO4, 0.7 g of MgSO4 · 7H2O, 0.5 g of NaCl, 1.0 g of KH2PO4, 0.4 g of anhydrous CaCl2, and 5.0 mg of FeCl3 · 6H2O.

Isolation of cytochrome c oxidase-deficient mutants. For complementation with 5-amino-4-methyl-N′-nitro-N-nitrosoguanidine (NTG) or ethyl methanesulfonate (EMS), the parent strain JM22 was treated as follows. Cells were grown in YPD medium on a rotary shaker at 28°C. When the cell density reached 1 × 10⁶ to 2 × 10⁶ cells per ml, either NTG or EMS was added at a final concentration of 50 μg/ml or 4% (vol/vol), respectively, to 5-ml samples of cells. Cultures containing NTG were incubated for 30 min on a rotary shaker at 28°C, and cultures containing EMS were incubated for 60 min under the same conditions. Cells were then harvested by centrifugation at 4,000 × g for 5 to 10 min and washed twice with an equal volume of sterile distilled water. Cells were suspended in an equal volume of YPD medium and sonicated for 1 min in a bath ultrasonic cleaner (Megasan) to break up clumps of cells. Cells were kept on ice until they were plated (see below).

Cells were mutagenized with MnSO4 essentially as described by Putrament et al. (24). Strain JM22 was cultured overnight in YPD medium and then inoculated at a final cell density of 1 × 10⁶ to 5 × 10⁶ cells per ml into YPD medium that had been adjusted to pH 6.0. MnSO4 was added to a final concentration of 8 mM, and cells were incubated overnight at 28°C. During this treatment, the density of the culture increased by only twofold, with no apparent loss in viability.

For outgrowth of mutant clones, mutagenized cells were diluted and plated onto YPD agar medium so as to produce 100 to 1,000 colonies per plate. Colonies were allowed to grow for 2 days at 28°C. Colonies that were deficient in cytochrome c oxidase activity were detected as follows. First, colonies were replica plated to Whatman 42 filter paper and permeabilized by allowing them to air dry (18). The dried colonies were rehydrated by briefly soaking the filter paper with 0.04 M potassium phosphate buffer (pH 6.7) containing 0.5% Tween 20. The wet filter paper was then transferred to a petri dish containing approximately 1 ml of a freshly prepared solution of 1% (wt/vol) N,N,N′,N′-tetramethyl-p-phenylenediamine dihydrochloride (TMPD). Care was taken not to immerse the filters and thereby cause the colonies to smear. After 2 to 5 min at room temperature, color development was complete. Colonies that contained active cytochrome c oxidase were stained blue, and cytochrome c oxidase-deficient mutants remained white or were stained light blue. Additional details of the procedure are presented below. Mutants detected in this manner were recloned twice and restested by the TMPD assay. Only stable mutants were kept.

Miscellaneous methods and chemicals. Quantitative cytochrome c oxidase assays were performed on mitochondrial preparations by the spectrophotometric assay (15). Cytochrome c oxidase activity is calculated as the first-order rate constant K (per minute) normalized to the amount of mitochondrial protein assayed. Yeast mitochondria were prepared from sphaeroplasts (15). Protein concentrations were determined by the method of Lowry et al. (13). Horse heart cytochrome c (type VI), NTG, and EMS were purchased from Sigma Chemical Co., St. Louis, Mo. TMPD was purchased from Eastman Kodak, Rochester, N.Y.

RESULTS

Rationale for use of an op1 parent strain. The isolation of respiration-deficient yeast mutants that have either nuclear mutations (pet) or nonpleiotropic mitochondrial yeast muta-
tions (mit) has been hampered in previous studies by the presence of pleiotropic mitochondrial deletion mutants (rho−) or mutants that lack mitochondrial DNA altogether (rho0). Time-consuming procedures (32) are required to distinguish pet and mit mutants from rho− and rho0 mutants, which are the most abundant class of respiratory-deficient mutants that arise in yeast populations. In addition, a serious problem often encountered when working with certain pet and mit mutants is the instability of the mitochondrial genome. For example, some pet mutants exist largely in the rho− state (25) and are therefore difficult to characterize.

To avoid these problems, we have made use of a parent strain that carries a nuclear mutation, op1, in the structural gene for the adenine nucleotide translocator of the inner mitochondrial membrane. Since rho− and rho0 mutations are lethal in an op1 strain (1, 12), respiratory-deficient mutants isolated in an op1 genetic background are either pet or mit. To use an op1 strain, which, like pet and mit mutants, scores as respiration defective because it is unable to grow on nonfermentable media, we use TMPD as a cytochrome c oxidase activity stain for yeast colonies. With this stain, it is possible to directly identify cytochrome c oxidase deficiency in any genetic background, including an otherwise respiration-deficient yeast strain such as an op1 mutant.

Development of a colony assay for cytochrome c oxidase activity. TMPD is a redox dye that has been widely used in polarographic assays of cytochrome c and cytochrome c oxidase activities (8, 9). The oxidation-reduction potential of TMPD (E°0 of about +0.26 [10]) allows it to donate electrons directly to the terminal portion of the electron transport chain. Upon oxidation of TMPD, a stable semiquinoidine radical is formed. This oxidation product is blue, whereas the reduced compound is colorless.

We attempted to use the redox-linked color change of TMPD to stain yeast colonies for cytochrome c oxidase activity and found that this was possible only if the yeast cells were permeabilized. To permeabilize yeast, we used the method of Moshowitz (18), in which yeast colonies are replica plated to filter paper, air dried, and then rehydrated.

After this treatment, yeast colonies with functional cytochrome c oxidase are stained dark blue within a few minutes of application of a 1% solution of TMPD (Fig. 1C, top row).

In Fig. 1 we demonstrate that yeast colonies are stained by TMPD specifically as a consequence of cytochrome c oxidase activity. The three strains in the top rows of Fig. 1 are D273-10B (wild type), JM22 (op1), and G2494 (box2-1). Note that the latter two strains fail to grow on the nonfermentable medium YPGE (Fig. 1B). The op1 mutation does not directly affect any portion of the electron transport chain, and the box2-1 mutation blocks electron transport at the cytochrome b portion of the chain. Both JM22 (op1) and G2494 (box2-1) contain functional cytochrome c oxidase (Table 1); as expected, they are stained dark blue by TMPD (Fig. 1C). Thus, it is clear that the TMPD reaction monitors the terminal (cytochrome c-cytochrome c oxidase) region of the electron transport chain and bypasses the cytochrome b and earlier regions of the chain. To demonstrate that it is the enzymatic activity of cytochrome c oxidase and not the mere presence of hemes c or a/a3 (or both) that is required for the TMPD staining reaction, an additional control was performed. When 1 mM KCN, which inhibits cytochrome c oxidase activity, was included during the rehydration and staining steps of the TMPD assay, the cytochrome c oxidase-proficient strains shown in Fig. 1 failed to be stained blue (data not shown).

Also shown in Fig. 1 are the results of staining mutants that have known deficiencies in cytochrome c oxidase. The three strains in the middle row carry mutations in the mitochondrial structural genes for cytochrome c oxidase subunits I, II, and III. As expected, they are not stained by TMPD (Fig. 1C). The 10 pet mutants, which are shown in the bottom two rows, were isolated and characterized by Tzagoloff and co-workers (33). Seven of these strains are not stained by TMPD, whereas three are stained light blue (bottom right of Fig. 1C). Note that these same three strains grow slowly on yeast extract-peptone-ethanol-glycerol medium (Fig. 1B) and must therefore have residual cytochrome c oxidase activity. To determine the level of sensitivity of the TMPD reaction and to correlate the degree of TMPD staining with the actual level of cytochrome c oxidase activity, we performed standard cytochrome c oxidase assays on isolated mitochondria from the 10 pet strains (Table 1). In general, the correlation is excellent. Based on these

FIG. 1. Specificity of the TMPD stain. (A) Sixteen yeast strains were patched onto YPD medium and grown at 37°C for 2 days. Strains, from left to right: D273-10B (WT), JM22 (op1), and G2494 (box2-1) in the top row; aM10-130 4D (ox13), M13-249 (ox11), and aM9-3 6C (ox12) in the second row; E2-116, E4-218, E11, E2-163, and E2-215 in the third row; E2-27, N8-128, E4-238, N7-211, and N9-8 in the fourth row. See Table 1 for additional information on these strains. (B) The strains from A were replica plated to 1% yeast extract–2% peptone–2% ethanol–3% glycerol and incubated for 2 days at 37°C. (C) The strains from A were replica plated to filter paper and stained with TMPD. See the text for additional details.
TABLE 2. Mutant frequencies

| Mutagen | % Survival | No. of colonies examined | TMPD negative | % of TMPD negative that are \( c^{+} \)
<table>
<thead>
<tr>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>100</td>
<td>1,660</td>
<td>0</td>
<td>&lt;0.06</td>
</tr>
<tr>
<td>EMS</td>
<td>45</td>
<td>4,024</td>
<td>60</td>
<td>1.5</td>
</tr>
<tr>
<td>NTG</td>
<td>9</td>
<td>2,566</td>
<td>50</td>
<td>1.9</td>
</tr>
<tr>
<td>MnCl(_2)</td>
<td>ND</td>
<td>310,940</td>
<td>421</td>
<td>0.13</td>
</tr>
</tbody>
</table>

The percent PET\(^+\) plus the percent MIT\(^-\) does not equal 100. The TMPD-negative mutants that are neither PET\(^+\) nor MIT\(^-\) are either dominant respiration-deficient mutants or mating-defective mutants. ND. Not determined.

data, we conclude that the threshold of cytochrome \( c \) oxidase activity that can be detected by the TMPD colony assay is somewhere between 3 and 8% of the activity of the wild-type strain, D273-10B. Strains that contain less than this amount of activity are not stained by TMPD. Strains that have as much as 23% of the wild-type level of cytochrome \( c \) oxidase activity are stained light blue and are distinguishable from the wild type in the TMPD colony assay. Strains that contain more than approximately 23% activity cannot be distinguished from wild-type strains.

Isolation of cytochrome \( c \) oxidase-deficient mutants. The method for isolation of cytochrome \( c \) oxidase-deficient mutants is diagrammed in Fig. 2. The parent strain JM22, which carries the \( opl \) mutation, was mutagenized with EMS, NTG, or MnSO\(_4\), as described above. After mutagenesis, the cells were washed and immediately plated onto the nonselective medium, YPD. The colonies of survivors were then replica plated to filter paper and stained with TMPD. Colonies that remained white in the TMPD assay were picked from the original YPD plate and restreaked. Each mutant was single colony purified twice and restreaked with TMPD each time. Only those isolates that yielded a consistent result were saved (about 30% of the original isolates).

The frequencies of stable TMPD-negative mutants obtained after mutagenesis with EMS, NTG, and MnSO\(_4\) are shown in Table 2. In these experiments, EMS and NTG treatments generated TMPD-negative mutants at frequencies of 1.5 and 1.9%, respectively. When these mutants were crossed to a rho\(^0\) tester strain to distinguish nuclear (pet) and mitochondrial (mit) mutations, the majority of mutants (92 and 98%, respectively) proved to be nuclear. To obtain a population of mutants enriched for the mit class, we used MnSO\(_4\) as a mutagen. The frequency of TMPD-negative mutants generated by MnSO\(_4\) treatment was only 0.13% (Table 2), but in contrast to the EMS and NTG experiments, the majority of these mutants (71%) were mit. Because mutagenized cells were not allowed a recovery and outgrowth period in liquid medium, but were instead plated onto solid YPD medium immediately after mutagenesis, each mutant isolated by the method diagrammed in Fig. 2 must be of independent origin. From the EMS and NTG mutagenesis experiments, we collected 123 independently derived pet mutants. From the MnSO\(_4\) mutagenesis, we collected 300 independently derived mit mutants. It is interesting that, after mutagenesis with either NTG or EMS, cytochrome \( c \) oxidase mutants were recovered at a frequency between 1 and 2%. This frequency, which is approximately equivalent to the rate of induction of auxotrophic mutants (17), is surprisingly high and suggests that a large number of nuclear genes is required for the biogenesis of functional cytochrome \( c \) oxidase.

**DISCUSSION**

For reasons that are not yet well understood, the \( opl \) mutation, which alters the nuclear gene for the adenine nucleotide carrier of the inner mitochondrial membrane, is lethal in combination with either rho\(^-\) or rho\(^0\) mutations (1, 12). Nevertheless, Kotylak and Slonimski (11) cleverly exploited this phenomenon in a method that they developed for the isolation of mit mutants. The advantage of the method is that, by isolating respiration-deficient mutants in the \( opl \) genetic background, rho\(^-\) and rho\(^0\) mutants are eliminated. This greatly facilitated the subsequent identification and analysis of mit mutants, which were of primary interest to them.

In this paper, we have described a new \( opl \) method for isolation of both pet and mit mutants that lack functional cytochrome \( c \) oxidase. The unique feature of this method, which allows us to directly identify \( opl \) strains that are cytochrome \( c \) oxidase deficient, is the use of the redox dye TMPD to specifically stain yeast colonies for cytochrome \( c \) oxidase activity. We explain this specificity of the TMPD colony staining reaction as follows. From studies on the mechanism of electron transfer from the artificial electron donor TMPD to the terminal portion of the electron transport chain, it is clear that cytochrome \( c \) is an important mediator between TMPD and cytochrome \( c \) oxidase (8, 9). In principle, therefore, colonies that fail to be stained blue by our TMPD colony staining procedure could be deficient in either cytochrome \( c \) or cytochrome \( c \) oxidase. However, \( S.\ cerevisiae \) encodes two isozymes of cytochrome \( c \), either one of which alone is sufficient to support growth on certain nonfermentable media (26, 27). Mutants that lack both isozymes of cytochrome \( c \) should be rare relative to cytochrome \( c \) oxidase-deficient mutants. Indeed, we have spectroscopically examined each of the 123 \( pet \) mutants that we isolated and found none that were specifically deficient in cytochrome \( c \) (data not shown). TMPD can also reduce cytochrome \( c_1 \) (20, 31). However, we expect that mutants deficient in cytochrome \( c_1 \) will be stained blue in the TMPD colony staining procedure, because TMPD can donate electrons directly to cytochrome \( c \) and thereby bypass the block at cytochrome \( c_1 \) in the electron transport chain.

The ability to stain yeast colonies for cytochrome \( c \) oxidase activity facilitates the isolation and characterization of mutants in several respects. First, TMPD effectively stains any yeast colony that has functional cytochrome \( c \) oxidase, regardless of the presence of other mutations that block electron transport or respiration. As we mentioned above, this has enabled us to develop an \( opl \) method for isolation of both pet and mit mutants that lack cytochrome \( c \) oxidase. Second, the TMPD colony staining reaction is complete within 2 to 5 min after application of the TMPD solution to yeast colonies. This permits a much more rapid identification of cytochrome \( c \) oxidase-deficient mutants than has been previously possible when either standard cytochrome \( c \) oxidase assays on isolated mitochondria or spectroscopic examination of individual colonies was necessary. Finally, because the TMPD assay is easy to perform, mutant strains can be frequently monitored for revertants. This ability to routinely check the phenotypes of mutants facilitates the storage and handling of these strains.

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LITERATURE CITED


