Ultrastructure and biochemical function of the mitochondria in respiratory-deficient mutant yeast induced by 4-nitroquinoline nitrogen oxide

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Abstract

1. A respiratory-deficient mutant strain of yeast was obtained from wild strain of Saccharomyces servisiae by treatment with 4-nitroquinoline N-oxide. Ultrastructure and function of the wild or mutant strains and the mitochondrial fractions isolated from these strains were examined by biochemical and electron microscopic analyses. 2. The frequency of the respiratory-deficient mutant strain in yeast induced with 10-6M 4-nitroquinoline N-oxide was about 40 %. 3. Respiratory-deficient mutant strain is incapable of reducing 2, 3, 5-triphenyltetrazolium chloride salt and to grow on lactate medium. In addition to this, the mutant has been found to have lost its ability to take up oxygen in sodium succinate and pyruvate. 4. 4-Nitroquinoline N-oxide in the concentration that induces a mutant of yeast cells or its kin inhibits the oxygen uptake in normal strain. 5. The normal strain of yeast is characterized by difference spectrum corresponding to cytochromes a+as, band c+CI respectively, whereas, the mutant strain contains almost no cytochromes a+ as, band C1 but contains normal or increased amount of cytochrome c. 6. Mitochondrial fraction isolated from mutant strain has largely lost its ability to oxidize succinate. On the other hand, NADH-, lactate-and cytochrome c-oxidase activities are reduced by about 1/17, 1/7 and 1/8 of that of normal strain, respectively. 7. Succinate dehydrogenase activity of mutant strain is almost zero. Moreover, this activity is not affected on the addition of phenazine methosulfate. NADH dehydrogenase activity of mutant strain is about 1/2 of normal strain. 8. The variations in mitochondrial structure of normal and mutant strain in the stationary phase have been followed with the aid of electron microscopy. In contrast to the normal strain, the mutant strain revealed distinct morphological changes in mitochondria, especially, the lack of cristae in its interior. The results have been interpreted to indicate that the mutant induced by 4-nitroquinoline N-oxide has a character of cyto. plasmic mutant.

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ULTRASTRUCTURE AND BIOCHEMICAL FUNCTION OF THE MITOCHONDRIA IN RESPIRATORY-DEFICIENT MUTANT YEAST INDUCED BY 4-NITROQUINOLINE NITROGEN OXIDE

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4-Nitroquinoline nitrogen oxide was synthesized by Otai et al. (1, 2) (1945), and demonstrated to be a powerful carcinogen by Nakahara and his colleagues (3). It has been also known that 4-nitroquinoline N-oxide, aside from its carcinogenic action, possesses various biological actions such as the induction of mutants in organism (4, 5), the ability to induce prophage (6), and the formation of characteristic intranuclear inclusion bodies in tissue culture cells (7).

On the other hand, it is well known that respiratory-deficient mutants of yeast are induced by various physical-chemical factors (8, 15) such as ultraviolet-light (16, 19), heat (18), anaerobic culture, etc. (19, 21). The deficiency of respiratory enzymes in the respiratory-deficient mutants is a hereditable character, and the ultrastructures of the cells undergo especially marked changes in mitochondrial profiles (22–28).

Mifuchi et al. (1963) (29) induced mutants with deficient respiration in Saccharomyces cerevisiae by 4-nitroquinoline N-oxide and reported about the toxohormone-like substance of its mutants. However, the mechanism of induction of mutant and the subsequent ultrastructural changes in yeast cells by 4-nitroquinoline N-oxide are not known yet. The investigation of biological mechanisms in the production of respiratory-deficient mutants in yeasts by 4-nitroquinoline N-oxide would be helpful for elucidating the problems involved in both carcinogenesis and inheritance. This study was conducted in order to dissolve the mechanism of production of respiratory-deficient mutant in yeast by this compound, and this paper describes ultrastructures and biochemical functions of the mitochondria of the respiratory-deficient mutant yeast.
MATERIALS AND METHODS

Yeast strain:
Saccharomyces cerevisiae (Hansen Kyokai, No. 7, IAM, 4518) was used in the present work. This strain was transplanted every other month on 1% glucose-agar plates.

Growth medium:
The solid medium used for cultivation of the cells was composed of 0.35% peptone, 0.4% yeast extract, 0.2% KH₂PO₄, 0.1% MgSO₄·6H₂O, 0.3% (NH₄)₂SO₄, 2% glucose, and 1.5% agar. The liquid medium consisted of the above medium minus agar.

Isolation of respiratory-deficient mutant strains and its identification:
Mutant strains were induced by the method of NAGAI (11, 14, 30). The respiratory-deficient mutant strains were prepared by cultivation of the same strain in the same liquid medium with final concentration of 10⁻⁴M 4-nitroquinoline N-oxide. The identification of mutant strains was done by the method of OGUR et al. (31). Namely, respiratory-deficient mutant strains were isolated by the 2,3,5-triphenyltetrazolium chloride (TTC) overlay and lactate medium technique (32, 33).

Growth of normal and mutant strain of yeasts:
The normal and mutant strains were maintained aerobically on agar slope for 48 hour at 30°C. The yeast cells were harvested, washed with cold water, and stored at 4°C until used.

Isolation of snail gut enzyme:
The snail gut enzyme was isolated by the method of ONISHI et al. (34). Namely, the shell of garden snails available in Japan was broken, and gut-juice sac taken out from digestion tube. After chopping by the Waring blender, centrifuged at 1,200 x g for 10 minutes at 4°C in the Kubota centrifuge, rotor No. 3. The supernatant was fractionated with ammonium sulfate (50% saturation) and dialyzed through collodion membrane at 4°C overnight.

Formation of spheroplasts and isolation of mitochondria:
The spheroplasts and mitochondria were prepared by the methods of ELIZABETH (35) and ONISHI et al. (34, 36). The harvested cells were suspended in 1 ml of the medium containing 1.5 M sorbitol, 0.5 mM EDTA, 10 mM Tris-HCl buffer (pH 6.5), 0.03 M 2-mercaptoethylamine, and 4 mg of snail gut enzyme per g of cells (wet weight). The suspension was thoroughly mixed in a Waring blender, and was incubated for a given time at 30°C with occasional stirring. After two-hour incubation, the cells were centrifuged at 1,000 x g for 10 minutes at 0°C in the Kubota centrifuge, with rotor No. 3. The pellets were resuspended in the incubation medium without the gut juice enzyme, and recentrifuged at 1,000 x g for 10 minutes at 0°C. This washing was repeated twice. Lysis of the spheroplasts was accomplished with 0.7 M sorbitol, 0.1 mM EDTA, and 10 mM Tris-HCl buffer (pH 6.5) by mixing the cells together with glass beads in a Waring blender at top speed for 4 minutes at 0°C. The cell debris and whole cells
were centrifuged at 1,000 × g for 5 minutes at 0°C, and resulting supernatant layer was centrifuged at 15,000 × g for 10 minutes at 0°C. The pellet containing the mitochondria was suspended in the preparation medium, and an identical centrifugation process was repeated once more. The pellet was taken as the fraction of mitochondria.

**Oxygen uptake of respiration with various substrates:**

The respiratory-inhibiting action upon normal yeasts by 4-nitroquinoline N-oxide and respiratory-deficient degree of mutant strains were measured in Warburg manometer with air gas phase at 30°C. The main chamber contained cell suspension (dry weight 0.5 mg), 25 mM phosphate buffer (pH 6.0), and 10 mM substrate (sucrose, sodium succinate, sodium pyruvate, sodium acetate, or lithium lactate). The center vessel contained 0.2 ml of 10 N KOH and filter paper. Then 4-nitroquinoline N-oxide was added to the side bulb and tripped into the reaction mixture after temperature equilibration, and oxygen consumption was measured every 30 minutes. In measuring the respiratory-deficient degree of mutant strains, the yeast was added to the side bulb, and tripped into the reaction mixture after temperature equilibration.

**Measurement of enzyme activities:**

The oxidase activities of succinate, NADH, and lactate systems were measured in the oxygen electrode (37) at 37°C. The medium contained 5 mM MgCl₂, 10 mM potassium phosphate buffer (pH 6.5), 10 mM KCl, 0.2 mM EDTA, and 1 mM sorbitol. To 2 ml of this medium was added an adequate amount of mitochondria suspension and substrate (5 mM sodium succinate, 2 mM NADH or 5 mM sodium lactate) to make the final volume 2.5 ml.

Cytochrome c-oxidase activity was measured in the oxygen electrode at 37°C. To 2 ml of the above medium were added adequate amounts of mitochondria suspension, 5 mM ascorbic acid, 0.01 mM tetramethyl paraphenylendiamine dihydrochloride, and 0.5 mg cytochrome c to make the final volume 2.5 ml.

Succinate and NADH dehydrogenase activities (38) were measured by reading the rate of decrease in absorbancy at 420 mJ of a mixture containing 1 mM KCN, 25 mM potassium phosphate buffer (pH 7.4), 0.1 mM EDTA, 0.5 mM ferricyanide, yeast mitochondria fraction, and 33 mM sodium succinate or 1 mM NADH. The final volume was 3.0 ml. In the case of addition of phenazine methosulfate, the final volume was made 3.0 ml by adding 0.01 ml of 1% phenazine methosulfate solution.

**Spectrophotometric measurement:**

Difference spectra were obtained with a Cary type autorecording spectrophotometer on a medium containing the following constituents: 0.3 ml of 1 M Tris-HCl buffer (pH 8.5), 0.3 ml of 20% cholate, 0.3 ml of 10% deoxycholate, and yeast mitochondria (protein 10 mg) of which the final volume was 3.0 ml. The resulting difference in absorbancy was through the wavelength interval between 500 and 650 mJ.

**Protein determination:**

Protein content of the pellet containing mitochondria was measured by the
biuret reaction in the presence of 0.3 % deoxycholate (39).

**Electron microscopy:**

The cells were harvested by centrifugation and the samples were prepared by the method of Linnen et al. (27). The cells were fixed in 10 volumes of 2 % potassium permanganate for two hours at 0°C and then in 10 volumes of a fresh permanganate solution overnight at room temperature. The preparation was washed with water and then post-fixed in a solution of 1 % uranyl nitrate-1 % potassium dichromate for two hours at 0°C to increase the contrast in the specimens. Fixed cells were subsequently washed with water, dehydrated in increasing concentrations of ethanol. The dehydrated material was embedded in Epon 812. Thin sections were cut on a Porter-Blum MT 2 ultra-microtome with a glass knife. Sections were stained with uranyl acetate and lead citrate. A Hitachi HU 11-D microscope was employed for the observations of sections.

**RESULTS**

**Induction and diagnosis of respiratory-deficient mutant by 4-nitroquinoline N-oxide**

Induced ratio of respiratory-deficient mutants in yeast shows about 40 % with 10^{-6}M 4-nitroquinoline N-oxide. In concentration higher than 10^{-6}M the growth is mostly suppressed. On the contrary, in concentration lower than 10^{-6}M the induced ratio of respiratory-deficient mutants shows a marked fall. Colonies of the mutant are much smaller than normal colonies, and they are given, therefore, the designation “petite colonies” to its form. These colonies are recognized as white colonies by the TTC-agar-overlay method, whereas the normal colonies are stained red. Also the normal strains can be grown on lactate-agar, but the mutant strain cannot grow on the same medium. These results show that the respiratory-deficient mutant induced by 4-nitroquinoline N-oxide differs from normal wild yeasts with respect to their morphological, physiological, and enzymatic characters.

**Inhibition of oxygen uptake of wild type of yeasts and respiratory-deficient mutant yeasts induced by 4-nitroquinoline N-oxide**

To confirm whether the mutant strains induced with 4-nitroquinoline N-oxide lose their oxygen uptake ability, experiments with such mutants were carried out with Warburg manometer. As a result it was found that the mutant strains have a very low capacity of oxygen uptake in various substrates as compared with wild strain, and especially, noteworthy is the fact that they have practically no capacity of oxygen uptake in substrates like sodium acetate and pyruvate (Fig. 1). This phenomenon has been interpreted to indicate that mutant strain lacks the respiratory enzymes.
Fig. 1 Oxygen uptake of normal and respiratory-deficient mutant yeasts by various substrates. — normal cells; —— mutant cells: substrate (○—○ sucrose, ●—● glucose, ×—× sodium acetate, Δ—Δ sodium pyruvate).

Fig. 2 Inhibition of oxygen uptake in normal yeast by the 4-nitroquinoline N-oxide with sucrose. — absence of 4-nitroquinoline N-oxide; —— presence of 4-nitroquinoline N-oxide (○—○, 10⁻⁵M; ●—●, 10⁻⁴M; Δ—Δ, 10⁻³M; ×—×, 0 M).

Figures 2 and 3 illustrate the inhibition of oxygen uptake with 4-nitroquinoline N-oxide on normal yeast cells in the medium containing sucrose and sodium succinate. The inhibitory ratio differs with a kind of substra-
Fig. 3 Inhibition of oxygen uptake in normal yeast by the 4-nitroquinoline N-oxide with sodium succinate. — absence of 4-nitroquinoline N-oxide; ---- presence of 4-nitroquinoline N-oxide (---, 0 M; ○, 10^{-4} M; , 10^{-5} M; , , 10^{-6} M).

tes and the concentrations of 4-nitroquinoline N-oxide. However it should be noted that 4-nitroquinoline N-oxide inhibits oxygen uptake in a similar concentration as that induced mutant.

Fig. 4 Difference spectra (reduced/oxidized) of mitochondria fractions of normal and respiratory-deficient mutant yeast. solid line, normal strain; dotted line, mutant strain; chain line, base line.
The difference spectrum of mitochondrial fractions prepared from wild and mutant yeasts

The difference spectra obtained after deoxycholate treatment of mitochondrial fractions prepared from normal and mutant yeast cells are shown in Figure 4. The spectrum obtained from the wild type yeast is represented by solid line. Absorption bands at 602, 562 and 553 m\(\mu\) indicate the presence of cytochrome \(a+a_s\), \(b\) and \(c+c_h\), respectively. On the contrary, absorption bands of cytochromes \(a+a_s\), \(b\) and \(c\) were not recognized in the mutant yeast. The results suggest that mutant strain induces ultrastructural changes in the mitochondrial cristae.

Enzymic activities of mitochondrial fraction from wild and mutant yeasts

Table 1 gives the activities of succinate, NADH, lactate, and cytochrome \(c\) oxidase in the mitochondrial fraction isolated from wild and mutant strains of yeast. The mitochondrial fraction isolated from mutant strain is found to have lost most of the ability to oxidize succinate when oxygen is the terminal electron acceptor. On the other hand, NADH, lactate and cytochrome \(c\) oxidase activities are reduced about 1/17, 1/7 and 1/8, respectively. Especially, NADH oxidase activity is significantly lower as compared with normal strain. Table 2 gives the activities of succinate and NADH dehydrogenase in the mitochondrial fraction isolated from wild and mutant strains of yeast. Succinate dehydrogenase activity

Table 1  Succinate, NADH, lactate and cytochrome \(c\) oxidase activities in the mitochondria fractions isolated from normal and mutant strains of yeast, \textit{Saccharomyces cerevisiae}

<table>
<thead>
<tr>
<th>Enzyme system</th>
<th>Activities</th>
<th>Normal yeast</th>
<th>Mutant yeast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Succinic oxidase</td>
<td>79</td>
<td>~0</td>
<td></td>
</tr>
<tr>
<td>NADH oxidase</td>
<td>280</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Lactate oxidase</td>
<td>72</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Cytochrome (c) oxidase</td>
<td>648</td>
<td>80</td>
<td></td>
</tr>
</tbody>
</table>

Table 2  Succinate- and NADH-dehydrogenase activities in the mitochondria fractions isolated from normal and mutant yeasts, \textit{Saccharomyces cerevisiae}

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Activities</th>
<th>Normal yeast</th>
<th>Mutant yeast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Succinate</td>
<td>66</td>
<td>~0</td>
<td></td>
</tr>
<tr>
<td>Succinate + Phenazine methosulfate</td>
<td>111</td>
<td>~0</td>
<td></td>
</tr>
<tr>
<td>NADH</td>
<td>120</td>
<td>28</td>
<td></td>
</tr>
</tbody>
</table>
of wild strain was increased to nearly two-fold by the addition of phenazine methosulfate. On the other hand, succinate dehydrogenase activity of mutant strain was mostly zero, and this activity was not affected by the addition of phenazine methosulfate. NADH dehydrogenase activity of mutant strain was likewise about 1/2 of the normal. It may be envisaged from the results that 4-nitroquinoline N-oxide inhibits the synthesis of the insoluble enzymes of the mitochondrial cristae.

Changes in the ultrastructures of respiratory-deficient mutant and normal yeast cells

Lack of respiratory enzymes in the mutant strain is supposed to induce ultrastructural changes in the mitochondria, especially, in cristae. Figs. 6 and 7 show electron micrographs of wild and mutant yeast cells grown in a 2% glucose medium for 48 hour at 30°C. In wild yeast, the cell wall, plasma membrane, nucleus, large vacuole, and mitochondria are observable. In contrast to the wild yeast, the cytoplasm of the mutant strain shows a clear morphological changes in mitochondria, especially, lack of cristae in the interior. Also, the increase of the characteristic vacuolar structure is seen in almost all the cells.

The results apparently indicate that respiratory-deficient mutant

![Diagram of electron transfer and oxidative phosphorylation systems](http://escholarship.lib.okayama-u.ac.jp/amo/vol23/iss5/1)

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Fig. 5 Diagrammatic representation of the electron transfer and oxidative phosphorylation systems of the respiratory-deficient mutant strain.

Fig. 6 The wild strain of *Saccharomyces cerevisiae* grown on 2% glucose medium for 48 hour at 30°C. The cell wall (CW), the plasma membrane (PM), mitochondria (M), vacuole (VAC), and nucleus (N) are apparent. The mitochondria has the well-developed cristae. (× 28,000)

Fig. 7 The cytoplasmic respiratory-deficient mutant grown on 2% glucose medium for 48 hour at 30°C. Remarkable changes are observed in the mitochondria, such as the decrease in both number and size as well as the underdevelopment or lack of the cristae. On the other hand, the increase in number and size of the vacuoles is seen. (× 28,000)
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strains induced by 4-nitroquinoline N-oxide are characterized by mitochondria with almost no cristae and with lack of cytochromes $a + a_n$, $b$ and $c$, and succinate dehydrogenase components (Figure 5). Thus, it may be reasonable to interpret that 4-nitroquinoline N-oxide inhibits the protein-synthesizing factor of the mitochondrial cristae.

**DISCUSSION**

The mutant strain of respiratory deficiency in yeast was first produced by Stier and Caster (40) by exposing yeasts to cyanide. A similar mutant was induced in yeast by ethylene oxide by Whelton and Phaff (41). Also, Ephrussi et al. (9) produced similar mutant strain by acriflavine and studied then in great detail. Since then the induction of mutant strains by ultraviolet-radiation, basic dyes, heavy metal salts, and respiratory enzyme inhibitors have been reported in many laboratories. The mechanism of action of these inducers is not known, but there are some speculations concerning the primary interaction between the agent molecules and nucleic acids of yeast cells (42). It is explained that the induction of the respiratory-deficient mutant is brought about by the action of drugs on the nucleus genes or cytoplasmic genes, which ultimately causes the failure of the respiratory enzyme formation (43). It is difficult to explain the action mechanism of induction of respiratory-deficient mutant by various factors whether such agents act directly on genes or indirectly to block cellular metabolisms. However, it is easily understandable from the nature of 4-nitroquinoline N-oxide that the interaction of 4-nitroquinoline N-oxide with nucleic acid of yeast cells may be responsible for the inhibition of the synthesis of nucleic acid. Mifuchi et al. (1963) (29) used for this purpose the carcinogenic agent, 4-nitroquinoline N-oxide. However, benzyrene and methylcholanthrene (44), or other carcinogenic agents have scarcely produced any respiratory-deficient mutants in yeast and bacteria. A number of chemical carcinogens of tern have not exhibited any mutagenic action. On the other hand, generally mutagens have not been shown to possess any carcinogenic activity. Therefore, the induction of respiratory deficiency by 4-nitroquinoline N-oxide might be specific for yeast cells.

It is noteworthy that 4-nitroquinoline N-oxide inhibits the oxygen consumption of normal yeast cell in the similar concentration as that induced mutant. This phenomenon has not yet been clarified as to whether the inheritable factors in the mother cell or new budding cells have a sensitive nature against agents. It is known that the great majority of new
budding cells are respiratory deficient, and that the mother cell still remains normal (43). This phenomenon has yet not been elucidated with respect to the question as to whether the inheritable factor in the mother cell transmitted indirectly in daughter cell or new budding cells have a sensitive nature against agents. Such mechanisms still remain unsolved, and it will be a matter to be solved in future.

From the experimental results, it seems most reasonable to consider that the respiratory-deficient mutant strain induced by 4-nitroquinoline N-oxide obviously indicates lack of respiratory enzymes. These respiratory enzymes are contained in mitochondria, and therefore, it is reasonable to assume that the ultrastructures of the inner mitochondrial membranes may necessarily undergo some changes. Up to the present, the respiratory-deficient mutants induced by various agents have been known to lack in cytochromes $a$, $b$ (16, 24, 28, 45), cytochrome oxidase, succinic dehydrogenase, NADH-cytochrome $c$ reductase, and $\alpha$-glycerophosphate dehydrogenase (43, 45—47), and to have a decrease in ubiquinone content (48). The respiratory-deficient mutant produced by 4-nitroquinoline N-oxide was recognized to be lacking in cytochromes $a + a_0$, $b$ and $c$, as well as in succinic dehydrogenase. In matters connected to this problem, it would be interest to examine structural units of the inner membrane of mitochondria. In general, it is well known that the lack of respiratory enzymes induces the ultrastructural changes in mitochondria. The mutant strain induced by 4-nitroquinoline N-oxide was observed to lack the cristae of mitochondria. This structural change differs according to the growth cycle of the yeast cells (25, 26), and have dealt with the influence of anaerobic conditions (27, 45, 49, 51), glucose concentration (25, 52, 58) on mitochondrial development. A large number of mitochondria in the cell of stationary phase have a well-developed cristae. There is a close correlation between the evolution of mitochondrial and metabolic changes (24, 28, 45, 47, 50). Noticeable phenomenon is structural changes between the cytoplasmic and chromosomal mutants. In the former, the cristae of mitochondria is not recognized. On this point, the mutant strain induced by 4-nitroquinoline N-oxide is supposed to have character of cytoplasmic mutant. It would be interest to know whether the lack of respiratory enzymes is correlated to a structural abnormality which has to be clarified.

Linnane et al. (52, 56) recently, from the effects of chloramphenicol on *Saccharomyces cerevisiae* and on a cytoplasmic respiratory-deficient mutant, they have suggested that chloramphenicol inhibits the protein-synthesizing system characteristic of the mitochondria. On the other hand, the mito-
chondrial DNA of yeast has recently been demonstrated by the chemical analysis (53, 54) and electron microscopy (57). It is suggested that there is a relationship between the mitochondrial DNA and the synthesis of the insoluble enzymes of the mitochondrial cristae (52, 58), and between the mitochondrial DNA and cytoplasmic genes (55). But, the interactions between 4-nitroquinoline N-oxide and nuclear genes, mitochondrial DNA and cytoplasmic genes were not made clear from the present experiment.

In any case, these matters may be helpful to solve the problems involved in the mitochondrial differentiation. Also, such investigations will certainly lead to a possibility which should not only help to solve biological problems — multiplication, differentiation and growth of the cell — but also be helpful to make clear the problem of mechanism of adaptation, and thus, contribute to the study of the cancer problems.

SUMMARY

1. A respiratory-deficient mutant strain of yeast was obtained from wild strain of Saccharomyces servisiae by treatment with 4-nitroquinoline N-oxide. Ultrastructure and function of the wild or mutant strains and the mitochondrial fractions isolated from these strains were examined by biochemical and electron microscopic analyses.

2. The frequency of the respiratory-deficient mutant strain in yeast induced with 10^{-6} M 4-nitroquinoline N-oxide was about 40%.

3. Respiratory-deficient mutant strain is incapable of reducing 2, 3, 5-triphenyltetrazolium chloride salt and to grow on lactate medium. In addition to this, the mutant has been found to have lost its ability to take up oxygen in sodium succinate and pyruvate.

4. 4-Nitroquinoline N-oxide in the concentration that induces a mutant of yeast cells or its kin inhibits the oxygen uptake in normal strain.

5. The normal strain of yeast is characterized by difference spectrum corresponding to cytochromes $a+a_s$, $b$ and $c+c_s$, respectively, whereas, the mutant strain contains almost no cytochromes $a+a_s$, $b$ and $c$, but contains normal or increased amount of cytochrome $c$.

6. Mitochondrial fraction isolated from mutant strain has largely lost its ability to oxidize succinate. On the other hand, NADH-, lactate-and cytochrome $c$-oxidase activities are reduced by about 1/17, 1/7 and 1/8 of that of normal strain, respectively.

7. Succinate dehydrogenase activity of mutant strain is almost zero. Moreover, this activity is not affected on the addition of phenazine methosulfate. NADH dehydrogenase activity of mutant strain is about 1/2
Respiratory-Deficient Mutant Yeast of normal strain.

8. The variations in mitochondrial structure of normal and mutant strain in the stationary phase have been followed with the aid of electron microscopy. In contrast to the normal strain, the mutant strain revealed distinct morphological changes in mitochondria, especially, the lack of cristae in its interior. The results have been interpreted to indicate that the mutant induced by 4-nitroquinoline N-oxide has a character of cytoplasmic mutant.

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