# CHARACTERIZATION OF RESPIRATION-DEFICIENT MUTANT OF SACCHAROMYCES CEREVISIAE LACKING CYTOCHROMES a AND c

T. Morita and I. Mifuchi
Department of Microbiology, Shizuoka College of Pharmacy, Shizuoka, Japan

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SUMMARY: A respiration-deficient yeast 58NW-46, which lacked the absorption of cytochromes a and c and showed characteristic absorption maxima at 529 and 557 mμ, was induced by treatment of haploid strain of Saccharomyces cerevisiae with carcinogenic agent 4-nitroquinoline 1-oxide. The mutant was demonstrated as a unique one which contained only cytochrome $b_2$, since the absorption maxima of mutant cells were identical with those of yeast cytochrome $b_2$ and the mutant had L-lactate dehydrogenase which was considered to be cytochrome $b_2$.

Typical respiration-deficient (RD) mutants of Saccharomyces cerevisiae lack cytochromes a and b, whether they are cytoplasmic or nuclear mutations. However, many types of RD mutants of yeast which had variously altered cytochrome patterns have been reported, including a deficiency in cytochrome a with the presence of cytochromes b and c (1, 2), a decrease in cytochrome c with a normal amount of cytochromes a and b (1), a deficiency in all cytochromes (3), etc.

As reported in the previous paper (4), various types of mutants were induced by treatment of S. cerevisiae with the carcinogenic agent 4-nitroquinoline 1-oxide (4-NQO). From these mutants of yeast, we have isolated an interesting RD mutant, strain 58NW-46, which lackes typical cytochrome patterns and has characteristic absorption maxima at 529 and 557 mμ.

In this investigation, spectrophotometric and enzymatic studies were carried out on the mutant showing absorption maxima at 529 and 557 mμ. The experimental results indicated that strain 58NW-46 was a unique RD mutant which had only cytochrome $b_2$ without all other cytochromes.

MATERIALS AND METHODS: Nuclear RD mutant 58NW-46 was isolated from various types of mutants induced by treatment of haploid strain, Saccharomyces cerevisiae $R_2$04A, with 4-NQO. Cytoplasmic RD mutant 58NW-1 was also induced by 4-NQO.

Each strain was cultured aerobically at 30°C for 16 hours with vigorous shaking in 500-ml flasks containing 100 ml of Ogur's glucose medium (5).

Harvested yeast cells were suspended in four volumes of 1/15 M phosphate buffer (pH 5.7). The absorption spectrum of the cell suspension was determined by Shibata's opal glass method (6) with a Hitachi Recording Spectrophotometer, EPS-2U. When measuring the spectrum, cytochromes in yeast cells were reduced by the addition of 0.3 mg/ml of $Na_2S_2O_4$ or oxidized by $H_2O_2$.

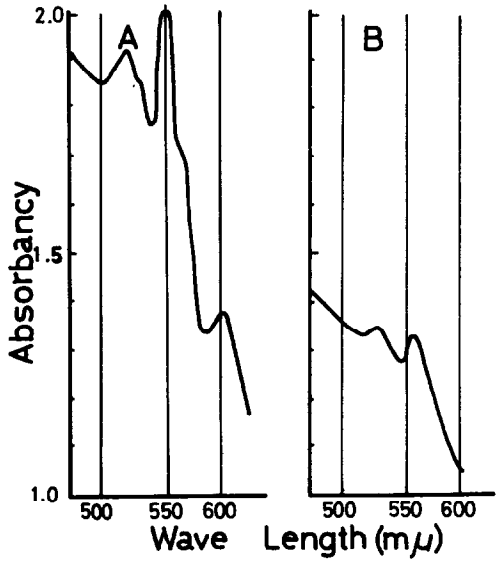
Cell free extracts of yeast used for the enzymatic tests were prepared as follows. Harvested yeast cells were washed three times with distilled water, suspended in the same volume of 0.2 M phosphate buffer (pH 7.0) and disintegrated by shaking with three volumes of glass beads for 30 sec at 4°C with a Braun cell homogenizer. The cell free extract obtained after centrifugation for 10 min at 1500 X g was analyzed for L-lactate:cytochrome c oxidoreductase (EC 1.1.2.3).

L-Lactate:cytochrome c oxidoreductase was assayed in 0.04 M phosphate buffer (pH 7.0), containing 0.08 mM ferricytochrome c, 1 mM KCN and 7 mM sodium L-lactate. The reac-

tion was started by the addition of the cell free extract at 30°C. Activities were calculated from the increase in absorbancy at 550 mμ and corrected for the endogenous reduction of ferricytochrome c measured under the same conditions but in the absence of the substrate. Specific activities were expressed as mμ moles of cytochrome c reduced per min per mg protein of the cell free extract. Protein was determined by the method of Lowry et al.(7).

RESULTS AND DISCUSSION: The absorption curve indicated that parent strain $R_2$04A had cytochromes a + $a_3$, b + $b_2$ and c as represented by a peak at 603 mμ, shoulders at 530 and 557 mμ,

Fig.1. Absorption spectra of parent strain and mutant strain 58NW-46 of S. cerevisiae




Aerobically cultured yeast cells in four volumes of 1/15M phosphate buffer(pH 5.7) were determined by Shibata's opal glass method. Spectrum was taken with 0.3 mg/ml of $Na_2S_2O_4$ as a reducing agent.
A; parent strain $R_2$04A B: RD mutant 58NW-46

and peaks at 520 and 550 mμ respectively (Fig.1-A). However, mutant strain 58NW-46 was shown to have characteristic absorption at 529 and 557 mμ (Fig.1-B). These absorption maxima were different from those of hemeless mutant of S. cerevisiae which had unusual absorptions at 538 and 575 mμ (3) or those of a mutant which had zinc porphyrins and showed absorption at 540 and 575 mμ (8). Accordingly, the absorption maxima of a whole cell suspension of strain 58NW-46 were compared with those of yeast cytochromes (Table 1). It was clear from this result that the absorption maxima of the mutant were identical with those of yeast cytochrome $b_2$, whether they were measured in a reduced or oxidized form.

It is known that yeast cytochrome $b_2$ is identical with yeast L-lactate dehydrogenase (9). A very small amount of the cytochrome can be detected by analyzing the presence of the enzyme. Consequently, L-lactate dehydrogenase activity of the cell free extract of strain 58NW-46 was assayed as L-lactate:

Table 1. Absorption maxima of whole cell suspensions of mutant strain 58NW-46 and of yeast cytochromes

| Samples | Absorption maxima (mμ) α | β | γ |
|---|---|---|---|
| 58NW-46 | 557 | 529 | 422 (412) |
| yeast cytochrome a* | 605 | | 444 (418) |
| b* | 564 | 530 | 430 (416) |
| $b_2$ | 557 | 528 | 422 (411) |
| c | 550 | 520 | 415 (408) |

Figures in the table indicate the absorption maxima(mμ) of cytochromes in reduced and oxidized(parenthesized figures). * Data were taken from ref.(11).

Table 2. L-Lactate:cytochrome c oxidoreductase activities of parent strain and RD mutants of S. cerevisiae

| Strain | L-Lactate:cyt.c oxidoreductase* |
|---|---|
| $R_2$04A | 27.6 ± 11.0 |
| 58NW-46 | 6.0 ± 3.3 |
| 58NW-1 | 0.4 ± 0.2 |

*Specific activities were expressed as mμ moles cytochrome c reduced/min/mg protein of the cell free extract. Figures in the table indicate mean value ± standard deviation of five experiments.

cytochrome c oxidoreductase (EC 1.1.2.3). As indicated in Table 2, strain 58NW-46 showed the enzyme activity at one fourth the level of parent strain $R_2$04A and was confirmed to have cytochrome $b_2$. Cytoplasmic RD mutant 58NW-1 was also shown to have a very small amount of enzyme and was considered to contain a definite amount of cytochrome $b_2$. Content of cytochrome $b_2$ in strain 58NW-1 was so small that it could not be detected from the absorption spectrum of a whole cell suspension of the mutant. The existence of cytochrome $b_2$ in RD mutants of S. cerevisiae has been reported by Gregolin (10), Sherman (1) and Sugimura (3), but in all cases they also contained cytochrome c.

The present investigation demonstrated that strain 58NW-46 was a unique RD mutant which lacked cytochromes a and c, and contained only cytochrome $b_2$. Detailed genetic and biochemical studies of this RD mutant and isolation of cytochrome $b_2$ from the mutant cells are now in progress.

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