MEMOIRS OF THE COLLEGE OF SCIENCE, UNIVERSITY OF KYOTO, SERIES B,
Vol. XXXIII No. 3 (Biology), March 1967

Studies on the Oxygen Uptake in Respiratory Deficient Mutants of Yeast

I. The Nature of Oxygen Uptake

by

Fumiko MIYAMOTO *

Department of Botany, Faculty of Science, Kyoto University

Abstract

Although the oxygen uptake of respiratory deficient (RD) mutants of yeast is very low, Q_{O_2} being less than 2 per cent in most strains, ample supply of oxygen remarkably increased their growth rate and cell yield. The oxygen uptake of vegetative RD mutants was found differing from that of normal strains in the following respects : Its rate was higher in the log phase than in the later period : it was enhanced by none of ethanol and various organic acids, except acetate, given as substrate, it was not inhibited by KCN, antimycin A or NaN_3 : and, although it was inhibited by CO, oxygen acceptor seemed to differ from normal type cells, since the uptake rate was dependent on oxygen partial pressure up to ca. 150 mm Hg, in contrast to ca. 10 mm Hg for normal cells. It is discussed that the growth increase of RD cells owing to oxygen uptake is to be ascribed to a role of molecular oxygen in the synthesis of some substances essential to the growth, rather than to energy relations.

Introduction

It is well known that respiratory deficient (RD) mutant cells are often produced in cultures of respiratory sufficient (RS) strains of yeast. These defective mutants possess no complete and functioning cytochrome system, nor any detectable activity of cytochrome oxidase. They are considered to proliferate using the energy obtained by means of fermentation (7, 8). According to KOTYK (5), however, resting RD cells take up a minute amount of oxygen and this uptake is cyanide resistant. Cyanide-resistant oxygen consumption was also remarked by MURAYAMA (6) in a respiratory normal, copper resistant yeast strain. It may be interesting to inquire into the role played by the oxygen

* present address, Department of Biology, Faculty of Education, Wakayama University, Wakayama

consumption not mediated by the ordinary terminal oxidizing enzyme system.

In the course of physiological investigations on RD mutants of yeast, the present author remarked that the growth rate of RD mutants was markedly reduced in the absence of air, notwithstanding that they consumed but little oxygen. The purpose of the presenr paper is to elucidate properties of this oxygen uptake by RD mutant cells. The possible physiological functions of the absorbed oxygen will be discussed.

Materials and Methods

The organisms used in experiments were thirteen diploid and haploid strains of *Saccharomyces cerevisiae* ; eight of them were respiratory deficient (RD) and five respiratory sufficient (RS). The genetic characters of the strains are listed in Table 1. Among the RD mutants, three were segregational mutants. The vegetative RD mutants were isolated in this laboratory from the corresponding RS strains through the *p*-nitrophenol treatment (5).

The culture medium contained sucrose 40.0g, peptone (Kyokutō) 3.5g, yeast extract (Wakō) 2.0g, KH_2PO_4 3.0g, and $MgSO_4 7H_2O$ 1.0g in 1 liter of deionized water, final pH being 5.2.

Inoculants were taken from precultures inoculated with shaking at 30°. In most experiments 2.5 ml of the aerobic culture, 24 to 28 hours old, was inoculated to 250 ml of the medium filled in a shaking flask of 500 ml capacity. Cultures were shaken or bubbled vigorously at 30° with air for aerobiosis, and nitrogen gas, washed with a sodium permanganate solution and Fieser's solution (4) was bubbled for anaerobiosis.

The growth was assayed at the proper intervals by using Coleman-Nephelocolorimeter, or by counting cells with Thomas' counting chamber. Dry weight of cells was determined after washed cells were dried for 72 hours or more at 98°.

Oxygen consumption was measured either manometrically or polarographically with a rotating platinun microelectrode.

Cells to be used were harvested by centrifugation from 24 to 28 hours' aerobic cultures, washed twice with M/15 phosphate buffer at pH 6.8, and resuspended in the same buffer.

Results

Effect of oxygen on the growth of RD mutants

When RD strains were cultured with vigorous aeration, they grew with a generation time of 1.8 hours in the most rapidly growing phase, the stationary phase was reached after 24~28 hours, as shown in Fig. 1. The growth rate was conspicuously lower under anaerobic conditions. And it was noted that the rate of anaerobic growth was very low when the inoculant cells had been cultured anaerobically. The more repeated the anaerobic preculturing the lower the growth rate, finally to exhibit no appreciable growth after many anaerobic culture passages.

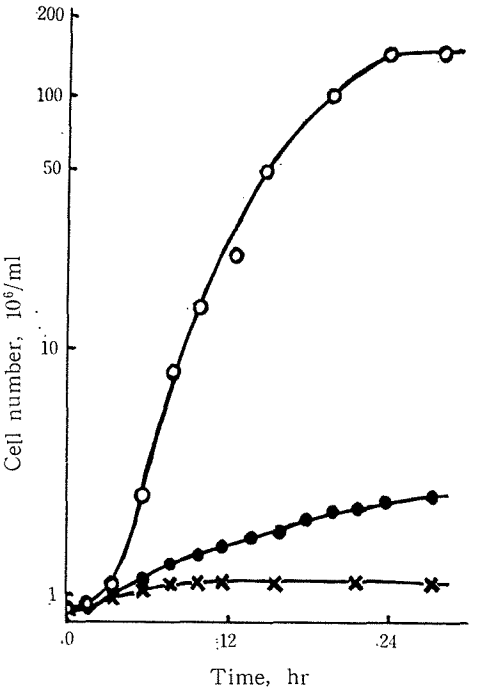

Fig. 1 Growth of a RD mutant under aerobic and anaerobic conditions.
○ : Culture with air-bubbling
● : aerobically precultured cells culture with nitrogen-bubbling
× : anaerobicaly precultured cells culture with nitrogen-bubbling

Table 1 Oxygen uptake of various strains of *Saccharomyces cerevisiae.*
Assay condition; 2mg (for RS strains) or 30~50mg (for RD strains) dry weight of cells, 150, μmoles of phosphate buffer pH 6.8 with or without 30 μmoles of glucose in 3.0ml at 30°.

Strain		Genetic Characters	Q_{O_2} (μl/hr/mg dry wt)	
			Endogenous	With glucose
Respiratory sufficient strains				
Diploid	A 101	ga^- pan^-	32.7	67.2
	A 102		42.0	80.6
	D 206		34.2	56.6
Haploid	B 5	is^-	25.3	64.9
	E 41	$ga^-is^-pan^-MG^-$	27.8	47.5
Vegetative respiratory deficient mutants				
Diploid	A 101P	ga^-pan^-	0.60	0.86
	A 102P		0.63	0.78
	D 206P		0.54	0.88
Haploid	B 5P	is^-	0.61	0.65
	E 41P	$ga^-is^-pan^-$ MG^-	0.50	0.61

Segregational respiratory deficient mutants				
Haploid	U 20	ga⁻ad⁻	0.70	1.84
	U 21	suc⁻ga⁻ur⁻le⁻ly⁻hi⁻tr⁻	7.73	10.50
	U 22	ga⁻ur⁻ly⁻tr⁻met⁻MZ⁻	0.98	2,37

For symbols see Microbial Genetics Bulletin, Suppl. to No 19, 1963

Oxygen uptake in various RD strains

Cells of all the RD strains used had oxygen consuming activities, as indicated in Table 1. However, the Q_{O_2} values of vegetative RD mutants with 0.01M glucose as substrate were about 1 to 1.6 per cent of their parent strains. The oxygen uptake values of segregational RD mutants were larger than those of vegetative RD mutants. Exceptionally large endogenous and exogenous values were obtained with U21, which will be shown later to be peculiar also in other respects.

Substrates for oxygen uptake

With RS strains, ethanol was a very effective substrate for oxygen uptake, followed by acetate and lactate, while pyruvate and malate were poor substrates (Table 2). In RD mutants, on the other hand, ethanol and organic acids, except acetate, did not increase oxygen uptake above the endogenous level.

Fumarate, succinate, α-ketoglutarate, glycerol, citrate, gluconate, butyrate, glyoxalate, ascorbate and glutamate (each 0.01M in final concentration), glucose-1-phosphate, glucose-6-phosphate and fructose-1, 6-diphosphate (each in 1.8×10^{-3}M) and casein tryptic hydrolyzate (0.2%), each had no effect upon the oxygen uptake of both RS and RD strains.

Table 2 Effect of various substrates on the oxygen uptake of RS and RD strains. Cells were preincubated in 1/15 M phosphate buffer at 30° under shaking for 120 minutes. Assay condition; 2mg(for the RS strain) or 50mg(for the RD strain) dry weight of cells. 150 μmoles of phosphate buffer pH 6.8. and 30 μmoles of a substrate in 3.0ml.

Substrate	Q_{O_2} (μl/hr/mg dry wt)	
	RS strain	RD mutant
Endogenous	7.4	0.44
Glucose	58.2	0.88
Lactate	13.7	0.45
Pyruvate	8.4	0.45
Acetate	19.7	0.57
Malate	8.2	0.48
Ethanol	55.2	0.44

Oxygen uptake in relation to growth phase

Oxygen consumption and cell number were determined at various growth phases of aerobic cultures of RS and RD strains. As is well known the respiratory rate of RS strains was low in the log phase and high in the stationary phase (Fig 2 B).

On the other hand, the rate of oxygen uptake of RD mutants was the highest in the log phase and became lower in the retardation phase, to fall to a low level at the stationary phase. (Fig 2 A)

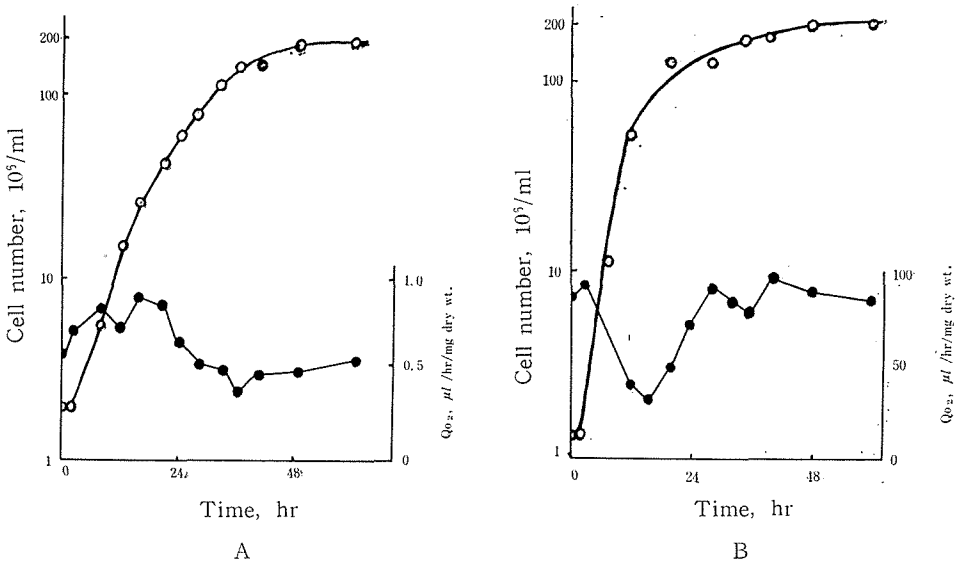

Fig. 2 Growth and oxygen consumption of a RD strain (A) and RS strain (B).
○ : Cell number, ● : Q_{O_2}
Cells were sampled at intervals from aerobic (shaking) cultures and cell number and oxygen consumption were determined. Assay conditions; 50mg of cells, 100 μmoles of phosphate buffer and 20μmoles of glucose in 2.0ml ; pH 6.8, 30°.

Effect of inhibitors

Effects of various inhibitors upon the oxygen uptake of RD and RS strains were tested and the results are summarized in Table 3. Cyanide at the concentration which decreased the respiration of RS strains by approximately 86% hardly inhibited that of RD strains, even to stimulate it in some cases. The cyanide resistant oxygen uptake was also observed in RS strains, 4 to 7 per cent of oxygen uptake remained in the presence of 10^{-3} M of cyanide, as shown in Fig. 3.

Among the inhibitors listed in Table 2, only carbon monoxide was found strongly inhibitory to the oxygen uptake of RD mutants. The inhibition by carbon monoxide was completely reversed by exposure to an incandescent lamp.

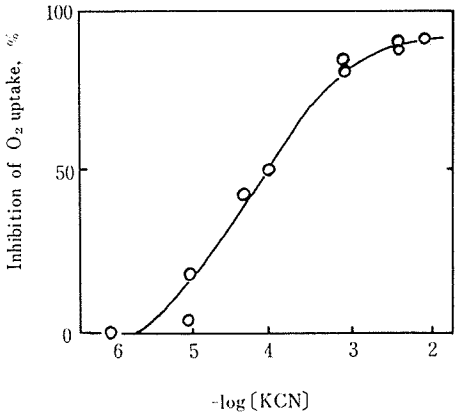

Fig. 3 Effect of cyanide on respiration of RS cells.

Table 3 Effect of various inhibitors on the oxygen uptake of RS and RD strains. Starvation of cells and assay conditions as in the legends to Table 2. Glucose in a final concentration of 1 / 100 M was given as substrate. After 30 minutes of equilibration an inhibitor was added from the side arm.

Inhibitor	Final conc.	per cent inhibition	
		RS strain	RD strain
Antimycin A	$5x10^{-7}$M	82.9%	14.1%
Amytal	$1x10^{-3}$M	6.8	12.7
NaN_3	$1x10^{-3}$M	63.8	9.4
CO dark	95% CO. 5% Air	93.7	67.2
〃 light	〃	29.5	0.0
KCN	$5x10^{-3}$M	92.2	—
〃	$1x10^{-3}$M	86.4	5.0
〃	$1x10^{-4}$M	60.6	0.0
〃	$1x10^{-5}$M	2.6	0.0
〃	$1x10^{-6}$M	0.0	0.0

Effect of oxygen tension

Oxygen consumption of RS and RD strains was measured manometrically, glucose as substrate. Manometer vessels were flushed at the beginning with mixtures of oxygen and nitrogen at various ratios. The results as detected in Fig. 4 showed that the oxygen uptake of RS strains was saturated by about 10mmHg of oxygen partial pressure, while that of RD strains was dependent on the oxygen pressure in a much wider range of it.

For more precise determination, an oxygen electrode was used. The results were as represented in Fig. 5, where the reciprocal of Q_{O_2} is plotted against the reciprocal of the calculated molar concentration of oxygen, as dissolved in the medium. The value corresponding to Michaelis constant (Km) of diploid and

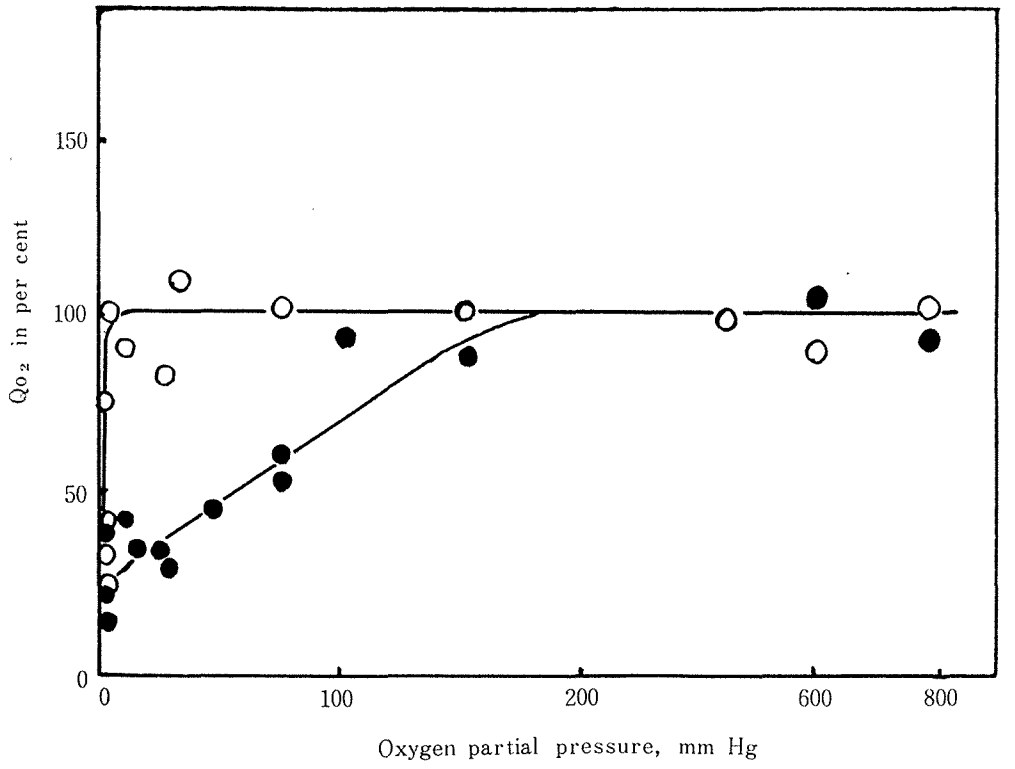

Fig. 4 Effect of oxygen tension on the oxygen uptake of RD and RS strains.
○ : RS strain, ● : RD strain
Reaction mixture, composed of 0.5～2mg(for RS strains) or 20～50mg (for RD strains) dry weight of cells, 20μmoles of glucose and 100μmoles of phosphate buffer pH 6.8 in 2ml, was preincubated in air at 30°. Then, gas phase was replaced with gas of particular oxygen tension and Q_{O_2} measured. Mean of Q_{O_2} of each strain obtained at oxygen tensions higher than 152 mmHg was referred to as 100%.

haploid RS strains was $7\sim8\times10^{-7}$ M, while that of D 206P and other vegetative RD strains was much larger, being approximately 1×10^{-4} M. Hence it seems that the affinity of cellular oxygen acceptor (s) to oxygen is much lower in vegetative RD mutants than in RS strains. The segregational RD mutants did not show lineality in the double reciprocal plotting, suggesting more complicated oxidation system of these mutants. A segregational strain, U 21, which has a high oxygen uptake activity, was distinct from other strains in that its oxygen uptake was inhibited by antimycin A and cyanide, and the difference spectra of a particulate fraction from cell extract had an absorption peak at 563 mμ when it was reduced by dithionite(Fig. 6). This peak is in the range of cytochrome b group.

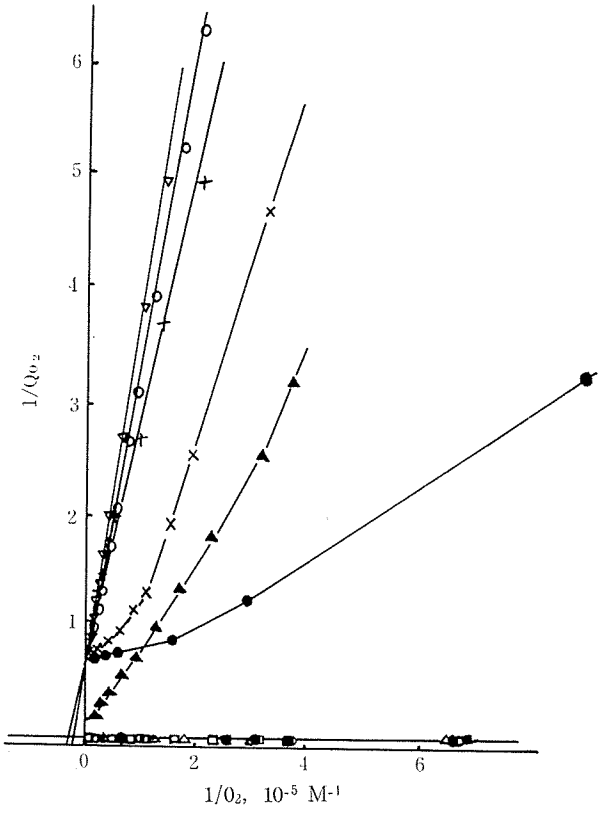

Fig. 5 Lineweaver-Burk plot of oxygen uptake of RS and RD strains against oxygen tension.

□ : A 101 ■ : D 206
△ : B 5 × : U20
▲ : U21 ● : U22
▽ : A101P ○ : D206P
+ : B5P

Q_{O_2} was determined by polarographic method at 30°. Assay conditions; Cells 1.5 mg (for RS strains) or 10~20mg (for RD strains), glucose 8.3μmoles and phosphate buffer at pH 6.8 200 μmoles in 3.0ml.

Dsicussion

The smallness of colonies of RD strains has often been ascribed to the low energy yield of fermentation on which they depend. Although RD mutants consume but small amounts of oxygen, biological significance of the oxygen consumption has not yet been elucidated. As shown in Fig. 1, the cell multiplication of RD strains was markedly accelerated by the presence of molecular oxygen. And when the amount of oxygen in the culture was limited the growth was proportional to it, as will be presented in the paper to follow. These facts suggest that oxygen plays an important role in the metabolism of RD strains.

Experimental results reported in this paper show that the oxygen uptake of RD mutants has properties different in many respects from that of RS strains. The respiratory rate of RS strains becomes higher after the log phase, accompanied by the well known (11) development of mitochondria. But in RD mutants, the rate of oxygen uptake was the highest during the log phase. YOTSUYANAGI (12)

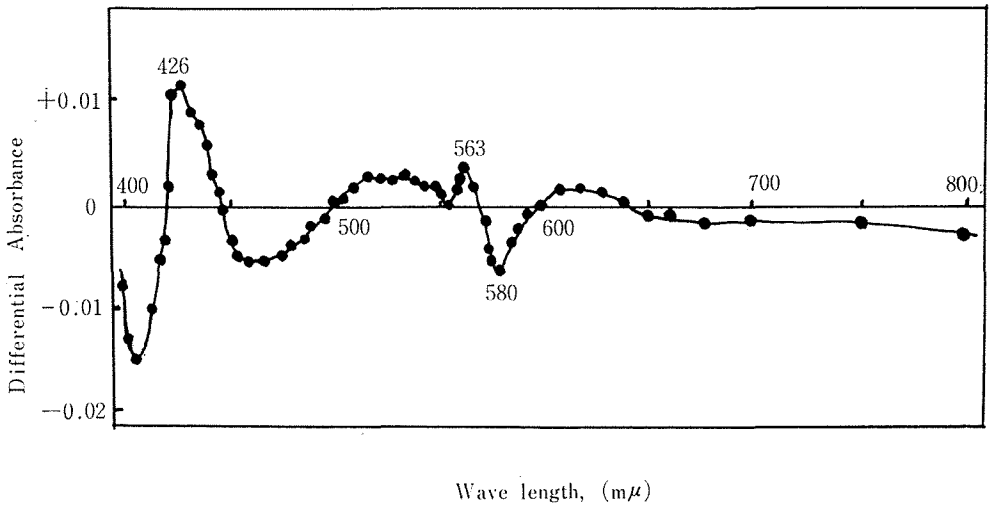

Fig. 6 Difference spectrum of particulate fraction from a RD mutant U 21.
Cells were disrupted by freezing-thawing and extracted with 0.6M KCl. Particulate fraction was collected by centrifugation at 100,000 g for 120 minutes from the supernatant by centrifugation at 25,000g for 40 minutes and suspended in 1/15 M phosphate buffer, pH 6.8. Reduced (by dithionite) minus oxidized difference spectrum is shown.

reported that chondriosomes, covered with double membranes but without cristae, are present in the RD mutant cell and that they become fully developed in structure and in number at the stationary phase of growth. He presumed that the organelle corresponds to the mitochondrion of RS strains. However, the phasic change in the activity of oxygen uptake does not correspond to the development of the reported structure in RD mutants.

Except carbon monoxide, inhibitors of the respiratory chain such as cyanide, azide and antimycin A scarcely inhibited the oxygen uptake of RD strains at the concentrations which considerably inhibited the respiration of RS strains. And as to the affinity to oxygen of cellular oxygen acceptor, the Km value for RD strains was larger by two figures than that for RS strains, which respire through the cytochrome oxidase system. Hence RD cells appears to use oxygen by some other system (s) than cytochrome oxidase.

When RD cells were cultured in the Warburg flask, about 7×10^{-7} moles of oxygen was consumed when the proliferation of 10^8 cells (dry weight 1.2mg) was increased by the presence of oxygen. The maximum possible energy produced by this amount of oxygen is apparently far short of that required for the enhanced growth. Organic acids and ethanol utilizable by RS cells as respiratory substrate did not increase the oxygen uptake by RD cells, except acetate (cf. Table 2).

Taking the above mentioned results into account, it is suggested that RD cells take up oxygen through some system (s) other than those for the respiration of RS strains and use it for some function (s) different from energy production, as for example, synthesis of trace substances which limit the growth in the absence of oxygen supply. For promoting the anaerobic growth of RS yeast, ANDREASEN and STIER (1, 2) recommended to supplement usual culture media with unsaturated fatty acids and ergosterol. In view of the facts that molecular oxygen is required for the synthesis of those compounds, i. e. that the synthesis is catalyzed by oxygenases (3, 9), it is tempting to assume that the oxygen taken up by RD mutants participates in the biosynthesis of those substances. Although it may appear characteristic of RD cells that their oxygen uptake is not sensitive to respiration inhibitors, especially to cyanide, the cyanide resistant oxygen uptake was also found in RS strains as shown in Fig. 3. Furthermore, Gohgi (unpublished) of our laboratory has observed that the Km value of anaerobically cultured RS cells was 1 $\times 10^{-4}$M, the same as for RD mutants, and that the value falls rapidly to the order of 10^{-7}M on introduction of oxygen. Hence RS cells seem to possess a system which is common to or resembling to that possessed by RD mutants, which remains even in anaerobically cultured cells.

One of the segregational RD mutants, U21, differs from other strains in the Lineweaver-Burk plot of oxygen uptake against oxygen concentration. This strain contains an oxidase whose absorption spectra are of the b type cytochrome, but it did not react with L-lactate. Properties of this oxidase are now under investigation.

Acknowledgement

The author expresses her sincere thanks to Prof. J. ASHIDA for his encouragement and advice. She is indebted to Dr. T. KATOH and Mr. T. GOHGI for their collaborations under which this work was carried out.

References

1. ANDREASEN, A. A. and T. J. B. STIER 1953 Anaerobic nutrition of *Saccharomyces cerevisiae* I Ergosterol requirement for growth in a defined medium. J. Cell. and Comp. Physiol., 41 : 23~36.
2. ——— & ——— 1953 Anaerobic nutrition of *Saccharomyces cerevisiae* II Unsaturated fatty acid requirement for growth in a defined medium. J. Cell. and Comp. Physiol., 43 : 271~282.
3. BLOOMFIELD, D. K. and K. BLOCH 1960 The formation of Δ^9 -unsaturated fatty acids. J. Biol. Chem., 235 : 337~344.
4. FIESER, L. F. 1955 Experiments in Organic Chemistry By D. C. Heath & Company 299.
5. KOTYK, A. 1961 Metabolism of the mutant *Saccharomyces cerevisiae* R12A II Endogenous metabolism. Folia. Microbiol., 6 : 171~174.
6. MURAYAMA, T. 1961 Studies on the metabolic pattern of yeast with reference to its copper resistance. I. Respiration and fermentation. Memoirs of Ehime Univ. Sect II (Sci) Ser. B (Biol), IV : 23~34.

7. RAUT, C. 1953 A cytochrome deficient mutant of *Saccharomyces cerevisiae* Exptl. Cell Res., 4 : 295~305.
8. SLONIMSKI, P. 1949 Action de l'acriflavine sur les levures IV Mode d'utilization du glucose par les mutant "petit colonie„ Ann. Inst. Pasteur, 76 : 510~530.
9. TCHEN, T. T. and K. BLOCH 1956 On the mechanism of cyclization of squalen. J. Am. Chem. Soc., 78 : 1516~1517.
10. YANAGISHIMA, N. 1957 Effect of nitrophenols and acriflavine inducing the w variation of yeast. J. Inst. Polytech. Osaka City Univ., Ser. D 8 : 45~51.
11. YOTSUYANAGI, Y. 1962 Etudes sur le chondriome de la levure. I Variation de l'ultrastructure du chondriome au cours du cycle de la croissance aérobie. J. Ultrast. Res., 7 : 121~140.
12. ——— 1962 Etudes sur le chondriosome de la levure. II Chondriomes des mutants á déficience respiratiore. J. Ultrastructure Res., 7 : 141~158.