Incorporation of Linoleate by a Halotolerant Yeast, *Saccharomyces rouxii*

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Oxygen absorption on linoleate by a halotolerant yeast, *Saccharomyces rouxii* IY-5 was examined with bovine serum albumin-linoleate complex. The maximum oxygen absorption was observed on the yeast cells harvested at the late log phase. At the initial stage of oxygen absorption on linoleate, it increased linearly with time or with cell concentration. From the incorporation assay of linoleate Km was 42.0 μm and Vmax was 2.17 nmole/mg min. Optimum pH of transport was 7.5. Examination by a Warburg manometer revealed that 1-14C-linoleate was degraded to 14CO2, which was the indication of the linoleate oxygen absorption by *S. rouxii* IY-5. Oxygen absorption on linoleate was measured by Biooxygraph with an oxygen electrode. Optimum pH of oxygen absorption was 4.0, which was different from that of incorporation. From the data of the substrate specificity, the oxygen absorption was remarkably high on free acids and methylesters of linoleate and linolenate as substrates. Glucose did not work to stimulate the oxygen absorption on linoleate though it was even a better substrate than linoleate. The cells grown in the media with 1 M NaCl showed high oxygen absorption on linoleate in the same or lower concentration on NaCl; while the cells grown in 2M NaCl showed high oxygen absorption under 2M NaCl.

Introduction

During the screening of free fatty acid-incorporating microorganisms from sybean fermented foods containing 10 to 12% salt, oxygen absorption on linoleate was seen in *Saccharomyces rouxii*1-3. *S. rouxii* is known as a halotolerant and predominant yeast in salted fermented foods4, but the incorporation and metabolism of FFA by *S. rouxii* and its subsequent flavor production would elucidate a certain function in the fermentation process.

Studies of uptake of FFA by microorganisms have been carried out: KLEIN et al.5 studied the uptake of FFA by *Escherichia coli*, TOSCANO and HARTLINE6 reported the octanoate transport by *Pseudomonas oleovorans*, and CALMS and DEAL7 reported the transport by *Nocardia asteroides*.

In this paper we report the linoleate incorporation by *S. rouxii* IY-5. Characteristics of the oxygen absorption and the NaCl effect on the oxygen absorption using BSA-linoleate complex as the substrate are also described.

Materials and Methods

Strain and culture *S. rouxii* IY-5 was isolated from a Japanese fermented food, miso. It was cultured at 30℃ with 100 ml of the following GC medium: Vitamin free casamino acids (Difco) 0.5g, glucose 5.0g, NaCl 15g, KH2PO4 1g, CaCl2·2H2O 0.1g, MgSO4·7H2O 0.5g, biotin 20μg, Ca-pantothenate 400μg, inositol 2,000μg, niacin 400μg, p-aminobenzoic acid 200μg, pyridoxine·HCl 400μg, riboflavin 200μg, thiamine 400μg, pure water 1 liter, pH 5.0.

The cells of the later log phase were harvested and centrifuged at 10,000 × g for ten min. The cells were washed twice with equal volume of pure water except the experiment of NaCl effect on oxygen absorption (see Table 7). In the latter experiment the saline water, containing the equal concentration of NaCl with that of the media, was used for washing respectively.
Then the cells were diluted to $7.2 \times 10^8$/ml.

**Chemicals** 1-14C-Linoleate was purchased from New England Nuclear (USA), and BSA was of fraction V of Daiichi Kagaku (Tokyo). The other chemicals were guaranteed reagents.

**Oxygen absorption test** The linoleate was expressed in the form of the rate of oxygen absorption (nmole/mg of dry cell•min) by means of Biooxygraph with Galvani type of oxygen electrode (Kyusui Kagaku, Tokyo).

In the reaction chamber, McIlvaine buffer (pH 4.0) 2.1ml, pure water 0.2ml, cell suspension 0.1ml, BSA-linoleate complex emulsion 0.3ml were added in this order, and oxygen absorption was recorded at 25°C. The incubation time was within a total of ten minutes.

Inhibitors, NaCl solution and others were added by using such solution in place of 0.2 ml pure water. BSA-linoleate complex solution was prepared as follows: first 22.4mg of BSA was dissolved in 0.9ml pure water and 0.1ml of linoleate was added (final concentration of linoleate of the complex was 35 mM, and the mixture was sonified by Bronson's sonifier at 50% pulse at 0°C for three min. BSA-linoleate complex solution was immediately used for the experiments.

**14CO2 production** 14CO2 production was measured by Warburg manometer. The substrate was prepared under the same conditions as BSA-linoleate complex solution for the oxygen absorption test except the addition of 1-14C-linoleate to unlabeled linoleate and the shaking condition. (BSA-linoleate complex solution contained 35 mM of linoleate and 13 μCi of 1-14C-linoleate.) The Warburg vessel contained 0.5ml yeast cell solution, 1.0ml McIlvaine buffer (pH 4.0), whereas the side arm contained 0.2ml BSA-linoleate complex solution. The center well had addition of 0.2ml 2N KOH or pure water. The total volume of reaction mixture, therefore, amounted to 2.2ml.

After 70 min incubation at 30°C, oxygen absorption was stopped with 0.5ml of 50% H2SO4 and radioactivity of KOH solution in the center well was measured by liquid scintillation counter after it was neutralized with 2N KOH.

**Incorporation test** The incubation mixture contained 0.1ml BSA-linoleate yeast cell solution and 0.9ml McIlvaine buffer (pH 7.5). For the incorporation test, BSA-linoleate solution contained 0.9mg linoleate and 1-14C-linoleate (13 μCi). The incubation was carried out at 25°C for 5 min. The incorporation assay, the mixture was shaken by means of shaking incubator (amplitude 150/min, stroke 3 cm). Portions (0.2 ml) were aspirated on membrane filter (type TM-2, Toyo, pore size 0.45 μm) and washed rapidly with 15ml of McIlvaine buffer (pH 7.5). Filtration and washing finished within 20 seconds. Membrane filters with cells transferred into Scintillator. Scintillator was composed of 5.5 g of 2,5-diphenyloxazole, 0.1 g of 1,4-bis-(2-(5-phenyloxazole) in the mixture of 333 ml Triton X-100 and 667 ml toluene. Radioactivity was counted by a scintillation counter after 16 hours when the filters and cells were dissolved in scintillator. The incorporated amount of linoleate was calculated from the radioactivity increased in five minutes of incubation.

**Results**

Relation between growth and oxygen absorption on linoleate. Fig. 1 shows the relation between the growth and linoleate oxygen absorption.

![Fig. 1. Growth and oxygen absorption of linoleate by S. rouxii IY-5.](image)

*--- Growth
**--- Oxygen absorption of linoleate*

The strain was grown on GC medium (See 'Materials and Methods'). The oxygen absorption was measured from the difference between the oxygen absorption with linoleate and that without linoleate.
absorption of *S. rouxii* IY-5. Oxygen absorption increased with growth in log phase: The cells attained the maximum oxygen absorption at the late log phase followed by the decreasing oxygen absorption.

**Kinetics of linoleate incorporation.** For the investigation of cellular incorporation of linoleate the following phenomena must be clearly distinguished and examined: (a) the absorption of linoleate to the cells, (b) the incorporation of linoleate into the cells, (c) the degradation or metabolism of the incorporated linoleate in the cells. Fig. 2 shows the time course of linoleate incorporation (2-a) and the relation between linoleate incorporation and cell concentration in the incubation mixture (2-b). Fig. 2 indicates that the radioactivities detected on the cells on the membrane filters increased linearly with time for the first ten minutes, and then they made no significant increase. Fig. 2-b shows the radioactivities increased with the concentration linearly, although the increase stopped in high concentration of cells.

**Effect of inhibitors on linoleate incorporation.** Inhibition by N-ethylmaleimide and p-chloromercuribenzoate shows that free sulfhydryl group (SH) was required for linoleate incorporation. Block by KCN, NaN₃ and 2,4-DNP, p-nitrobenzoic acid shows that linoleate incorporation was dependent on energy generating system. The results of Fig. 2 and Table 1 show that increase of radioactivity on the membrane filter was caused not by adsorption but by active transport dependent on energy.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Concentration (mM)</th>
<th>Relative inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-Ethylmaleimide</td>
<td>1.0</td>
<td>100</td>
</tr>
<tr>
<td>PCMB</td>
<td>0.001</td>
<td>77.6</td>
</tr>
<tr>
<td>Iodoacetamide</td>
<td>1.0</td>
<td>64.9</td>
</tr>
<tr>
<td>KCN</td>
<td>1.0</td>
<td>96.0</td>
</tr>
<tr>
<td>NaN₃</td>
<td>1.0</td>
<td>55.1</td>
</tr>
<tr>
<td>2,4-DNP</td>
<td>1.0</td>
<td>100</td>
</tr>
<tr>
<td>p-Nitrobenzoic acid</td>
<td>1.0</td>
<td>100</td>
</tr>
<tr>
<td>CCCP</td>
<td>0.1</td>
<td>98.7</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>

The cells of *S. rouxii* IY-5 was incubated with BSA-linoleate complex containing 14C-linoleate (13 µCi/ml) for 5 min and the incorporation was measured from the difference between the radioactivity at 5 min and that at 0 min.

2,4-DNP; 2,4-Dinitrophenol.

CCCP; *m*-Chloro-carbonyl cyanide phenylhydrazone.

PCMB; p-Chloromercuribenzoate.

**Substrate dependence of linoleate incorporation.** Fig. 3 shows the kinetics of linoleate incorporation. From the double reciprocal plot of Lineweaver and Burk, \( K_m \) was equal to 42.0 \( \mu M \) and \( V_{max} \) was equal to 2.17 nmole/mg·min.

**Effect of pH on linoleate incorporation.** Fig. 4 shows the linoleate incorporation at various pH. Optimum pH was 7.5 and the incorporation was less than 20% of that at the maximum pH between pH 3 and 5.

**Effect of pH on oxygen absorption on linoleate as a substrate.** Examination was made whether linoleate could be metabolized after the incorporation. The oxygen absorption was measured by means of Biooxygraph with an oxygen electrode. At first oxygen absorption was measured at pH 7.5 which was optimum pH of the incorporation; only low oxygen absorption
Fig. 3. Substrate dependence of linoleate incorporation by *S. rouxii* IY-5

The concentration of linoleate as the substrate was varied between 8.1 and 65 μM. The other conditions of incorporation were the same as the standard method. Double reciprocal plot of Lineweaves and Burk was shown to obtain K_m and V_max.

Fig. 4. Effect of pH on incorporation of linoleate by *S. rouxii* IY-5

The standard incorporation assay method was used except pH or buffer. See "Materials and Methods".

*○—○...McIlvaine buffer
×---×...Tris maleate buffer*

was detected. From the investigation of pH effect on oxygen absorption (Fig. 5), it was found that the maximum oxygen absorption was at pH 4.0 and oxygen absorption decreased as pH became higher; there was a second maximum at pH 7.0. It is not clear whether the peaks detected at pH 4.0 and 7.0 are due to the same reason or not.

Fig. 5. Effect of pH on the oxygen absorption of linoleate by *S. rouxii* IY-5.

The standard oxygen absorption assay method was used except that pH of McIlvaine buffer was changed. See the "Materials and Methods".

14CO_2 production caused by linoleate degradation. We examined whether the increase of oxygen absorption was due to the direct metabolism of linoleate or to the stimulation of the metabolism of some other compounds by linoleate. On the assumption that if linoleate is intracellularly metabolized, CO_2 will finally be produced, CO_2 production was measured by Warburg manometer with 14C-linoleate as the substrate. Fig. 6 shows the time course of oxygen absorption and CO_2 production when linoleate was the substrate. CO_2 was produced more in the vessel with linoleate than in the vessel without linoleate.

The result is shown in Table 2. No 14CO_2 production was detected in vessels 1 and 2 with no addition of linoleate*. No significant 14CO_2 production was detected because the center well vessel 3 contained pure water in place of KOH. KOH solution in vessel 4 showed radioactivity.

Substrate specificity of oxygen absorption. Table 3 shows the substrate specificity of oxygen absorption.

*While it was detected in vessels 3 and 4 with addition of linoleate.*
absorption. The fatty acids constituting soybean neutral lipids were chosen here. When relative oxygen absorption on linoleate was taken as 100, linolenate showed the same relative oxygen absorption, while free oleate and stearate showed 58 and their methylesters showed 42 and 32, respectively. Both free palmitate and myristate showed only 5% and their methylesters showed 32 and 26%, respectively.

**Effect of carbon sources on linoleate uptake.** Table 4 shows the oxygen absorption on carbon sources by *S. rouxii* IY-5. If linoleate oxygen absorption was carried out by Warburg manometer. The reaction mixture in the Warburg vessel contained 0.5 ml of the cell suspension of *S. rouxii* IY-5 (96.2 mg wet weight), 1.0 ml of McIlvaine buffer. The center well contained 0.2 ml of 2 N KOH or pure water. The side arm contained 0.2 ml of linoleate as BSA-linoleate complex. The total volume was 2.2 ml. The preliminary incubation was carried out for 10 min and then the cell suspension and the substrate solution were mixed, and subsequently the incubation was carried out for 70 min. The reaction was stopped with 0.5 ml of 50% H2SO4 and after neutralization the radioactivity in KOH solution was measured by Liquid Scintillation counter (See Table 2)

Fig. 6. Oxygen absorption and CO2 production on linoleate by *S. rouxii* IY-5

- O — O2 adsorbed (+Linoleate)
- ● — O2 adsorbed (−Linoleate)
- △ — CO2 produced (+Linoleate)
- x — CO2 produced (−Linoleate)

The experiment was carried out by Warburg manometer. See the footnote of Fig. 6.

(a) BSA-linoleate complex containing 1-14C-linoleate was added in the vessels as substrates.

(b) KOH was the solution contained in the center well.

(c) CO2 which was absorbed by KOH in the center well.

<table>
<thead>
<tr>
<th>Vessel</th>
<th>Linoleate (1)</th>
<th>KOH (b)</th>
<th>14CO2 produced (c) (dpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>−</td>
<td>−</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>−</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>−</td>
<td>352</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
<td>19,380</td>
</tr>
</tbody>
</table>

The oxygen absorption and 14CO2 production by *S. rouxii* IY-5 on linoleate as the substrate

Table 2. Oxygen Absorption and 14CO2 Production by *S. rouxii* IY-5 on Linoleate as the Substrate

**Effect of carbon sources on linoleate uptake.** Table 4 shows the oxygen absorption on carbon sources by *S. rouxii* IY-5. If linoleate oxygen absorption was carried out by Warburg manometer. See the footnote of Fig. 6.

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(c) CO2 which was absorbed by KOH in the center well.
Table 4. Oxygen Absorption on Linoleate and Carbon Sources by S. rouxii IY-5

<table>
<thead>
<tr>
<th>Carbon source</th>
<th>Concentration ((\times 10^{-4} M))</th>
<th>Relative oxygen absorption (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>0.6</td>
<td>169</td>
</tr>
<tr>
<td>Fructose</td>
<td>6.0</td>
<td>52.9</td>
</tr>
<tr>
<td>Ethanol</td>
<td>18.0</td>
<td>31.0</td>
</tr>
<tr>
<td>Succinate</td>
<td>9.0</td>
<td>0</td>
</tr>
<tr>
<td>Linoleate</td>
<td>3.5</td>
<td>100</td>
</tr>
<tr>
<td>Control(^{(a)})</td>
<td>—</td>
<td>0</td>
</tr>
</tbody>
</table>

See the footnote of table 3 about the method.

\(^{(a)}\) Pure water was added in place of the solution of carbon sources.

Absorption was taken as 100, oxygen absorption was 169 on glucose and 52.9 on fructose. Oxygen absorption on ethanol was only 31.

According to Table 5, relative oxygen absorption of glucose to linoleate was 167 and the total of both oxygen absorption was 267, while the mixture of linoleate and glucose showed 215% of relative oxygen absorption. Therefore glucose had no apparent effect on the enhancement of linoleate oxygen absorption.

Effect of metabolic inhibitors on oxygen absorption. Table 6 shows effect of metabolic inhibitors on linoleate. Potassium cyanide and sodium azide, CCCP and 2, 4-DNP, inhibitors of electron transport and uncouplers, showed strong inhibition. Inhibition by N-ethylmaleimide and PCMB showed enzymes and/or compounds with SH groups were relative with the oxygen absorption.

Effect of NaCl on oxygen absorption. Fig. 7 shows the effect of NaCl on oxygen absorption on linoleate. The experiment was carried out with the yeast cells harvested from the GC medium with 1.5% (0.26M) NaCl. The relative oxygen absorption was kept constant under less than 0.1M. One to 2M of NaCl led lower oxygen absorption and 2M NaCl gave less than 40% of maximum oxygen absorption, 0 to 2M of KCl in place of NaCl respectively gave the same results as the equal concentration of NaCl. Therefore the effect of NaCl was dependent on the osmotic pressure.

For the investigation of this phenomenon,

Table 5. Effect of Glucose on Oxygen Absorption on Linoleate

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Concentration ((\times 10^{-4} M))</th>
<th>Oxygen absorption (nmole O$_2$/mg-min)</th>
<th>Relative oxygen absorption (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linoleate</td>
<td>3.5</td>
<td>6.9</td>
<td>100</td>
</tr>
<tr>
<td>Glucose</td>
<td>6.0</td>
<td>11.3</td>
<td>167</td>
</tr>
<tr>
<td>Linoleate+Glucose(^{(a)})</td>
<td>3.5, 6.0</td>
<td>14.6</td>
<td>215</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>0.0</td>
<td>0</td>
</tr>
</tbody>
</table>

See the footnote of Table 3 about the method.

\(^{(a)}\) Mixture of BSA-linoleate and glucose was added as substrates.

Table 6. Effect of Metabolic Inhibitors on Oxygen Absorption on Linoleate by S. rouxii IY-5

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Concentration ((mM))</th>
<th>Relative inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp. 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-Ethylmaleimide</td>
<td>1.0</td>
<td>51.7</td>
</tr>
<tr>
<td>Iodoacetamide</td>
<td>1.0</td>
<td>8.5</td>
</tr>
<tr>
<td>10.0</td>
<td>27.3</td>
<td></td>
</tr>
<tr>
<td>Monooiodoacetic acid</td>
<td>1.0</td>
<td>7.4</td>
</tr>
<tr>
<td>KCN</td>
<td>1.0</td>
<td>64.8</td>
</tr>
<tr>
<td>NaN$_2$</td>
<td>1.0</td>
<td>97.3</td>
</tr>
<tr>
<td>2, 4-DNP</td>
<td>1.0</td>
<td>88.6</td>
</tr>
<tr>
<td>Control</td>
<td>—</td>
<td>0</td>
</tr>
</tbody>
</table>

Exp. 2

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Concentration ((mM))</th>
<th>Relative inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCCP</td>
<td>0.001</td>
<td>22.3</td>
</tr>
<tr>
<td>PCMB</td>
<td>0.1</td>
<td>100</td>
</tr>
<tr>
<td>Control</td>
<td>—</td>
<td>0</td>
</tr>
</tbody>
</table>

2, 4-DNP: 2, 4-Dinitrophenol
CCCP: m-Chloro-carbonyl cyanide phenylhydrazone
PCMB: p-Chloromercuribenzoate
See the "Materials and Methods" about the method. Inhibitors were added instead of 0.2 ml of pure water of the reaction mixture.
Fig. 7. Effect of NaCl and KCl on oxygen absorption on linoleate by S. rouxii IY-5

The standard oxygen absorption assay method was used except that NaCl or KCl was added in incubation mixture. The yeast cells were harvested from the GC medium with 1.5% NaCl.

The oxygen absorption of various cells which were harvested from the GC medium with 0.01M to 2M NaCl was investigated (Table 7). The oxygen absorption of the cells harvested from 0.01M GC medium was constant between 0.01 and 0.1M NaCl of incubation mixtures. Oxygen absorption, however, was negligible under 2M NaCl.

The cells from the GC media with 0.1 to 1.0M NaCl showed the same amounts of linoleate oxygen absorption under the same or lower concentration of NaCl in incubation mixture. Under 2M NaCl the oxygen absorption decreased to one third. The cells harvested from the medium with 2M NaCl, on the contrary, showed the low oxygen absorption with no NaCl and high oxygen absorption under 1M or 2M NaCl.

Discussion

Chen9) reported the formation of BSA-FFA complex. Spector et al.10) described that 14C-labeled fatty acids was transferred rapidly from albumin to Ehrlich ascites tumor cells. Samuel et al.11) applied BSA-FFA complex to uptake and metabolism of FFA by cultured cardiac cell from chick embryo. We investigated the linoleate uptake of a halotolerant yeast, S. rouxii with BSA-linoleate complex.

After harvesting the cells from the media with 0.25M NaCl, the cells were washed with pure water. In the preliminary experiments, cells washed with pure water showed no appreciable difference in oxygen absorption on linoleate as the substrate from the cells washed with 0.25M saline water. And after washing the former cells had the same viability on plate agars as the latter cells. From the data of Fig. 3, Km = 42.0 fM of S. rouxii IY-5 on linoleate was valid when compared with 7 fM of Ps. oleovarance6) on octanoate and 870 fM of N. asteroides7) on palmitate. The relative oxygen absorption

Table 7. Effect of NaCl in Media on Oxygen Absorption on Linoleate by S. rouxii IY-5

<table>
<thead>
<tr>
<th>NaCl</th>
<th>Media (M)</th>
<th>Reaction mix. (M)</th>
<th>Oxygen absorption (nmole O2/mg. min)</th>
</tr>
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<td></td>
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<td>0</td>
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After the harvest, the cells were respectively washed with the same concentration of saline water as those of media. The experiments were carried out by the standard oxygen absorption method though NaCl of the reaction mixture was dissolved in the McIlvaine buffer.
of other fatty acids and carbon sources, suggested that the oxygen absorption was nutritionally significant.

As shown in Figs. 4 and 5, the optimum conditions of oxygen absorption and incorporation of linoleate did not coincide. It was presumably because oxygen absorption contained metabolic process by enzymes other than incorporation.

Calmes and Deal reported that transport of saturated fatty acids had two or three chain length specificities, for short- or long-chained fatty acids. They described N. asteroides had the same transport system for fatty acids from C12:0 to C18:0. We used McIlvaine buffer in oxygen absorption assay. S. rouxii IY-5 showed no oxygen absorption on citrate as the substrate. In fact, according to Lodder, S. rouxii strains did not assimilate citrate, and also S. rouxii IY-5 did not.

In Warburg manometric assay, ¹⁴CO₂ was trapped in KOH solution. This fact means that ¹⁴C-linoleate was degraded in the cells to produce ¹⁴CO₂. Namely linoleate was not only absorbed on the cell surface but it was metabolized within the cell.

S. rouxii IY-5 showed specifically strong oxygen absorption on linoleate and linolenate. The lower oxygen absorption on oleate and stearate, and further lower oxygen absorption on palmitate and myristate elucidated that S. rouxii should have the different mechanism of fatty acid oxygen absorption from Ps. oleovorans and N. asteroides.

According to Table 6, the linoleate oxygen absorption was inhibited by inhibitors of electron transport and oxidative phosphorylation, while it had no requirement of extracellular energy source.

This fact envisioned that linoleate oxygen absorption by S. rouxii could be carried out with energy accumulated in the cells and with energy produced by the metabolism of linoleate (Fig. 6 and Table 1).

Oxygen absorption on linoleate of S. rouxii was influenced by structural and physiological changes by extracellular osmotic pressure (Fig. 7 and Table 7). The cells grown under low concentration of NaCl showed high oxygen absorption, while the cells growth under as high as 2M NaCl showed high oxygen absorption with the presence of 1M or 2M NaCl.

This fact describes that a halotolerant yeast, S. rouxii could maintained great oxygen absorption under the same circumstances as the yeast had grown. Therefore it is suggested that oxygen absorption on linoleate should have an important meaning not only of nutrition and growth but also in the subsequent fermentation.

Further investigation of the relation between NaCl and oxygen absorption on linoleate will be reported in another paper.

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References

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耐塩性酵母サッカロミセス・ルキシーによるリノール酸化合物のとりこみ

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耐塩性酵母サッカロミセス・ルキシーIY-5 によるリノール酸化合物の酸素吸収を牛血清アルブミンとリノール酸との化合物を用いて調べた。酸素の最大吸収は対数期後期の細胞で認められた。リノール酸化合物への酸素吸収の初期の段階では、酸素吸収は時間もしくは細胞数に比例して増加した。リノール酸化合物のとりこみ実験から、Km は 42.0 μM, Vmax は 2.17 nmole/mg・min, 移行の最適 pH は 7.5 であった。ソーラルグラフ実験による密着の結果、1-14C-linoleate は分解して 14CO2 を生成した。このことは S. rouxii IY-5 によるリノール酸化合物の酸素吸収を明らかにしている。リノール酸化合物への酸素吸収は酸素電極を備えたビオキサグラフで測定した。酸素吸収の最適 pH は 4.0 で、それはリノール酸のとり込みのそれとは異っていた。基質移動性のデータから、酸素吸収がリノール酸とリノレン酸の遊離酸かメチルエステルの場合に著しく高いことがわかった。リノール酸化合物よりもよい基質であるにもかかわらず、ダルコースはリノール酸の酸素吸収で刺激的に作用しなかった。1M の食塩濃度の培地に生育した酵母細胞はこれと同じもしくは低食塩のリノール酸化合物の高い酸素吸収を示したが、2M の食塩濃度の培地に生育した細胞は 2M の食塩濃度で高い酸素吸収を示した。