Lipid Composition and Lipid Molecular Species of *Lipomyces starkeyi* Cultivated in Medium Containing 1,2-Propanediol

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In the previous paper,¹⁾ *Lipomyces starkeyi* IFO 0678 was cultivated in the medium containing glucose and 1,2-PD ("glucose-1,2-PD medium") to investigate the formation of diol lipids. It was found that the growth pattern of *L. starkeyi* in this medium was diauxic-like and that the consumption of glucose preceded 1,2-PD. An increase in O.D.₆₆₀ was observed at the 1,2-PD consuming stage. However, it was uncertain whether the increase in O.D.₆₆₀ was due to increased cell numbers or due to expansion of cell bodies. Variations of lipid composition, fatty acid composition and molecular species at different growth stages have not been made clear. It is of interest to examine the relationship between carbon source utilization and variations of lipid composition of the yeast at different growth stages. In this paper, the cell numbers, lipid content and lipid molecular species at different growth stages were compared.

L. starkeyi IFO 0678 was cultivated in the medium containing 2.5% each of glucose and 1,2-PD as carbon sources in a manner described in the previous paper.¹⁾ Aliquots of cells were harvested at 72 hr, when glucose was under consumption and the 1,2-PD was under slight consumption; at 120 hr, when glucose was nearly exhausted and the consumption of 1,2-PD was starting; and at 180 hr, when 1,2-PD was being actively consumed. The cells harvested were lyophilized and supplied for lipid analyses.²⁾

As previously described,¹⁾ the growth pattern of *L. starkeyi* in the "glucose-1,2-PD medium" was diauxic-like, and the O.D.₆₆₀ for 72 hr, 120 hr and 180 hr were 28.0, 36.0 and 37.7, respectively. However, cell counts by the haematometer were 3.1×10^8 , 3.3×10^8 and 3.4×10^8 , respectively. It was also observed by microscope that considerable numbers of rod-shaped

cells with long diameters of about 20 μ or longer were present along with normal spherical cells (4 ~ 10 μ in diameter) both at 120 hr and 180 hr. The dry weights of the cells for 72 hr, 120 hr and 180 hr were 5.2 mg/ml (1.67 mg/ 10^8 cells), 6.4 mg/ml (1.94 mg/ 10^8 cells) and 8.0 mg/ml (2.35 mg/ 10^8 cells), respectively. The results mentioned above suggest that the increase in optical densities was not due to cell multiplication, but due to cell expansion, i.e., cell multiplication did not occur under the 1,2-PD consumption, i.e., cell multiplication did not occur under the 1,2-PD consumption. A similar finding was presented by Morishita and Tanaka³⁾ with *Saccharomyces ellipsoideus* cultured in the medium containing ethyleneglycol as the carbon source.

The lipid contents of *L. starkeyi* at different growth stages showed interesting results. The lipid contents were 250 μ g/ 10^8 cells (150 mg/g of dry cells) for 72 hr, 155 μ g/ 10^8 cells (80 mg/g of dry cells) for 120 hr, and 306 μ g/ 10^8 cells (130 mg/g of dry cells) for 180 hr, respectively. The fact that lipid content at 120 hr was lower than at 72 hr suggests that the lipid once accumulated in the cells during the stage of glucose consumption was again utilized as an energy source and/or as a carbon source at 120 hr when the assimilation of 1,2-PD was not yet active.

Lipid composition of *L. starkeyi* at different growth stages are given in Table I. As the viable cell numbers at 120 hr and 180 hr were uncertain and the shape of the cells were not homogeneous at those stages, the values are expressed as mg/g of dry cells in Table I and Table II. The lipid of this yeast consisted of sterol ester, triglyceride, free fatty acid, diglyceride, ergosterol, trace amount of monoglyceride and phospholipid. Among these compounds, triglyceride was the major component throughout the culture period. The variations in lipid composition given in Table I would support the speculation that the lipid once accumulated was utilized again at the stage when glucose was nearly exhausted but 1,2-PD was not actively consumed yet. The content of triglyceride at 120 hr was reduced to about half of that of 72 hr. On the other hand, at 180 hr when 1,2-PD was utilized as the energy source, the lipid content increased again. However, it is noteworthy that the contents of free fatty acid, diglyceride and also phospholipid increased remarkably at 180 hr. This probably reflects the active syntheses of these compounds by a *de novo* synthetic mechanism.

The major fatty acids of the total lipid fraction were palmitic acid and oleic acid which together occupied more than 80% of the weight of total fatty acids throughout the culture stage. Palmitoleic acid, linoleic acid and stearic acid were the remaining fatty acids. An increase in the content of the odd number fatty acids was not found even at the growth stage consuming 1,2-PD. Fatty acid compositions in the total lipid and simple lipid did not vary significantly throughout

Abbreviations used: O.D.₆₆₀, optical density at 660 nm; 1,2-PD, 1,2-propanediol; TLC, thin-layer chromatography; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; PG, phosphatidylglycerol; PL, phospholipid.

TABLE I. LIPID COMPOSITION OF *L. starkeyi* GROWN IN THE "GLUCOSE-1,2-PROPANEDIOL MEDIUM"^{a)}

Lipid	72 hr		120 hr		180 hr	
	mg/g cell	(wt%)	mg/g cell	(wt%)	mg/g cell	(wt%)
Sterol ester	trace	(trace)	2.2	(2.8)	1.6	(1.2)
Triglyceride	133.0	(88.7)	66.6	(83.2)	83.3	(64.1)
Free fatty acid	6.0	(4.0)	5.6	(7.0)	13.0	(10.0)
Diglyceride	2.7	(1.8)	1.7	(2.1)	11.6	(8.9)
Ergosterol	2.1	(1.4)	1.1	(1.4)	2.3	(1.8)
Monoglyceride	trace	(trace)	0.4	(0.5)	trace	(trace)
Phospholipid	5.9	(3.9)	2.3	(2.9)	14.3	(11.0)

^{a)} Identification of the individual lipid was carried out by the method described in the previous paper.²⁾

TABLE II. POSITIONAL DISTRIBUTION OF FATTY ACIDS IN TRIGLYCERIDES OF *L. starkeyi* GROWN IN THE "GLUCOSE-1,2-PROPANEDIOL MEDIUM"^{a)}

Carbon number	Fatty acid			72 hr		120 hr		180 hr	
	C-1 or	C-3	C-2	mg/g cell (wt%)		mg/g cell (wt%)		mg/g cell (wt%)	
48	16:0,	16:0	16:1	0.8	(1)	7.3	(3)	9.1	(11)
50	16:0,	16:0	18:1	33.6	(25)	24.6	(33)	30.7	(37)
52	16:0,	18:1	18:1 }	72.2	(54)	27.3	(51)	34.1	(41)
	16:0,	18:1	18:2 }						
54	18:1,	18:1	18:1 }	26.3	(20)	7.9	(15)	9.9	(12)
	18:1,	18:1	18:2 }						

^{a)} Positional distribution of fatty acids in triglyceride was determined by subfractionation of triglycerides on argentation TLC with benzene-ethyl ether (80:20, v/v) as the developing solvent and by subsequent analysis on gas chromatograph-mass spectrometer, as reported earlier.²⁾

TABLE III. COMPOSITIONS OF MOLECULAR SPECIES OF MAJOR PHOSPHOLIPIDS IN *L. starkeyi* GROWN IN THE "GLUCOSE-1,2-PROPANEDIOL MEDIUM"^{a)}

Carbon number	Fatty acid		72 hr	120 hr	180 hr
	C-1	C-2	wt%	wt%	wt%
<i>Phosphatidylcholine</i>					
32	16:1	16:1	2	trace	trace
34	16:0	18:1	20	16	16
	16:1	18:1	20	20	14
36	18:1	18:1	32	36	40
	18:1	18:2	20	26	28
	18:2	18:2	6	2	trace
<i>Phosphatidylserine</i>					
32	16:0	16:0	2	2	7
	16:0	16:1	2	2	2
34	16:0	18:1	82	92	88
	16:0	18:2	10	2	3
<i>Phosphatidylethanolamine</i>					
32	16:0	16:1	1	trace	1
34	16:0	18:1	18	10	16
	16:1	18:1	28	20	18
36	18:1	18:1	30	54	51
	18:1	18:2	10	10	16
	18:2	18:2	12	5	trace

^{a)} Compositions of molecular species of phospholipids were determined by the method described in the previous paper.^{2,6)}

the growth stage. On the other hand, significant differences in fatty acid composition of the phospholipid fraction among the three different growth stages were observed. The proportion of oleic acid at 120 hr (66.7 wt%) was considerably higher than that at 72 hr (50.5 wt%). As the content of phospholipid at 120 hr decreased, the high proportion of oleic acid decreased remarkably.

Positional distributions of fatty acids in triglyceride at different growth stages are shown in Table II. The composition of triglyceride at 120 hr did not decrease evenly, but the decrease rate of triglycerides with total acyl carbon numbers of 52 and 54 containing C_{18:0}-C_{18:1}-C_{18:1} and C_{18:1}-C_{18:1}-C_{18:1}, respectively, was higher than triglycerides with total acyl carbon numbers of 48 and 50. This result also supports the finding that a lipase similar to that found in *Geotrichum candidum*⁴⁾ would participate in this yeast, as mentioned previously.⁵⁾

The phospholipid compositions for 72, 120 and 180 hr were PC (48, 41 and 43 wt% of total PL respectively), PE (26, 34 and 27 wt% of total PL respectively), PS (26, 25 and 27 wt% of total PL respectively), PI (2, trace and 6 wt% of total PL respectively) and trace amount of PG. Although the content of individual phospholipids was altered by the growth stages, the kind of phospholipid did not change. The composi-

tions of molecular species of major phospholipids at different growth stages are shown in Table III. Comparing the compositions of molecular species for the individual phospholipids, considerable differences were present not only between the phospholipid species, but also among different growth stages in the same phospholipid. However, 1-C_{16:0}-2-C_{18:1} occupied the major molecular species in PS at all stages examined. The fact that the molecular species of PS in *L. starkeyi* is almost exclusively 1-C_{16:0}-2-C_{18:1} in any conditions^{2,5)} suggests the unique role of 1-C_{16:0}-2-C_{18:1} phosphatidylserine in the cell membrane. Appreciable morphologic changes and the occurrence of diol lipid in the phospholipid fraction¹⁾ at 1,2-PD adaptation stage and 1,2-PD utilizing stage present an interesting pro-

blem for study on the regulatory mechanisms of cell membrane in the mixed carbon source.

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