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Effects of Cultivation Temperature on Fatty Acid Composition in *Rhodotorula gracilis*

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The lipid content of *Rhodotorula gracilis* decreases when the cultivation temperature is increased from 27 to 35°C. In parallel cultures run at the same temperature, increased lipid content implies an increased percentage of fatty acids in the lipid fraction.

Fatty acid composition varies with cultivation temperature. Oleic acid increases, linoleic acid decreases, and linolenic acid increases strikingly with decreasing temperature, while the saturated fatty acids remain unchanged.

The temperature during the first cultivation phase in which normal cells are produced, has some influence on the fatty acid composition. This influence persists during the second growth phase in which lipid is accumulated, but the temperature during this phase has a much more distinct effect on the formation of unsaturated acids, especially linolenic acid.

An important characteristic of microbial fat production is the possibility of changing fatty acid composition by altering culture conditions. Although microbial fat production is not in commercial use owing to high costs, it may be possible to make it economical if the amount of valuable lipid components can be increased by selection of both microorganisms and culture conditions. Especially important is the fatty acid composition of the fat, and above all, the levels of polyunsaturated fatty acids.

Of the various factors which may influence the fatty acid composition of microbial fat to a greater or lesser extent, temperature is thought to be the most important. In the plant kingdom, the fatty acids of a plant grown in a warmer district are more saturated than those of the same plant when grown in a cooler district.[1] Temperature may similarly be a significant factor in the microbial world. It should be noted that the composition of microbial lipid varies with the extent of lipid accumulation. Generally speaking, an increase of the lipid content of microorganisms is accompanied by a decrease in the nitrogen content. That this is so in the case of *Rhodotorula gracilis*

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was shown some twenty years ago by Enebo *et al.*[2] Therefore, differences in fatty acid composition must always be related to cells of almost the same nitrogen or lipid contents.

As microbial lipid usually accumulates under conditions of nitrogen deficiency, the culture period can be divided into two phases, one (the protein phase) in which cells of normal nitrogen content (about 10 %) are produced, and a second (the fat phase) in which nitrogen deficient cells of greater lipid content are formed. It would be interesting to know if the temperatures of both phases have effects upon the composition of the accumulated lipid, because lipid synthesis occurs mainly in the latter phase, while nitrogenous cell substances are thought to be synthesized mostly in the former phase, apart from some reorganization in the latter phase. According to Meyer and Broch,[3] 100 000 $\times$ *g* particles separated from *Torulopsis utilis* cells and incubated microaerobically at 19°C for a few days, have a greater capacity for desaturating oleate to linoleate than similar particles of the cells incubated at 30°C. This suggests that the incubation temperature irrespective of whether growth is occurring or not, has some effect on the synthesis of linoleic acid, and that a most important condition for obtaining a desired fatty acid composition might be the temperature in the latter phase, although that in the former phase might also be of some significance.

METHOD

Rhodotorula gracilis (our laboratory strain) was used as the test organism. Medium I was used in the seed culture, and Medium II in the main culture for fat accumulation. Five liter jar fermentors were used for the main cultures. Since Medium II contains 0.5 g/l urea, the nitrogen content of cells is about 8 % when the cell concentration reaches about 3 g/l, but after that nitrogen deficient cells are formed as the cell concentration increases. By continuing cultivation under conditions of nitrogen starvation, two kinds of samples, one containing cells of about 4 % and the other cells of about 2 % nitrogen content, were obtained. The cell concentration was roughly determined with hematocrits during the cultivation and the cell nitrogen content was determined by a semimicro Kjeldahl method.

Cells were separated by centrifugation, washed twice with distilled water, and partially dehydrated on porous plates. The cakes of cells thus obtained were dried in a desiccator, and then the dry cakes were powdered.

Table 1. Composition of media.

	Medium I	Medium II
Glucose	50 g	50 g
Urea	5 g	0.5 g
KH_2PO_4	4 g	1 g
$MgSO_4 \cdot 7H_2O$	3 g	1 g
Fe (as $FeSO_4$)	2 ppm	2 ppm
Distilled water	1 l	1 l
pH	4.5	4.5

Table 2. Effect of cultivation temperature on lipid and fatty acid content.

No.	Temp.	N, % (in dry cells)	Lipid, % (in dry cells)	Fatty acid, % (in lipid)
I	35°C	3.07 2.00	39.9 51.7	81.7 83.2
II	27°C	1.98 1.51	62.8 68.2	79.8 85.2
III	20°C	1.93 1.52	63.9 67.9	78.4 84.2
IV	20°C	3.86 2.05	30.4 60.6	73.9 86.6

Extraction of lipid was carried out with ether, after disintegration of the dry cells with 2 % HCl in an autoclave at 120°C for one hour.

The fatty acids were determined by gas chromatography, after saponification of the crude lipid extract and methylation of the fatty acids.

RESULTS AND DISCUSSION

1. *Effects of the cultivation temperature on the lipid content of the cells and the fatty acid content of the lipid.* The cultivation temperature has effects both on the lipid content of the cells and on the fatty acid content of the lipid. At a nitrogen level of about 2 % the lipid content was 62—63 % at both 20°C and 27°C but only 52 % at 35°C. No difference in lipid content was evident at 20°C and 27°C in the case of cells containing 1—5 % nitrogen. Obviously an increase in temperature above 27°C markedly influences the cell lipid content.

In parallel cultures run at the same temperature increased lipid content also implied an increased percentage of fatty acids in the lipid.

2. *Effects of the cultivation temperature on the fatty acid composition.* The effects of the cultivation temperature on the fatty acid composition are shown in Tables 3 and 4. Table 3 shows the difference between the cells containing about 4 % and 2 % nitrogen content grown at 35°C, 27°C, and 20°C. When the nitrogen content is about 4 %, it can be seen that the content of linoleic acid is lower, the lower the temperature of cultivation (17.8 % at 35°C, 11.8 % at 27°C, 10.4 % at 20°C). When the nitrogen content decreases to about 2 %, distinct differences in the content of linolenic acid appear in addition to the decrease of linoleic acid. The content of linolenic acid increases with decreasing temperatures (1.87 % at 35°C, 2.07 % at 27°C, 5.00 % at 20°C). This can also be seen when comparing III-3 and III-8 in Table 4 (1.54 % at 35°C, 4.50 % at 20°C). Oleic acid shows a slight tendency to increase as the temperature decreases.

Table 3. Effect of cultivation temperature on fatty acid composition (% of total fatty acids).

No.	II-1	II-2	II-3	II-4	II-5	II-6
Temp.	35°C	27°C	20°C	35°C	27°C	20°C
N %	4.00	4.18	3.73	2.08	2.06	1.90
C_{12}	0.08	0.05	0.10		0.04	0.04
C_{14}	1.51	1.06	0.74	1.72	1.35	0.80
C_{15}	0.04	0.06	0.10		0.40	
C_{16}	21.3	14.4	22.2	21.0	22.3	21.9
$C_{16}F_1$	1.50	2.05	2.02	1.51	1.40	1.25
C_{17}	0.38	0.38	0.64	0.36	0.47	0.33
$C_{17}F_1$	0.19	0.22			0.20	0.19
C_{18}	9.52	11.8	7.87	10.4	11.2	9.64
$C_{18}F_1$	44.8	52.9	53.0	46.6	50.3	48.9
$C_{18}F_2$	17.8	11.8	10.4	16.8	9.3	8.8
$C_{18}F_3$	2.44	4.02	2.89	1.87	2.07	5.00
C_{20}	0.23	0.88			0.35	3.11

$C_{18}F_1$ = oleic acid
$C_{18}F_2$ = linoleic acid
$C_{18}F_3$ = linolenic acid

Table 4. Effect of change in cultivation temperature on fatty acid composition (% of total fatty acids).

No.	III-1	III-2	III-3	III-4	III-5	III-6	III-7	III-8
Protein phase[a]	35°C				20°C			
Fat phase[a]	35°C	20°C	35°C	20°C	35°C	20°C	35°C	20°C
N %	4.41	3.81	1.81	1.98	3.98	4.02	1.98	1.94
C_{12}	0.15	0.04	0.13	0.08	0.11	0.10	0.06	0.30
C_{14}	1.55	0.95	1.73	0.95	1.43	1.02	1.53	1.20
C_{15}	0.37	0.22	0.32	0.67	0.63	0.19	0.11	
C_{16}	23.3	23.6	22.4	21.4	25.4	22.0	23.8	23.4
$C_{16}F_1$	2.44	1.89	2.56	1.67	2.11	2.04	1.27	
C_{17}	0.74	0.42	0.86	0.28	0.42	1.07		
$C_{17}F_1$	1.11	0.63	0.86	0.20				
C_{18}	8.52	10.5	9.42	8.60	11.5	11.2	12.2	8.40
$C_{18}F_1$	48.3	45.1	45.7	50.4	48.1	45.6	47.5	51.4
$C_{18}F_2$	10.3	12.0	12.8	10.2	8.5	13.1	10.9	10.8
$C_{18}F_3$	1.62	3.12	1.54	2.58		2.43	1.70	4.50
C_{20}	0.89	1.53	1.35	2.38	1.90	1.17	1.02	

[a] see the text.

Table 4 shows the effects of changing the temperature between the two phases. When the cell concentration reached about 3 g/l, the culture was divided into two and the resultant cultures were cultivated at different temperatures.

The effect of changing the temperature of the protein phase is indistinct when the temperature of the fat phase is 35°C as can be seen from III-3 and III-7, but is comparatively clear, when the temperature of the fat phase is only 20°C, especially with respect to linolenic acid content as seen from III-4 and III-8.

On the other hand, the effect of changing the temperature of the fat phase is rather distinct with respect to linolenic acid content, whether the temperature of the protein phase is 35°C (III-3, III-4) or 20°C (III-7, III-8). When the temperature of the protein phase is 20°C, the differences in linolenic acid content are particularly distinct as can be seen from III-7 and III-8 (1.70 % and 4.50 %). In conclusion, the temperatures of both phases have been shown to have some effect on the fatty acid composition, but that of the fat phase is especially significant. Generally speaking, the contents of oleic acid and linolenic acid increase, those of palmitic acid and stearic acid remain almost unchanged, and that of linoleic acid decreases slightly with decreasing temperature.

Bass *et al.*[3] found that the content of oleic acid decreased and that of linoleic and linolenic acids increased with decreasing growth temperature in *Rhodotorula gracilis*. Marr and Ingraham [4] examined the fatty acid content of *Escherichia coli*, and found that the contents of unsaturated acids increased slightly with decreasing growth temperature.

These results together with the results obtained here support the view that microorganisms generally contain a larger proportion of unsaturated fatty acids if they are grown at low temperatures.

According to Ng *et al.*[5] the fatty acid composition of *Escherichia coli* changes markedly even in the lag period when the temperature was lowered from 40°C to 10°C. This result and those reported in this paper suggest that temperature treatment of cells during growth or possibly even after growth, may be of great importance in increasing the yield of desirable components in microbial fats.

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