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Very Long-Chain Fatty Acids in Yeast

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Novel fatty acids ranging from 20 to 30 carbons have been found in *Saccharomyces cerevisiae*. These comprise 1 to 2% of the total fatty acid fraction.

It is well known that the yeast *Saccharomyces cerevisiae* synthesizes 16 and 18 carbon fatty acids. We have now determined that this yeast produces fatty acids ranging up to 30 carbons.

Wild-type yeast (strain X2180) was grown in minimal medium (2% glucose and yeast nitrogen base, Difco) at 30 C on a shaker. Growth flasks were removed at various stages in the yeast growth cycle. The centrifuged, lyophilized cells were extracted with $CHCl_3$:MeOH (2:1). The evaporated extract was saponified with 10% NaOH in aqueous MeOH at reflux temperatures for 2 h. The mixture was extracted with petroleum ether to remove non-saponifiables. We backwashed this organic fraction with dilute aqueous $NaHCO_3$ to remove long-chain fatty acids (4). The acidified aqueous fractions were extracted again with petroleum ether. The petroleum ether fractions were evaporated and the fatty acids esterified with 14% BF_3-MeOH (Applied Science, State College, Pa.). Blanks were employed at each step of the isolation so that eventually we found and eliminated all sources of contamination. After this was achieved, a blank carried through the whole procedure would show no gas chromatographic peaks in the fatty acid ester region. Microscope observation revealed no bacterial contamination.

By using a Perkin-Elmer model 900 gas chromatograph equipped with a flame ionization detector, we separated the fatty acid esters on a 10 foot (30.48 cm), 3% SE30, $^{100}/_{20}$ Chrom Q, ⅛ inch (0.32 cm) column. The temperature was programmed for 200 to 290 C (12 C per min) and then held constant at 290 C. To detect the small quantities of the long-chain esters, we injected moderately large samples and turned up the sensitivity after 18:0 (from attenuation 512 to attenuation 8). Standard fatty acid esters were cochromatographed to give tentative identifications. In some cases samples were collected from the gas chromatograph for introduction into the high-resolution mass spectrometer; in others the whole fatty acid ester fraction was injected into the low-resolution gas chromatograph-mass spectrometer. Spectra of standard fatty acid esters were compared with spectra of the unknown esters, for example, see Fig. 1. Though positive identification is possible for the saturated series (2), mass spectral analysis identifies only the number of double bonds in an unsaturated fatty acid ester, but not their position or orientation without derivatization. Low-resolution mass spectra were taken using a modified Perkin-Elmer 270 mass spectrometer with an all glass Watson-Biemann separator. It was operated on a cyclic scan mode at a resolution of 1,000 with a scan rate of 3 s per decade. The ionizing potential was 70 eV. Data were acquired under the LOGOS data system (3). High-resolution spectra were determined in real-time with a GEC-AEI-MS-902 mass spectrometer operated at 50 eV with a source temperature of 180 C (1).

All solvents were nanograde quality and were checked for contamination by gas chromatography. Distilled water was purchased from Alhambra Co. and was found to be uncontaminated with fatty acids. Standard fatty acid esters were purchased from Applied Science Laboratories.

In the analysis of the total fatty acid composition of wild-type yeast, many fatty acids having chains longer than 18:0 (Table 1) appear, and among these 26:0 is the most striking (Table 2). The relative quantities of the various fatty acids do not exhibit dramatic changes during the growth cycle. However, although 26:0 and 28:0 increase as the cultures age, 22:0 and the longer unsaturated fatty acids decrease. If long-chain fatty acid ($>C_{18}$) production resulted from faulty regulation of the chain-elongating mechanism, we might reasonably expect, on a proba-

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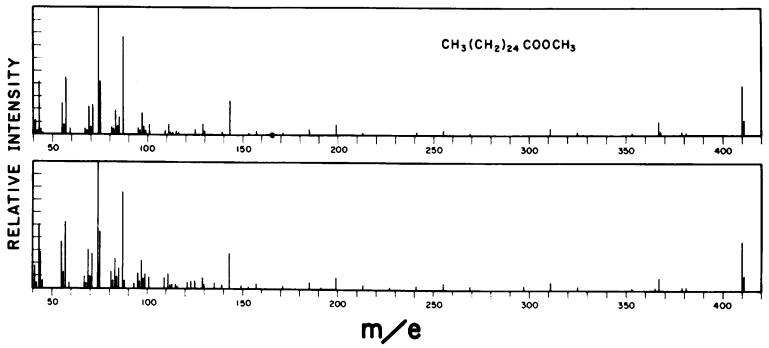

FIG. 1. *Mass spectra of authentic methyl cerotate, molecular weight 410 (above) and unknown (below).*

TABLE 1. *Percentages of total fatty acids in yeast at various stages in its growth cycle*[a]

Fatty acid	Mid-log (16 h)	Late log (23 h)	Early stationary (38 h)	Late stationary (62 h)
C_{10}–C_{14}	4	4	2	3
15:1[b]	Trace	0.08	0.2	0.2
15:0[c]	Trace	0.3	0.8	0.5
16:1	56	50	53	56
16:0	14	14	10	6
18:1	22	25	28.5	28
18:0	3	5	4	4
$>C_{18}$	1	1.5	1.5	2

[a] Fatty acids comprise about 1% of yeast dry weight. Weight percentages were determined by simple triangulation. No correction was made for detector response.

[b] 15:1, C_{15} fatty acid with one double bond.

[c] 15:0, Saturated unbranched C_{15} fatty acid.

TABLE 2. *Weight (%) of fatty acids with chain lengths longer than 18 carbons*[a]

Fatty acid	Mid-log (16 h)	Late log (23 h)	Early stationary (38 h)	Late stationary (62 h)
C_{19}[b]	2	1	1	1.5
20:1	7	3	7	5
20:0	7	3	5	5
22:1	2	1.5	2	1
22:0	7	5.5	2.5	2
23:1[b]	3	2	1	0.5
24:1	1	1	1	TR
24:0	5	4	2	2
25:1[b]	2	1	1	TR
25:0	4	1	4	4
26:1[b]	3	TR[c]	4	TR
26:0	38	64	49	58
27:1[b]	2	1	0.5	TR
27:0	6	4	5	6
28:1[b]	3	1	TR	TR
28:0	4	5	12.5	12
29:0	1	1	1	1
30:0	1	0.5	0.5	2
C_{31}[b]	1	0.5	0.5	TR
C_{32}[b]	1	0.5	0.5	TR
C_{33}[b]	TR	TR	TR	TR
C_{34}[b]	TR	TR	TR	TR

[a] The double-bond positions were not determined.

[b] Tentative assignment by GC retention time.

[c] TR, Trace.

balistic basis, that chain length and quantity synthesized would be inversely related. For example, molecules with chain lengths of C_{20}, C_{22}, and C_{24} should be more abundant than C_{26} molecules. However, the finding that C_{26} chains are more prevalant than C_{20}, C_{22}, and C_{24} chains does not support this view. Moreover, these fatty acids are not products only of stationary or dying populations. To assure ourselves that the yeast did not merely absorb these compounds from the medium, we extracted media by using our standard procedure and detected no fatty acids with chains longer than 18 carbons. Further, to test the notion that yeast accumulates and excretes these fatty acids as an aspect of secondary metabolism, we extracted the supernatant fluid after the cells were grown up and centrifuged. At best, the culture fluid extracts contained only very low levels of material chromatographing in the fatty acid range.

Fatty acids longer than 18 carbons, though comprising only 1 to 2% of the total fatty acids, may nonetheless have a key role in specific membrane functions, such as transport. Or since biological control mechanisms are often mediated by molecules representing only a small percentage of the total concentration, these fatty acids may be implicated as hormonal or regulatory agents.

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LITERATURE CITED

1. Burlingame, A. L. 1970. Developments and applications of real-time high resolution mass spectrometry, p. 104–115. *In* K. Ogata and T. Hayakawa (ed.), Recent developments in mass spectroscopy. University Park Press, Baltimore.
2. McCloskey, J. A. 1970. Mass spectrometry of fatty acid derivatives, p. 369–437. *In* F. D. Gunstone (ed.), Topics in lipid chemistry, vol. 1. Logos Press, London.
3. Smith, D. H., R. W. Olsen, F. C. Walls, and A. L. Burlingame. 1971 Real-time mass spectrometry. Anal. Chem. **43**:1796–1806.
4. Van der Veen, J., B. F. Medwadowski, and H. S. Olcott. 1968. Losses of fatty acids during the saponification extraction of small samples. Lipids **3**:189–190.