SHORT COMMUNICATIONS

Microbiological Synthesis of Wax Esters by *Euglena gracilis*[1]

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Euglena gracilis **(ATCC 12716) grown on yeast-malt extract medium synthesized wax esters. Saturated even-numbered acids and alcohols (C_{12} to C_{18}) were major constituents, with traces of odd-numbered fatty acids and alcohols. Tetradecanoic acid and tetradecanyl alcohol were the predominant components, and tetradecanyl tetradecanoate was the predominant wax ester.**

In our survey of microorganisms (1) capable of modifying fats and oils, we have observed the synthesis of wax esters by *Euglena gracilis* (ATCC 12716). *Acinetobacter* sp. grown on n-alkanes has been reported to synthesize wax esters (2). *Euglena gracilis* (streptomycin-bleached mutant) produces wax esters when grown aerobically and then maintained under anaerobic conditions (3). There is considerable interest in alternate sources for liquid wax esters, because importation of sperm whale oil products into the U.S. is prohibited, and in solid wax esters, which are domestically in short supply. In this report, the analysis of wax esters synthesized by *Euglena gracilis* is given.

EXPERIMENTAL

Fermentations were carried out in 300-ml flasks containing 50 ml sterilized medium inoculated with *Euglena gracilis* (ATCC 12716). The synthetic medium consisted of (in g/l): yeast extract, 3; malt extract, 3; peptone, 5; glucose, 10. The cells were cultured at 28 C under 500 ft candles of light for 11 days. Aerobic conditions were maintained by shaking the flasks once daily, and good growth was observed at the end of fermentation. The fermented contents from five flasks were filtered to yield 3.5 g wet cells. About 0.7 g of crude lipid was obtained after extraction of cells with chloroform-methanol (2:1). Purified wax esters (0.4 g, 11.4% based on wet weight) were obtained by passing the crude lipids through a small silica column (pasteur capillary pipette) with hexane as the eluant. Saponification of the esters and recovery of alcohols were performed in accordance with AOCS method Ca 6b-53 (4), except that samples were refluxed for 2–3 hr as suggested by Spencer and Tallent (5). The aqueous layer was acidified and extracted with ether to obtain fatty acids which were then methylated with diazomethane. Capillary gas chromatography (GC) of wax esters was carried out with a Hewlett-Packard 5890 instrument equipped with flame ionization detectors (FID) and a 15-m × 0.32-mm i.d. fused silica column coated with SPB-1 (Supelco Inc., Bellefonte, Pennsylvania). The column was temperature-programmed from 150 to 275 C at 5 C/min with helium flowing at 21 cm/sec.

[1]Part of a presentation at the American Oil Chemists' Society Annual Meeting in May 1988 in Phoenix, AZ.

RESULTS AND DISCUSSION

Thin layer chromatography (TLC) of purified wax esters on silica gel with benzene as developing solvent showed only one spot. GC analysis (Table 1) showed C_{28} as a major component with C_{24}, C_{26}, C_{30} and C_{32} as minor components. Trace amounts of other esters with 20 to 36 carbon atoms were present. GC analysis of fatty acids and alcohols (Table 2) showed only saturated fatty acids and alcohols with myristic acid and myristyl alcohol as major components. The C_{28} wax ester therefore was composed primarily of myristyl myristate. Only minor amounts of odd-numbered fatty acids and alcohols were found.

Odd-numbered fatty acids and alcohols are major components when fermentations are carried out with autotrophically grown *Euglena gracilis* cells in the dark under anaerobic conditions (6). With a streptomycin-bleached mutant (3), wax ester synthesis occurred only when aerobically grown cells were maintained under anaerobic conditions. In the present work, wax esters composed predominantly of even-numbered fatty acids and alcohols were found in the aerobically grown cells.

TABLE 1

Composition of Wax Esters by GC

Carbon no.	Area %	Carbon no.	Area %
20	0.9	28	41.5
22	2.2	29	1.1
23	0.3	30	7.4
24	15.1	31	0.4
25	0.9	32	11.6
26	13.8	34	1.0
27	1.7	36	2.0

TABLE 2

Composition of Fatty Acids and Alcohols from Wax Esters by GC

	Area %	
Carbon no.	Fatty acid	Alcohol
10	0.2	0.1
12	13.4	11.8
13	5.8	3.9
14	68.7	62.9
15	—	3.1
16	7.4	17.1
18	2.2	1.1
19	2.3	—

The absence of unsaturation in wax esters precludes their use as sperm whale oil substitute. It is not known whether fermentation conditions such as growth temperature or the nature of the carbon source will influence the unsaturation of wax esters, as has been the case with *Acinetobacter* sp (7). However, the wax esters of *Euglena gracilis* might find use as re;acement for spermaceti, a solid wax composed of saturated esters with chain length varying from 26 to 34 carbons (8), or to augment other solid waxes such as bees wax.

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