Effect of Substrate on the Fatty Acid Composition of Hydrocarbon-Utilizing Filamentous Fungi[1]

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The fatty acid pattern in hydrocarbon-utilizing filamentous fungi was determined after growth on acetate, propionate, n-alkanes (C_{13} to C_{15}), and alk-1-enes (C_{14} to C_{18}). The fatty acid profile of *Cunninghamella elegans* and *Penicillium zonatum* after growth on acetate shows a predominance of even-carbon fatty acids (C_{16}, $C_{18:1}$, $C_{18:2}$), whereas cells grown on propionate showed significantly higher levels of odd-carbon fatty acids (C_{15}, C_{17}, $C_{17:1}$). Growth on n-alkanes resulted in the incorporation of fatty acids homologous to the growth substrate. *Cunninghamella elegans* grown on the alk-1-enes from C_{14} to C_{18} incorporated the unsaturated substrate into cellular fatty acid after oxidation at the saturated end of the molecule. Regardless of substrate these fungi contain, predominantly, fatty acids 18 carbons in length.

The effect of substrate on the fatty acid composition of microorganisms has received considerable attention in recent years. Studies suggest that the mono-terminal oxidation products of some n-alkane and alk-1-ene substrates can be incorporated into the cellular lipids of bacteria (5, 6, 15, 16, 28), actinomycetes (4), and yeasts (9, 12, 19, 21, 26). Normal hydrocarbon substrates of a chain length near that of the fatty acids generally present in microorganisms (C_{14} to C_{18}) are most readily incorporated into the cellular lipids, e.g., 85% of the fatty acids in *Mycobacterium rhodochrous* grown on n-pentadecane are 15 carbons in length (5). Analysis of the fatty acids in *M. rhodochrous* after growth on alk-1-enes suggests that the substrate is oxidized at the methyl carbon and that either α-oxidation or β-oxidation of the fatty acid occurs (6). Thus, the cellular lipids of this organism grown on heptadec-1-ene contains heptadec-16-enoic acid, hexadec-15-enoic acid, and pentadec-14-enoic acid. Substrates that are metabolized via some 3-carbon intermediates (propionate) contain predominantly odd-chain fatty acids, and those that are degraded through a 2-carbon intermediate (acetate, isopropanol) contain mostly even-chain-length fatty acids (28).

The utilization of hydrocarbon substrates by filamentous fungi has received considerable attention (2, 7, 8, 13, 14, 17, 22, 24) since the

observation by Miyoshi in 1895 that fungi can attack paraffinic hydrocarbons (18). The direct incorporation of hydrocarbon substrates into the lipids of filamentous fungi has received little attention (23), although it has been suggested (3) that filamentous fungi do not incorporate substrate hydrocarbons directly and without degradation to the acetate level.

In the course of studies on the mineralization of various crude oils by microorganisms present in littoral areas, we isolated several fungi that were capable of an extensive and rapid growth on hydrocarbons (2). The effect of various substrates on the fatty acid composition of two of these, *Cunninghamella elegans* and *Eupenicillium zonatum* Hodges and Perry sp. nov. (10), is the subject of this report.

MATERIALS AND METHODS

Microorganisms. The filamentous fungi utilized in this study were strains of *Cunninghamella elegans* and *Penicillium zonatum*. These organisms were isolated by enrichment culture with crude oil as substrate by methods previously described (2, 24) and can utilize, as sole source of carbon and energy, all n-alkanes tested from C_9 through C_{32} and a wide array of long-chain alkenes, ketones, and fatty acids. *C. elegans* and *P. zonatum* are effective in degradation of paraffinic, asphaltic, and mixed-base crude oil (2).

Media and growth conditions. The fungi were cultured on L-salts medium (5) supplemented with the appropriate carbon and energy source. Solid and liquid substrates were added at 0.2%. The inoculum was prepared by aseptically homogenizing a 10-day plate culture in 50 ml of sterile saline. After inoculation the flasks were incubated at 25 C for 10 days in

stationary culture. After growth the mycelial mat was recovered and washed three times with chloroform to remove residual substrate.

Fatty acid analysis. The fatty acids were recovered from mycelial mats as the methyl ester, subjected to gas liquid chromatography, and identified as previously described (5, 6). The area of each chromatographic peak (percentage by weight) was determined by the method of Carroll (1). The tentative identification of other fatty acids was determined by comparison with the methyl esters of linoleic; linolenic; docosa-5,13-dienoic; eicosa-11,14,17-trienoic; and eicosa-11,14-dienoic acid (Applied Science Laboratories, State College, Pa.).

The abbreviations for fatty acids throughout this paper are as follows: n-hexadecanoic acid, C_{16}; unsaturated other than in terminal position, n-hexadecenoic acid, $C_{16:1}$ (-dienoic acid $C_{16:2}$, etc.); unsaturated in terminal position, 15-hexadecenoic acid, $C_{16:\omega}$; and branched fatty acid, BrC_n.

RESULTS

The fatty acid composition of *C. elegans* and *P. zonatum* was determined after growth on acetate and propionate (Table 1). Because the BrC_{19} fatty acid is derived from an even-carbon-chain fatty acid ($C_{18:1}$) by methylation (D. H. King and J. J. Perry, unpublished data), it is evident that the fungi grown on acetate contain over 93% even-carbon-chain acids. Growth on propionate resulted in a significant increase in odd-chain-length fatty acids (38 odd per 62 even in *C. elegans* and 42 per 58 in *P. zonatum*). The major fatty acids obtained from acetate-grown fungi are 16 and 18 carbons in length. Similar fatty acid profiles were obtained with glucose or crude oil as the growth substrate. Growth with propionate as substrate did not result in a marked change in total C_{18} fatty acids present, but 17-carbon fatty acids increased and those 16 carbons in length decreased in percentage composition.

The fatty acid profile in *C. elegans* and *P. zonatum* (Table 2) grown on n-tridecane, n-tetradecane, and n-pentadecane reflects the chain length of the substrate. Growth on n-alkanes of even-carbon length yielded cells that contained virtually 100% even-number fatty acids. The ratio of odd per even fatty acids after growth on n-tridecane was 25:75 and 30:70 in *C. elegans* and *P. zonatum*, respectively, and for n-pentadecane was 54:46 and 55:45 for *C. elegans* and *P. zonatum*, respectively.

C. elegans grown on C_{14} to C_{18} alk-1-enes contained significant amounts of ω-unsaturated fatty acid of equivalent chain length to that of the growth substrate (Table 3). The amount varied from 15.5% $C_{14:\omega}$ in tetradec-1-ene-grown cells to 28% $C_{16:\omega}$ in hexadec-1-ene-

TABLE 1. *Fatty acid composition of Cunninghamella elegans and Penicillium zonatum after growth on acetate and propionate*[a]

Fatty acid	Cunninghamella elegans		Penicillium zonatum	
	Acetate	Propionate	Acetate	Propionate
C_{13}	Tr[b]	2.2	Tr	3.1
C_{14}	1.5	Tr	2.3	Tr
C_{15}	Tr	4.0	Tr	5.3
C_{16}	28.4	7.4	27.1	6.2
C_{17}	2.8	20.4	2.5	22.1
$C_{17:1}$	ND[c]	11.0	ND	11.5
C_{18}	4.2	5.0	7.2	5.2
$C_{18:1}$	14.2	18.5	13.8	16.1
$C_{18:2}$	31.1	27.5	33.4	26.2
$C_{18:3}$	6.4	2.7	6.1	2.5
BrC_{19}[d]	4.1	ND	3.1	ND
$C_{20:2}$	2.2	1.2	2.3	1.5
$C_{20:3}$	1.1	Tr	1.4	Tr
$C_{22:2}$	Tr	Tr	Tr	Tr

[a] Recorded as percentage of the total fatty acids present. The organisms were grown in stationary culture at 25 C for 10 days. Acetate (Na) and propionate (Na) were added at 0.2% (wt/vol).
[b] Tr, Trace.
[c] ND, None detected.
[d] BrC_{19}, 10-methyl branched.

TABLE 2. *Fatty acid composition of Cunninghamella elegans and Penicillium zonatum after growth on various n-alkanes*[a]

Fatty acid	Cunninghamella elegans			Penicillium zonatum		
	Tridecane	Tetradecane	Pentadecane	Tridecane	Tetradecane	Pentadecane
C_{13}	13.1	Tr[b]	1.2	16.2	Tr	2.2
C_{14}	Tr	23.2	1.1	Tr	24.5	Tr
C_{15}	6.2	Tr	23.2	7.4	Tr	23.4
C_{16}	17.3	21.6	4.0	15.7	21.9	3.0
C_{17}	3.8	Tr	21.8	4.2	Tr	23.2
$C_{17:1}$	1.3	ND[c]	6.0	1.3	ND	5.8
C_{18}	2.9	3.5	1.3	2.5	4.2	1.1
$C_{18:1}$	9.7	3.8	12.3	8.5	3.2	11.7
$C_{18:2}$	26.1	22.1	22.8	22.1	24.1	23.0
$C_{18:3}$	12.1	10.2	1.5	15.2	8.4	2.3
BrC_{19}[d]	3.1	2.4	1.1	2.1	1.7	1.3
C_{20}	Tr	1.7	Tr	Tr	2.1	Tr
$C_{20:2}$	1.8	2.3	1.8	1.7	1.8	1.9
$C_{20:3}$	1.2	1.4	Tr	1.5	1.6	Tr
$C_{22:2}$	Tr	1.0	Tr	Tr	1.2	Tr

[a] Recorded as percentage of the total fatty acids present. The organisms were grown in stationary culture at 25 C for 10 days. Substrates were added at 0.2% (vol/vol).
[b] Tr, Trace.
[c] ND, None detected.
[d] BrC_{19}, 10-methyl branched.

TABLE 3. *Fatty acid composition of Cunninghamella elegans after growth on various 1-alkenes*[a]

Fatty acid	Growth substrate				
	Tetra-decane	Penta-decane	Hexa-decane	Hepta-decane	Octa-decane
C_{13}	Tr[b]	8.1	Tr	Tr	7.2
C_{14}	3.3	1.8	1.2	4.2	Tr
$C_{14:\omega}$	15.5	ND[c]	Tr	ND	Tr
C_{15}	Tr	4.8	6.3	6.2	5.2
$C_{15:\omega}$	ND	25.8	ND	3.1	ND
C_{16}	13.7	2.3	6.2	5.5	5.4
$C_{16:1}$	3.5	4.4	3.4	2.1	4.4
$C_{16:\omega}$	ND	ND	28.0	ND	Tr
C_{17}	Tr	Tr	Tr	4.2	1.7
$C_{17:1}$	Tr	ND	ND	2.6	Tr
$C_{17:\omega}$	ND	ND	ND	18.2	ND
BrC_{18}[d]	Tr	1.2	Tr	6.1	3.0
C_{18}	1.2	2.2	4.3	Tr	4.2
$C_{18:1}$	12.3	16.4	6.1	13.5	3.4
$C_{18:\omega}$	ND	ND	ND	ND	17.1
$C_{18:2}$	18.0	12.5	18.9	4.2	18.1
$C_{18:3}$	17.2	14.5	7.8	16.1	15.4
BrC_{19}	4.1	3.0	3.5	6.2	1.7
C_{20}	1.5	Tr	7.3	2.1	7.1
$C_{20:2}$	2.8	1.1	3.2	1.4	1.3
$C_{20:3}$	3.1	Tr	2.2	Tr	2.1
$C_{22:2}$	1.1	Tr	ND	Tr	Tr

[a] Recorded as percentage of the total fatty acids present. The organisms were grown in stationary culture at 25 C for 10 days. Substrates were added at 0.2% (vol/vol).

[b] Tr, Trace.

[c] ND, None detected.

[d] BrC_{18}, BrC_{19}, 10-methyl branched.

grown cells. The only evidence of chain shortening was with heptadec-1-ene where 3.1% of the total fatty acids was $C_{15:\omega}$ as compared with 18.2% $C_{17:\omega}$.

DISCUSSION

The presence in both fungi of C_{16}, C_{18}, and C_{20} fatty acids with a predominance of those of 16 and 18 carbons in length is comparable to reports by other workers (20, 25, 27). The high levels of fatty acid of even-chain length in both fungi after growth on acetate or glucose suggests that these fatty acids are a result of de novo synthesis via the malonyl pathway. The marked increase in odd-chain fatty acid in *C. elegans* (38%) and *P. zonatum* (42%) after growth on propionate indicates that propionate acts as a primer for odd-chain fatty acids. Similar results have been reported for other organisms including *Bacillus subtilis* (11) and *Mycobacterium vaccae* (28).

The incidence of fatty acids of chain length equivalent to the substrate in the cellular lipids

of fungi grown on *n*-alkanes suggests that the mono-terminally oxidized substrate can be directly incorporated into fungal lipids. Thus, eukaryotic filamentous fungi can incorporate *n*-alkanes to some extent in the same manner as the prokaryotic bacteria and actinomycetes. There is evidence (9) that the fatty acid synthesizing system in yeasts utilizing *n*-alkanes as sole growth substrate is completely suppressed. The cellular fatty acids are synthesized by lengthening of the mono-terminally oxidized alkane substrate by 2-carbon units. Comparison of the fatty acid profile in *n*-tridecane-, *n*-tetradecane-, and *n*-pentadecane-grown fungi suggests that both chain lengthening and de novo fatty acid synthesis occur. The presence, in *C. elegans*, of C_{15} (6.2%) and C_{17} (3.8%) with *n*-tridecane and C_{17} (21.8%) with *n*-pentadecane as substrate suggests the addition of 2 or 4 carbons to the fatty acid precursor. Although chain lengthening apparently occurs in yeasts (9) and these filamentous fungi, it is not evident in bacteria (5, 6; D. H. King and J. J. Perry, Abstr. Annu. Meet. Amer. Soc. Microbiol. 1973, p. 184). The consistent level of 18-carbon fatty acids in *n*-tetradecane- and *n*-pentadecane-grown fungi (42% in *n*-tetradecane and 39 and 38% in *n*-pentadecane-grown cells of *C. elegans* and *P. zonatum*, respectively) suggests that these fatty acids might be a product of de novo synthesis. If they are not, some mechanism for 1 carbon addition to the substrate would be required.

The direct incorporation of C_{14}, C_{15}, C_{16}, C_{17}, and C_{18} alk-1-enes after mono-terminal oxidation to ω-unsaturated fatty acids suggests that these also might partially inhibit de novo fatty acid synthesis. There was however, considerably less total incorporation of ω-unsaturated fatty acids in the filamentous fungi than in bacteria (6).

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