Effect of Substrate on the Fatty Acid Composition of Hydrocarbon- and Ketone-utilizing Microorganisms¹

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The fatty acid pattern in hydrocarbon- and ketone-utilizing bacteria after growth on various substrates was examined. The fatty acid composition of one hydrocarbon-utilizing organism (*Mycobacterium* sp. strain OFS) was investigated in detail after growth on *n*-alkanes, 1-alkenes, ketones, and *n*-alcohols. *n*-Alkanes shorter than C₁₃ or longer than C₁₇ were not incorporated into cellular fatty acids without some degradation. Strain OFS incorporated C₁₄ to C₁₇ 1-alkenes into cellular fatty acids as the ω -monoenoic fatty acid. Methyl ketones were incorporated into strain OFS after removal of one- or two-carbon fragments from the carbonyl end of the molecule. An organism isolated by enrichment on methyl ketones was incapable of *n*-alkane utilization but could grow on, although not incorporate, ketones or long chain *n*-alcohols into cellular fatty acids.

Recently, we published a report on the fatty acid pattern in three hydrocarbon-utilizing bacteria after growth on acetate, propane, and C₁₃ to C₁₇ *n*-alkanes (2). After growth on C₁₃ to C₁₇ *n*-alkanes, the major fatty acids in the cells of one of these bacteria (*Mycobacterium* sp. strain OFS) were of a chain length equivalent to the growth substrate. Isotopic competition experiments suggested that these *n*-alkanes were incorporated into the cellular fatty acids after α -oxidation and without degradation to the acetate level.

This investigation is concerned with the incorporation of *n*-alkane growth substrates shorter than C₁₃ and longer than C₁₇ into the cellular fatty acids of strain OFS. The effect of various long chain 1-alkenes, ketones, and *n*-alcohols as growth substrate on the fatty acid pattern of strains OFS and an *Achromobacter* sp. that had been isolated by enrichment on a methyl ketone was also ascertained.

MATERIALS AND METHODS

Microorganisms. The organisms used in this study are designated as follows: OFS, a *Mycobacterium* sp. (2); JOB5, a *Brevibacterium* sp. (2); 7EIC, a *Mycobacterium* sp. (3, 6); and A-64, a gram-negative short rod isolated on 2-undecanone and tentatively assigned

to the genus *Achromobacter*. These organisms and those reported in previous papers (2, 3, 6) are under investigation and their identification will be the subject of a separate report.

Media and growth conditions. The growth conditions and basal medium were the same as previously described (2). The C₁₂, C₁₃, C₁₄, C₁₆, and C₁₈ 1-alkenes (obtained from Humphrey Chemical Co., New Haven, Conn.) and the C₁₅ and C₁₇ 1-alkenes (obtained from Aldrich Chemical Co., Milwaukee, Wisc.) were added directly to sterilized media at 0.17% (v/v). 7-Tridecanone (C_{13-7-one}), obtained from the Aldrich Chemical Co., 3-tetradecanone (C_{14-3-one}), 2-pentadecanone (C_{15-2-one}), and 9-heptadecanone (C_{17-9-one}), obtained from K & K Laboratories, Inc., Plainview, N. Y., were added at 0.17% (v/v). The alcohols *n*-tetradecanol and *n*-hexadecanol (obtained from Humphrey Chemical Co.) were added at 0.03% (v/v).

The methyl esters of fatty acids were prepared, chromatographed, and identified as previously described (2). The fatty acids presumably derived from 1-alkenes were identified by comparing the retention time of the methyl esters with ω -pentadecenoic acid (K & K Laboratories, Inc.) and the value for ω -octadecenoic acid (4).

The abbreviations for fatty acids throughout this paper are as follows: *n*-pentadecanoic acid, C₁₅ fatty acid; *n*-pentadecenoic acid unsaturated in other than a terminal position, C_{15:1} fatty acid; omega (ω) unsaturated *n*-pentadecenoic acid, C_{15: ω} fatty acid; and branched fatty acid, Br-C_n.

RESULTS

Mycobacterium sp. (OFS) was grown on the C₁₁, C₁₂, C₁₈, C₂₀, C₂₄, and C₂₈ *n*-alkanes, and the

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TABLE 1. Fatty acid composition of *Mycobacterium* sp. (strain OFS) after growth on various *n*-alkanes^a

Fatty acid	Growth substrate					
	C ₁₁	C ₁₂	C ₁₈	C ₂₀	C ₂₄	C ₂₈
C ₁₁	2.1	5.1	ND ^b	ND	ND	0.4
C ₁₂	0.4	4.1	ND	ND	0.1	0.8
C ₁₃	1.1	Tr ^c	ND	ND	0.3	3.1
C ₁₄	3.3	7.0	2.9	3.0	3.0	6.0
C ₁₅	2.9	1.4	1.3	Tr	10.4	21.1
C _{15:1}	0.4	0.1	0.4	Tr	2.5	3.7
C ₁₆	30.6	27.5	28.3	22.0	21.8	15.8
C _{16:1}	13.8	18.1	15.5	18.0	13.8	10.6
C ₁₇	1.2	0.5	0.4	ND	ND	3.0
C _{17:1}	2.0	0.6	0.5	Tr	ND	8.5
C ₁₈	0.8	ND	1.9	Tr	ND	ND
C _{18:1}	20.7	17.5	26.3	30.0	21.6	13.8
C ₁₉	ND	ND	ND	ND	0.3	ND
Br-C ₁₉	20.7	18.1	22.5	27.0	26.2	13.2

^a Recorded as per cent of the total fatty acids present. The cells were grown for 72 hr at 27°C on a rotary shaker. The substrate was added at 0.66%.

^b ND = none detected.

^c Tr = trace.

fatty acids were extracted from the cells and subjected to chromatographic analysis (Table 1). The fatty acids resulting from growth on these substrates were primarily C₁₆, C_{16:1}, C_{18:1}, and Br-C₁₉. The overall fatty acid pattern was similar to that obtained after growth on acetate (2), except for the appearance of a high concentration of C₁₅ saturated fatty acid in the cells grown on C₂₄ and C₂₈ *n*-alkanes. Growth of strain OFS on long chain *n*-alcohols (C₁₂ to C₁₈) resulted in an incorporation pattern similar to that observed with the homologous *n*-alkane; e.g., 57.8% of the fatty acid in *n*-tetradecanol-grown cells was the C₁₄ fatty acid.

Two other strains, 7EIC and JOB5, were grown on C₁₃ and C₁₇ *n*-alkanes, and the relationship between length of substrate and the fatty acid pattern in these organisms was compared with that of acetate- and propane-grown cells (Table 2). The fatty acid patterns in propane- and acetate-grown cells of strain JOB5 were incorporated in the previous report (2). The predominant fatty acids in the acetate-grown JOB5 cells were C₁₆ and C_{18:1}. Cells grown on propane also had significant levels of C₁₅, C₁₇, and C_{17:1} fatty acids. Growth of strain JOB5 on C₁₃ *n*-alkane resulted in an increase in *n*-tridecanoic acid and growth on C₁₇ *n*-alkane caused an increase in C₁₅ and C₁₇ fatty acids. Similar results were obtained on analysis of strain 7EIC.

The cellular fatty acid composition of strain

TABLE 2. Fatty acid composition of *Mycobacterium* sp. (strain 7EIC) and *Brevibacterium* sp. (strain JOB5) after growth on various substrates^a

Fatty acid	Growth substrate					
	Strain JOB5		Strain 7EIC			
	C ₁₃	C ₁₇	C ₁₃	C ₁₇	Acetate	Propane
C ₁₁	2.9	ND ^b	1.5	ND	ND	0.4
C ₁₂	0.2	0.6	1.2	0.8	0.4	0.4
C ₁₃	19.6	0.9	28.7	1.2	0.1	0.1
C ₁₄	3.5	0.4	1.4	0.4	1.1	1.5
C ₁₅	5.2	35.2	2.8	23.7	ND	1.8
C _{15:1}	0.5	2.7	0.2	1.0	ND	0.3
C ₁₆	24.1	3.4	22.5	1.2	36.1	29.4
C _{16:1}	6.3	1.4	4.5	ND	13.9	11.0
C ₁₇	4.1	19.7	1.5	43.5	ND	6.6
C _{17:1}	2.7	30.1	11.8	27.0	0.2	2.6
C ₁₈	3.0	ND	1.6	ND	0.6	3.5
C _{18:1}	18.0	3.3	15.5	1.2	19.5	29.2
Br-C ₁₉	9.9	2.3	6.8	ND	28.1	13.2

^a Recorded as percentage of the total fatty acids present. The cells were grown on a rotary shaker for 72 hr at 27°C. The substrate was added at 0.66% for *n*-alkanes, 0.2% for acetate (Na), and 50:50 (v/v) for propane.

^b ND = none detected.

OFS was determined after growth on the C₁₂ to C₁₈ 1-alkenes (Table 3). There was no evidence that C₁₂ and C₁₃ 1-alkenes are incorporated into the fatty acids of strain OFS without degradation. Cells grown on C₁₄, C₁₅, C₁₆, and C₁₇ 1-alkenes incorporated the unsaturated substrate into cellular fatty acid after oxidation of the saturated end. This was particularly evident in C₁₆ 1-alkene-grown cells where 46% of the total fatty acid was the ω -unsaturated fatty acid. Cells grown on C₁₇ 1-alkene contained C₁₅ and C₁₅ ω -unsaturated fatty acids. The amount of unsaturated substrate incorporated into cellular fatty acids of strain OFS was much less (4.7%) for 1-octadecene than with C₁₄ to C₁₇ 1-alkenes.

The fatty acid composition of strain OFS cells after growth on 2-pentadecanone, 3-tetradecanone, 7-tridecanone, and 9-heptadecanone was determined (Table 4). With the exception of C_{15-2-one}, the results are similar to those obtained with strain OFS after growth on acetate. In cells grown on C_{15-2-one}, there was a marked increase in C₁₃ and C₁₄ fatty acids.

The fatty acid pattern in an organism which was isolated by enrichment on methyl ketones (A-64) and which grows on long chain *n*-alcohols but is incapable of *n*-alkane utilization was also investigated. Results are presented in Table 5. The fatty acid pattern in methyl ketone-grown

TABLE 3. Fatty acid composition of *Mycobacterium* sp. (strain OFS) after growth on various *l*-alkanes^a

Fatty acid	Growth substrate						
	C ₁₂	C ₁₃	C ₁₄	C ₁₅	C ₁₆	C ₁₇	C ₁₈
C ₁₁	1.0	ND ^b	ND	3.4	0.4	ND	ND
C ₁₂	0.5	0.6	0.5	0.5	0.6	1.7	8.4
C ₁₃	0.6	0.8	1.7	1.3	1.4	0.3	ND
C ₁₄	5.4	7.1	8.4	2.8	3.6	1.4	0.9
C _{14:1ω}	ND	ND	27.4	ND	ND	ND	ND
C ₁₅	3.2	7.8	ND	9.2	9.5	16.9	3.2
C _{15:1}	0.4	ND	ND	ND	1.7	ND	ND
C _{16:1ω}	ND	ND	ND	39.5	ND	13.2	ND
C ₁₆	29.1	28.8	20.4	17.6	5.3	6.1	13.5
C _{16:1}	17.8	23.6	14.0	14.1	6.1	3.9	5.6
C _{16:2ω}	ND	ND	ND	ND	46.0	4.1	28.8
C ₁₇	1.1	ND	ND	1.2	ND	ND	ND
C _{17:1}	2.2	ND	ND	0.8	18.0	15.0	ND
C _{17:2ω}	ND	ND	ND	ND	ND	15.4	2.6
C ₁₈	0.3	ND	ND	0.9	ND	ND	ND
C _{18:1}	13.3	11.8	10.7	8.7	2.8	22.0	5.8
C _{18:2ω}	ND	ND	ND	ND	ND	ND	4.7
C ₁₉	4.1	ND	ND	ND	0.8	ND	19.4
Br-C ₁₉	21.0	19.5	16.9	ND	3.8	ND	7.1

^a Recorded as per cent of the total fatty acids present. The cells were grown for 72 hr at 27 °C on a rotary shaker. The substrate was added at 0.17%.

^b ND = none detected.

TABLE 4. Fatty acid composition of *Mycobacterium* sp. (strain OFS) after growth on various straight chain ketones^a

Fatty acid	Growth substrate			
	C _{12-25-one}	C _{14-8-one}	C _{16-7-one}	C _{17-9-one}
C ₁₁	0.6	1.5	ND ^b	2.3
C ₁₂	0.9	7.6	0.2	0.7
C ₁₃	24.6	1.8	0.2	0.7
C ₁₄	13.7	4.3	2.6	1.7
C ₁₆	7.7	4.7	4.0	2.8
C _{16:1}	Tr ^c	0.9	0.6	ND
C ₁₆	18.0	20.8	26.8	29.2
C _{16:1}	9.7	12.7	18.7	13.9
C ₁₇	5.4	3.2	2.5	3.1
C _{17:1}	8.0	3.8	5.2	3.3
C ₁₈	ND	2.1	0.4	2.1
C _{18:1}	10.0	15.6	12.5	24.7
C ₁₉	ND	ND	ND	ND
Br-C ₁₉	ND	20.5	26.3	15.5

^a Recorded as per cent of the total fatty acids present. The cells were grown for 72 hr at 27 °C on a rotary shaker. The substrate was added at 0.03% (w/v).

^b ND = none detected.

^c Tr = trace.

cells of A-64 did not differ significantly from the pattern in the organism after growth on glucose or acetate. Growth of this isolate on C₁₄ and C₁₆ *n*-alcohols also yielded cells with a fatty acid

TABLE 5. Fatty acid composition of *Achromobacter* sp. (strain A-64) after growth on various substrates^a

Fatty acid	Growth substrate						
	C _{13-2-one}	C _{15-2-one}	C _{14-3-one}	Acetate	Glucose	C _{14-ol}	C _{16-ol}
C ₁₁	ND ^b	1.8	2.8	0.9	ND	1.8	5.0
C ₁₂	1.0	0.8	18.2	0.5	0.3	ND	3.3
C ₁₃	1.0	0.5	2.3	0.2	0.2	2.7	2.5
C ₁₄	5.2	6.4	8.2	6.0	5.0	9.1	4.6
C ₁₅	0.3	1.4	2.9	Tr ^c	Tr	ND	ND
C _{15:1}	0.4	Tr	0.7	Tr	Tr	ND	ND
C ₁₆	20.6	42.2	28.5	21.0	22.9	20.4	18.7
C _{16:1}	33.0	10.6	8.2	34.2	34.6	28.7	29.2
C ₁₇	1.2	2.4	1.7	ND	ND	ND	ND
C _{17:1}	3.5	10.1	5.8	2.8	1.8	1.8	ND
C ₁₈	0.8	0.8	1.2	0.4	0.7	2.0	ND
C _{18:1}	33.0	23.0	19.5	34.0	34.5	33.5	36.6

^a Recorded as per cent of the total fatty acids present. The cells were grown for 72 hr at 27 °C on a rotary shaker. The substrate was added at 0.2% for acetate (Na) and glucose. The *n*-alcohols and ketones were added at 0.03% (w/v).

^b ND = none detected.

^c Tr = trace.

composition similar to that in acetate-grown A-64 cells.

DISCUSSION

Previous results suggested that C₁₃ to C₁₇ *n*-alkanes are incorporated into the fatty acids of *Mycobacterium* sp. strain OFS without degradation to the acetate level. The upper and lower limit of the size of *n*-alkanes that can be so incorporated has been investigated by growing the organism on *n*-alkanes of a chain length shorter than C₁₃ and longer than C₁₇. The similarity of the fatty acid pattern of cells grown on these *n*-alkanes to that of acetate-grown cells suggested that the fatty acids are synthesized from the degradation products and are not a result of direct substrate incorporation. Earlier work demonstrated that strain OFS contains significant amounts of C₁₅ fatty acid after growth on propane, *n*-pentadecane, and other alkanes of odd-chain length but not when grown on even-chain length *n*-alkanes (2). Results with C₁₁ and C₁₂ *n*-alkanes did not demonstrate any correlation between odd-chain length substrates and the production of odd-chain length fatty acids, as was observed with acetate and propane as substrates. There was no measurable amount of C₂₀, C₂₄, or C₂₈ fatty acids in cells grown in *n*-alkanes of these chain lengths. The incorporation of C₁₃ and C₁₇ *n*-alkanes into the fatty acids of 7E1C and JOB5 suggested that the phenomenon of direct incorporation of C₁₃ to C₁₇ *n*-alkanes is not confined to strain OFS. The *n*-alcohol results with strain OFS indicated that *n*-alcohols behave in a similar

manner to *n*-alkanes of equivalent chain length. The synthesis of fatty acids of chain length equivalent to various *n*-alkane and 1-alkene substrates has been demonstrated in various other organisms including *Candida lipolytica* (7).

The fatty acid composition of C_{12} and C_{13} 1-alkene-grown cells of strain OFS is similar to that of strain OFS cells grown on acetate, except for some increase in the C_{14} and C_{15} saturated fatty acid content. Cells grown on C_{14} , C_{15} , and C_{16} 1-alkenes contained significant amounts of the ω -unsaturated fatty acid of equivalent length to the growth substrate. The amount varied from 27.4% ω -tetradecenoic acid in 1-tetradecene-grown cells to 46.0% ω -hexadecenoic acid in 1-hexadecene-grown cells. There was a decreased amount of $C_{18:1}$ in the 1-hexadecene-grown cells, with a corresponding increase in $C_{17:1}$. When 1-heptadecene served as substrate, the major fatty acids were C_{15} , ω -pentadecenoic acid ($C_{15:\omega}$), C_{16} , $C_{16:1}$, ω -hexadecenoic acid ($C_{16:\omega}$), $C_{17:1}$, ω -heptadecenoic acid ($C_{17:\omega}$), and $C_{18:1}$. It is interesting to speculate on the origin of the C_{15} , C_{16} , and C_{17} fatty acids in 1-heptadecene-grown cells. The saturation of olefinic hydrocarbon substrates has been demonstrated by Huybregtse and Van Der Linden (5). The $C_{17:1}$ and C_{15} acids could have come from the α -olefinic substrate after saturation. The C_{15} would result from β -oxidation and the $C_{17:1}$ by desaturation of the C_{17} molecule. Oxidative attack on the saturated end of the substrate would yield the $C_{17:\omega}$, and β -oxidation at the same end would yield the $C_{15:\omega}$ fatty acids. Steward et al. (9) demonstrated that the initial attack on α -olefins by a gram-negative coccus was on the saturated end. Bruyn (1) found that the unsaturated end of α -olefins is the point of initial oxidation in *C. lipolytica*, resulting in the formation of 1,2 diols. One-carbon removal from such a diol could yield the C_{16} saturated fatty acid and two-carbon removal the C_{15} fatty acid. Desaturation of the saturated C_{16} fatty acid could yield the $C_{16:1}$. Markovetz et al. (8) isolated 13-tetradecenoic acid from 1-tetradecene-grown cells of *Pseudomonas aeruginosa*, suggesting that the methyl group is attacked directly and a one-carbon fragment is selectively removed. This mechanism could account for the presence of the $C_{16:\omega}$ fatty acid in 1-heptadecene-grown cells of strain OFS.

The 1-octadecene-grown cells contained substantial amounts of $C_{16:\omega}$ (28.8%) and some $C_{17:\omega}$ (2.6%) and $C_{18:\omega}$ (4.7%) fatty acids. The $C_{18:\omega}$ would arise from oxidation of the saturated end of the molecule and the $C_{17:\omega}$ and $C_{16:\omega}$ fatty acids by the mechanisms already discussed.

Growth of strain OFS on $C_{15-2-one}$ yielded cells with 24.6% C_{13} and 13.7% C_{14} fatty acids. These fatty acids could result from a 1,2 diol mechanism similar to the α -olefin oxidation mechanism as proposed by Bruyn (1). Low levels of Br- C_{19} were present in $C_{15-2-one}$ -grown cells of strain OFS; this finding is similar to results obtained with this organism on C_{15} *n*-alkane and 1-pentadecene. Utilization of $C_{13-7-one}$, $C_{14-3-one}$, and C_{17-one} as growth substrate did not result in a fatty acid pattern significantly different from that of acetate-grown cells. These results suggested that for ketones other than methyl ketones the fatty acids are synthesized from degradation products of the substrate, and the ketones do not influence the qualitative fatty acid content of the cell.

The results with *Achromobacter* sp. (strain A-64) suggested that there is little correlation between the structure of the ketone substrate and the fatty acid composition of cells. Additional studies with C_{14} and C_{16} *n*-alcohols demonstrated that in this organism there is little direct incorporation of substrate into the cellular fatty acids.

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