JOURNAL OF BACTERIOLOGY, Nov. 1974, p. 987-989

Vol. 120, No. 2
Printed in U.S.A.

Metabolism of *n*-Butane and 2-Butanone by *Mycobacterium vaccae*

W. E. PHILLIPS, JR., AND J. J. PERRY

Department of Microbiology, North Carolina State University, Raleigh, North Carolina 27607

Received for publication 13 August 1974

n-Butane was metabolized in *Mycobacterium vaccae* (JOB5) via terminal oxidation. This organism metabolized 2-butanone through propionate (or propionyl coenzyme A). Subterminal oxidation in *M. vaccae* was apparently limited to propane.

The dominant pathway for propane oxidation in *Mycobacterium vaccae* (JOB5) is a subterminal attack to produce acetone (2, 6). Acetone is further metabolized through acetol and cleaved to acetate and a one-carbon intermediate. Studies on the metabolism of longer chain alkanes by other bacteria suggest that terminal oxidation is the dominant route of attack (1, 5). This investigation was initiated to determine the site of initial oxidative attack on *n*-butane by *M. vaccae*. Since 2-butanone would be the product of subterminal oxidation of *n*-butane, the metabolism of this and related substrates is reported.

The fatty acid composition of hydrocarbon-utilizing microorganisms often reflects the pathway of oxidation of the substrate (3, 4). *M. vaccae* cells were grown on various substrates in L-salts (2, 3, 6) and analyzed for fatty acid composition by gas-liquid chromatography (3, 4). The results are presented in Table 1. *M. vaccae*, grown on propionate, and 2-butanone contained significant levels of fatty acids having odd-carbon number chain length, whereas less than 1.0% of the fatty acids in those grown on *n*-butane, butyrate, β-hydroxybutyrate, and acetate had odd-carbon number chain lengths. These data suggested that 2-butanone may be metabolized to propionate, yielding the precursor necessary for odd-carbon number fatty acid synthesis. The absence of significant amounts of odd-carbon number fatty acids in *n*-butane-grown cells suggested that *n*-butane was not oxidized through propionate. The similarity of the fatty acid composition in *n*-butane, butyrate, β-hydroxybutyrate, and acetate-grown cells implies that terminally oxygenated intermediates are involved in *n*-butane oxidation by *M. vaccae*.

M. vaccae was grown on various substrates and assayed for isocitrate lyase activity (EC 4.1.3.1) (2, 6). Isocitrate lyase was induced in *M vaccae* by growth on *n*-butane, butyrate, β-hydroxybutyrate, and acetate (Table 2). If *n*-butane is metabolized through terminal oxidation to butyrate, further to β-hydroxybutyrate, and cleaved to yield acetate, growth on these substrates should result in the induction of isocitrate lyase. The presence of isocitrate lyase in cells grown on these substrates suggest that *n*-butane may be metabolized to acetate through an initial terminal oxidation. Isocitrate lyase was absent from cellular extracts of *M. vaccae* after growth on propionate and 2-butanone, suggesting that acetate is not an intermediate in the oxidation of these compounds.

M. vaccae has previously been shown (2, 6) to metabolize propionate via the methylmalonyl-succinate pathway, which involves the carboxylation of propionate to methylmalonate with subsequent isomerization to succinate (7). *M. vaccae* was grown on *n*-butane, butyrate, acetate, 2-butanone, and propionate. The cells were recovered in late log phase and placed in the presence of the growth substrate and $NaH^{14}CO_3$. Sodium arsenite was added to effect an accumulation of any pyruvate produced during the oxidation of the substrate. If metabolism of the substrate occurred via propionate, the addition of $NaH^{14}CO_3$ to the reaction mixture should result in the formation of radioactive pyruvate. The results in Table 3 demonstrate that no significant radioactivity was incorporated into the pyruvate produced by *M. vaccae* during oxidation of *n*-butane, acetate, or butyrate. A significant amount of radioactivity was incorporated into pyruvate produced by *M. vaccae* metabolizing 2-butanone and propionate. These results further substantiate that the site of initial oxidation of *n*-butane by *M. vaccae* is terminal and that propionate is not an intermediate in the metabolism of *n*-butane.

TABLE 1. *Fatty acid composition of M. vaccae cells after growth on various substrates*[a]

Fatty acid	Growth substrate					
	n-Butane	Butyrate	β-Hydroxy-butyrate	Acetate	2-Butanone	Propionate
C_{12}	Tr	Tr	ND	ND	Tr	Tr
C_{13}	ND	ND	ND	Tr	Tr	1.2
C_{14}	4.8	5.9	3.0	3.1	6.1	2.4
C_{15}	ND	ND	ND	Tr	3.1	12.1
C_{16}	29.1	32.2	34.1	33.1	24.9	13.9
$C_{16:1}$	16.0	16.7	16.5	14.5	14.5	6.1
C_{17}	Tr	Tr	Tr	Tr	4.3	13.0
$C_{17:1}$	ND	ND	ND	ND	5.2	27.2
C_{18}	7.3	3.3	ND	ND	4.1	1.4
$C_{18:1}$	42.8	41.9	46.4	49.3	37.8	18.7
$C_{19:1}$	ND	ND	ND	ND	ND	4.0
Even-carbon	100.0	100.0	100.0	100.0	87.4	42.5
Odd-carbon	Tr	Tr	Tr	Tr	12.6	57.5

[a] Recorded as percentage of the total fatty acids present. The cells were grown on a rotary shaker to late log phase (approximately 72 h). Tr, Trace (<1.0%); ND, none detected.

TABLE 2. *Isocitrate lyase levels in cell extracts of M. vaccae after growth on various substrates*

Growth substrate[a]	Sp act[b]
n-Butane	0.7
Butyrate	0.7
β-Hydroxybutyrate	0.5
Acetate	0.8
2-Butanone	0
Propionate	0

[a] Cells were recovered in late log phase of growth (approximately 72 h).

[b] Results expressed in units per milligram of protein in which 1 U is the amount of enzyme necessary for the cleavage of 1 μmol of isocitrate in 10 min at 30 C.

TABLE 3. *Relative [^{14}C]carbon dioxide incorporation into pyruvate produced by nonproliferating M. vaccae during the oxidation of various substrates*[a]

Oxidation substrate	Cell growth on:[b]				
	n-Butane	Butyrate	Acetate	2-Butanone	Propionate
Malate	0	0	0	0	0
Malate + $NaAsO_2$	52	28	30	47	39
n-Butane	0				
n-Butane + $NaAsO_2$	75				
Butyrate		7			
Butyrate + $NaAsO_2$		21			
Acetate			0		
Acetate + $NaAsO_2$			26		
2-Butanone				353	
2-Butanone + $NaAsO_2$				3,895	
Propionate					6
Propionate + $NaAsO_2$					1,243

[a] Each vessel contained 50 μmol of substrate (except *n*-butane, 50:50 *n*-butane-air mixture) and 10 μCi of $NaH^{14}CO_3$. $NaAsO_2$ (4 μmol) was added to one flask for each substrate. Cells suspended in L-salts (pH 7.5) were added. Final volume was 3.2 ml and incubation was at 30 C for 150 min.

[b] Values, corrected for background and endogenous counts, are expressed in counts per min in pyruvate per milligram (dry weight).

From these data, it is concluded that 2-butanone oxidation occurs via propionate, which is further metabolized by the methylmalonyl-succinate pathway.

This investigation was supported by grant GB-23815 from the National Science Foundation.

LITERATURE CITED

1. Baptist, J. N., R. K. Gholson, and M. J. Coon. 1963. Hydrocarbon oxidation by a bacterial enzyme system. I. Products of octane oxidation. Biochim. Biophys. Acta **69**:40-47.
2. Blevins, W. T., and J. J. Perry. 1972. Metabolism of propane, *n*-propylamine, and propionate by hydrocarbon-utilizing bacteria. J. Bacteriol. **112**:513-518.
3. Dunlap, K. R., and J. J. Perry. 1967. Effect of substrate on the fatty acid composition of hydrocarbon-utilizing microorganisms. J. Bacteriol. **94**:1919-1923.
4. Dunlap, K. R., and J. J. Perry. 1968. Effect of substrate on the fatty acid composition of hydrocarbon and ketone-utilizing microorganisms. J. Bacteriol. **96**:318-321.
5. Stewart, J. E., R. E. Kallio, D. P. Stevenson, A. C. Jones, and D. O. Schissler. 1959. Bacterial hydrocarbon oxidation. I. Oxidation of *n*-hexadecane by a gram-negative coccus. J. Bacteriol. **78**:441-448.
6. Vestal, J. R., and J. J. Perry. 1969. Divergent metabolic pathways for propane and propionate utilization by a soil isolate. J. Bacteriol. **99**:216-221.
7. Wegener, W. S., H. C. Reeves, R. Rabin, and S. J. Ajl. 1968. Alternate pathways of metabolism of short-chain fatty acids. Bacteriol. Rev. **32**:1-26.