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Production of Methyl Ketones from Secondary Alcohols by Cell Suspensions of C_2 to C_4 *n*-Alkane-Grown Bacteria

CHING T. HOU,* RAMESH PATEL, ALLEN I. LASKIN, NANCY BARNABE, AND IRENE BARIST

Corporate Research Science Laboratories, Exxon Research and Engineering Company, Linden, New Jersey 07036

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Nineteen new C_2 to C_4 *n*-alkane-grown cultures were isolated from lake water from Warinanco Park, Linden, N.J., and from lake and soil samples from Bayway Refinery, Linden, N.J. Fifteen known liquid alkane-utilizing cultures were also found to be able to grow on C_2 to C_4 *n*-alkanes. Cell suspensions of these C_2 to C_4 *n*-alkane-grown bacteria oxidized 2-alcohols (2-propanol, 2-butanol, 2-pentanol, and 2-hexanol) to their corresponding methyl ketones. The product methyl ketones accumulated extracellularly. Cells grown on 1-propanol or 2-propanol oxidized both primary and secondary alcohols. In addition, the activity for production of methyl ketones from secondary alcohols was found in cells grown on either alkanes, alcohols, or alkylamines, indicating that the enzyme(s) responsible for this reaction is constitutive. The optimum conditions for in vivo methyl ketone formation from secondary alcohols were compared among selected strains: *Brevibacterium* sp. strain CRL56, *Nocardia paraffinica* ATCC 21198, and *Pseudomonas fluorescens* NRRL B-1244. The rates for the oxidation of secondary alcohols were linear for the first 3 h of incubation. Among secondary alcohols, 2-propanol and 2-butanol were oxidized at the highest rate. A pH around 8.0 to 9.0 was found to be the optimum for acetone or 2-butanone formation from 2-alcohols. The temperature optimum for the production of acetone or 2-butanone from 2-propanol or 2-butanol was rather high at 60°C, indicating that the enzyme involved in the reaction is relatively thermally stable. Metal-chelating agents inhibit the production of methyl ketones, suggesting the involvement of a metal(s) in the oxidation of secondary alcohols. Secondary alcohol dehydrogenase activity was found in the cell-free soluble fraction; this activity requires a cofactor, specifically NAD. Propane monooxygenase activity was also found in the cell-free soluble fraction. It is a nonspecific enzyme catalyzing both terminal and subterminal oxidation of *n*-alkanes.

Leadbetter and Foster (18) were the first to demonstrate the bacterial formation of methyl ketones from *n*-alkanes, a unique alpha-oxidation without changes in the carbon skeleton. Cell suspensions of propane-grown *Mycobacterium smegmatis* 422 oxidize propane or butane mainly to their corresponding methyl ketones with less than 1% neutral volatile substances, presumably *n*-propanol. Vestal and Perry (26), based on their findings with [2-^{14}C]propane and the presence of isocitrate lyase in propane-grown *Mycobacterium vaccae* JOB5, suggested that propane is not metabolized via terminal oxidation.

We found that cell suspensions of methylotrophic bacteria catalyzed the oxidation of gaseous *n*-alkanes and 2-alcohols to their corresponding methyl ketones (13, 23). The enzyme responsible for the oxidation of secondary alcohols is a novel NAD-linked enzyme, which oxidizes secondary alcohols to methyl ketones specifically (9, 14, 22). NAD-dependent primary alcohol dehydrogenases from liver and baker's yeast have been well studied (3). They oxidize preferentially primary alcohols, with a lower rate for secondary alcohols (about 10% of their ethanol activity). NAD(P)-dependent primary alcohol dehydrogenases were also reported in *Pseudomonas* species (16, 20, 25), *Escherichia coli* (6), a *Leuconostoc* species (7), and *Rhizopus javanicus* (28). However, these enzymes were active only toward short-chain primary alcohols (28), long-chain primary alcohols (6, 7, 20), or long-chain hydroxy fatty acids, with some activity on medium-chain-length secondary alcohols containing a hydroxy group at the center of the carbon chain (20). Recently, alcohol dehydrogenase with a noticeable preference for second-

ary alcohols was also reported from *Comamonas terrigena* (2) and *Thermoanaerobium* species (4, 16) grown on carbohydrates.

In the course of our continuing studies on microbial oxidation of gaseous hydrocarbons (8, 10–12), we have isolated many C_2 to C_4 alkane-utilizing bacteria and have studied their oxidation of gaseous alkenes and gaseous alkanes. In the companion paper (12), we demonstrated that a propane monooxygenase system from various propane-grown bacterial strains oxidized gaseous *n*-alkenes to their corresponding 1,2-epoxides. This paper describes the isolation of 19 new C_2 to C_4 alkane-utilizing microbes from lake water and oil samples. It also describes the formation of methyl ketones from their corresponding secondary alcohols by resting-cell suspensions of these new cultures as well as by known cultures which have been adapted to utilize propane as the sole carbon and energy source. The optimum conditions for in vivo production of methyl ketones from 2-alcohols were compared among selected strains: *Brevibacterium* sp. strain CRL56, *Nocardia paraffinica* ATCC 21198, and *Pseudomonas fluorescens* NRRL B-1244. Methyl ketone production was also demonstrated in cell-free systems.

MATERIALS AND METHODS

Bacterial strains. Cultures that were known to be able to grow on liquid alkanes were selected from either the American Type Culture Collection (Rockville, Md.) or from the Northern Regional Research Laboratories (Peoria, Ill.); all were able to grow also on C_2 to C_4 alkanes as the sole source of carbon and energy. Nineteen organisms used in these studies were newly isolated strains from lake water from Warninanco Park, Linden, N.J. and from soil and lake water samples from Bayway Refinery, Linden, N.J. (isolated by an enrichment culture technique). The organisms were maintained on mineral salt plates in a dessicator jar under an atmosphere of propane and air (1:1, vol/vol) at 30°C.

Growth of organisms. Small-scale cultures of gaseous *n*-alkane-utilizing organisms were grown at 30°C in 300-ml flasks containing 50 ml of mineral salt medium (18) with C_2 to C_4 *n*-alkane and air (1:1, vol/vol) as the sole carbon and energy source. Flasks were fitted with a rubber stopper with a glass tube and clamps for gassing. The gaseous phase of the flasks was evacuated and replaced with a gas mixture of C_2 to C_4 *n*-alkane and air (1:1, vol/vol). Cultures were incubated at 30°C on a rotary shaker at 200 rpm.

Larger-scale cultures of C_2 to C_4 *n*-alkane-utilizing organisms were grown in 2.8-liter flasks containing 800 ml of mineral salts medium (18) with gaseous *n*-alkane as sole source of carbon and energy. A 50-ml 24-h culture was used to inoculate the large flasks.

Chemicals. Gaseous hydrocarbons were obtained from Matheson Gas Products (East Rutherford, N.J.). Liquid alkanes, alcohols, and methyl ketones were purchased from Matheson, Coleman and Bell Manufacturing Co. (Norwood, Ohio). BF_3-methanol was purchased from Supelco, Inc., Bellefonte, Pa. Other chemicals were purchased from Sigma Chemical Co. (St. Louis, Mo.).

Preparation of cell-free soluble fraction. Cell suspensions of twice-washed cells at 4°C were disintegrated by a single passage through a French pressure cell (American Instruments Co., Silver Spring, Md.) at 20,000 lb/in^2. Disintegrated cell suspensions were centrifuged at 15,000 × *g* for 15 min to remove unbroken cells. The supernatant solution was then centrifuged at 40,000 × *g* for 60 min, and the supernatant solution therefrom was again centrifuged at 80,000 × *g* for 60 min, yielding the soluble fraction.

Assay for methyl ketone production. Cells were harvested during exponential growth by centrifugation at 10,000 × *g* for 15 min. Cells were washed twice with 50 mM sodium phosphate buffer, pH 7.0, and the final pellet was suspended in a small amount of fresh buffer. A 0.5-ml sample of this washed cell suspension was placed in a 10-ml vial at 4°C which was sealed with a rubber cap. Five microliters of substrate 2-alcohol was added. The vials were then incubated at 30°C on a rotary shaker at 300 rpm. A 3-μl sample was removed with a syringe and assayed by gas-liquid chromatography (GLC), using a stainless steel column (6.1 m by 3.2 mm) packed with 10% Carbowax 20M on 80/100 Chromosorb W (Supelco). The column temperature was maintained isothermally at 130°C and the carrier gas flow rate was 30 ml of helium per minute. The products, methylketones, were identified by retention time comparisons and cochromatography with authentic standards. The amounts of the products that accumulated were determined from peak areas by using a standard curve which had been constructed with authentic samples. Duplicate measurements were performed for each assay. In the whole-cells system, protein was determined by the biuret method (17) after the sample was boiled with 1.0 N NaOH for 30 min.

For the cell-free system, the enzyme activity was measured with a fluorescence spectrophotometer (model MPF 44A; The Perkin-Elmer Corp., Norwalk, Conn.) by following the formation of reduced NAD (excitation, 340 nm; emission, 460 nm). The formation of reduced NAD was also followed with an absorption spectro-photometer at 340 nm. The assay system in 3 ml contained: potassium phosphate buffer (pH 7.0), 150 μmol; NAD, 1 μmol; a given amount of enzyme preparation; and secondary alcohol, 10 μmol. The reaction was started by the addition of substrate. Protein was determined by the method of Lowry et al. (19).

RESULTS

Oxidation of secondary alcohols by C_2 to C_4 *n*-alkane-grown bacteria. Cell suspensions of C_2 to C_4 *n*-alkane-grown bacteria oxidized secondary alcohols to their corresponding methyl ketones. After incubation, the reaction mixture was centrifuged to remove the cells. The product methyl ketones were found to have been accumulated extracellularly. Control experiments with heat-killed cells indicated that the methyl ketones were produced enzymatically. The rates of production of methyl ketones (acetone and 2-butan-

TABLE 1. Production of methyl ketones from 2-propanol and 2-butanol by cell suspensions of C_2 to C_4 *n*-alkane-grown bacteria

Strain[a]	Production rate (μmol/h per mg of protein) of:	
	Acetone from 2-propanol	2-Butanone from 2-butanol
Propane grown		
Arthrobacter petroleophagus ATCC 21494	10.3	10.7
Arthrobacter simplex ATCC 21032	6.8	6.7
Arthrobacter sp. strain CRL60 = NRRL B-11315	2.2	5.0
Acinetobacter sp. strain CRL67 = NRRL B-11313	2.4	3.5
Acinetobacter calcoaceticus ATCC 19140	8.4	6.7
Actinomyces sp. strain CRL66 = NRRL 11314	2.1	3.5
Alcaligenes sp. strain ATCC 15525	14.4	7.3
Brevibacterium insectiphilum ATCC 15528	11.5	9.3
Brevibacterium sp. strain ATCC 14649	9.8	8.8
Brevibacterium fuscum ATCC 15993	18.8	18.2
Brevibacterium sp. strain CRL52 = NRRL B-11318	3.3	6.2
Brevibacterium sp. strain CRL56 = NRRL B-11319	16.0	16.8
Brevibacterium sp. strain CRL61 = NRRL B-11320	2.1	5.2
Corynebacterium sp. strain CRL63 = NRRL B-11321	8.5	7.5
Hydrogenomonas sp. strain ATCC 17697	8.2	8.0
Mycobacterium album ATCC 29676	10.8	9.2
Mycobacterium rhodochrous ATCC 29672	10.3	9.9
Mycobacterium sp. strain CRL51 = NRRL B-11322	3.1	4.8
Mycobacterium sp. strain CRL62 = NRRL B-11323	2.8	4.0
Nocardia neoopaca ATCC 21499	13.8	14.9
Nocardia paraffinica ATCC 21198	15.4	16.8
Nocardia sp. strain CRL55 = NRRL 11325	2.1	5.1
Nocardia sp. strain CRL57 = NRRL 11326	5.8	7.2
Nocardia sp. strain CRL64 = NRRL 11327	2.0	5.2
Pseudomonas crucurae NRRL B-1021	10.8	12.5
Pseudomonas fluorescens NRRL B-1244	12.9	15.3
Pseudomonas multivorans ATCC 17616	12.8	11.4
Pseudomonas sp. strain CRL53 = NRRL B-11329	6.2	4.9
Pseudomonas sp. strain CRL58 = NRRL B-11331	10.0	9.4
Pseudomonas sp. strain CRL65 = NRRL B-11332	6.2	4.2
Ethane grown		
Arthrobacter sp. strain CRL68 = NRRL B-11316	0.98	3.2
Brevibacterium fuscum ATCC 15993	7.8	8.4
Brevibacterium sp. strain CRL61 = NRRL B-11320	2.1	2.5
Mycobacterium sp. strain CRL69 = NRRL B-11324	1.6	3.0
Nocardia paraffinica ATCC 21198	5.8	6.0
n-Butane grown		
Arthrobacter sp. strain CRL70 = NRRL B-11317	2.8	4.7
Brevibacterium sp. strain CRL61 = NRRL B-11320	3.0	4.1
Brevibacterium fuscum ATCC 15993	8.0	9.5
Nocardia paraffinica ATCC 21198	5.8	6.0
Pseudomonas sp. strain CRL71 = NRRL B-11333	2.1	2.8
Others		
Brevibacterium sp. strain CRL61 = NRRL B-11320		
Ethanol grown	1.2	4.2
Propanol grown	2.2	5.7
Butanol grown	2.5	4.0
Ethylamine grown	3.0	4.1
Propylamine grown	2.1	3.2
Glucose grown	0.3	0.5

[a] CRL numbers designate newly isolated cultures from our laboratory.

one) from 2-propanol and 2-butanol by cell suspensions of C_2 to C_4 *n*-alkane-grown bacteria are shown in Table 1. The production of methyl ketones was found in all of the strains tested, including newly isolated and known cultures. Dehydrogenation activity was found in cells of both known and newly isolated strains grown on either alkanes, alcohols, alkylamines, or glucose, indicating that the enzyme(s) responsible for this reaction is constitutive. (Table 1 lists

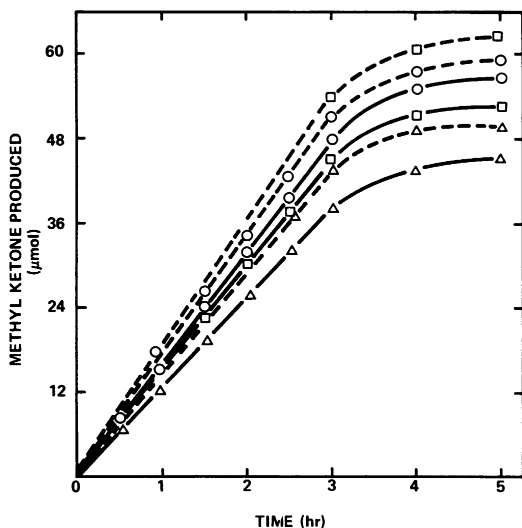

FIG. 1. Time courses for the production of acetone or 2-butanone from 2-propanol or 2-butanol by cell suspensions of propane-grown *Brevibacterium* sp. strain CRL56, *P. fluorescens* NRRL B-1244, and *N. paraffinica* ATCC 21198. Cells (1 mg) were incubated with substrate at 30°C in a vial as described in the text. Acetone (solid line) or 2-butanone (broken line) were detected by GLC. Symbols: ○, strain CRL56; △, strain NRRL B-1244; □, strain ATCC 21198.

only the data for *Brevibacterium* sp. strain CRL61.)

The optimum conditions for the production of methyl ketones from 2-propanol and 2-butanol by cell suspensions were compared among selected strains, *Brevibacterium* sp. strain CRL56, *N. paraffinica* ATCC 21198, and *P. fluorescens* NRRL B-1244, grown on propane.

Time course of methyl ketone production. The reaction was linear during the first 3 h (Fig. 1). The rate of production of methyl ketone decreased upon further incubation. Therefore, the production of methyl ketone was measured within 1 h whenever the effect of a variable was tested.

Effect of temperature and pH on methyl ketone production. Although the optimum temperature for the production of acetone or 2-butanone from propane or *n*-butane for all three strains was around 35 to 40°C, the optimum temperature for the production of methyl ketone from 2-propanol or 2-butanol by these microbes was rather high, around 60°C.

pH studies were done with sodium phosphate buffer (0.05 M) for pH 5.5 to 8.0 and Tris buffer (0.05 M) for pH 8.0 to 10.0. A pH around 8.0 to 9.0 was found to be the optimum for acetone or 2-butanone formation from secondary alcohols. Authentic samples of acetone or 2-butanone (final concentration, 4 μmol/ml) were added to heat-killed cell suspensions at pH 5.5, 7.0, and 10.0 to test for nonenzymatic degradation of methyl ketones at these pH values. Methyl ketone concentration in these suspensions did not decrease during 3 h of incubation, indicating that nonenzymatic oxidation of methyl ketone was negligible under these assay conditions.

Substrate specificity. The substrate specificity of cell suspensions of the three selected strains grown on propane was studied (Table 2). For comparison, the oxidation of *n*-alkanes by these strains is also shown. Among the secondary alcohols, 2-propanol and 2-butanol were oxidized at higher rates.

Further oxidation. The production of methyl ketone from *n*-alkane or secondary alcohol by resting-cell suspensions of propane-grown bacteria indicates that an enzyme system for the subterminal oxidation of propane does exist in these cells. However, the possibility that the propane monooxygenase system is also a nonspecific enzyme, catalyzing both terminal and subterminal oxidations, cannot be ruled out. Therefore, the possibility was investigated that 1-propanol was produced (terminal oxidation) and was further oxidized instantaneously into aldehyde or acid and could not be detected by GLC under our assay conditions. 1-Propanol, 2-propanol, or acetone (0.3 to 0.4 μmol/ml of cell suspension) was incubated with cell suspensions (0.5 mg of cells per ml) of both viable and heat-killed cells of propane-grown strains ATCC 21198 and CRL56. The rates of disappearance of substrates were followed by GLC. Cell suspensions of heat-killed cells did not oxidize any of these substrates. With viable cells, however, all of the 1-propanol added disappeared within 6 min of incubation (Fig. 2). When the reaction

TABLE 2. Production of methyl ketones from various *n*-alkanes or secondary alcohols by cell suspensions of selected strains grown on propane[a]

Substrate	Methyl ketone production (μmol/h per mg of protein)		
	Brevibacterium sp. strain CRL56	*N. paraffinica* ATCC 21198	*P. fluorescens* NRRL B-1224
Propane	2.0	2.0	1.0
n-Butane	2.6	4.4	1.2
n-Pentane	0.20	0.6	0.08
n-Hexane	0.06	0.01	
2-Propanol	16.0	15.4	12.9
2-Butanol	16.8	16.8	15.3
2-Pentanol	1.2	1.3	2.4
2-Hexanol	0.01	0.02	0.13

[a] Product methyl ketones were identified by GLC retention time comparisons and cochromatography with authentic standards.

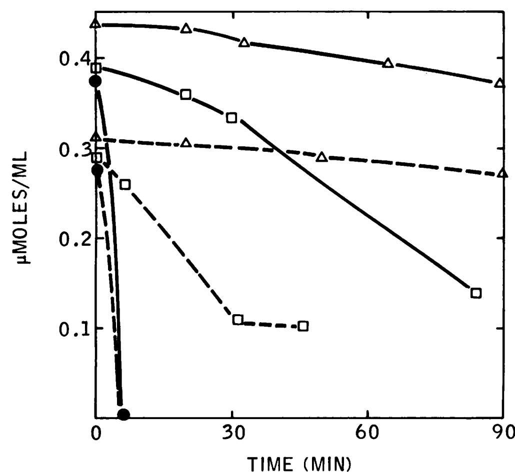

FIG. 2. Oxidation of 1-propanol, 2-propanol, and acetone by resting-cell suspensions of propane-grown *Brevibacterium* sp. strain CRL56 and *N. paraffinica* ATCC 21198. 1-Propanol, 2-propanol, or acetone (0.3 to 0.4 μmol) was incubated with resting-cell suspensions (0.5 mg of cells per ml). The disappearance of the substrates was followed by GLC. Solid line for strain CRL56, broken line for strain ATCC 21198. Symbols: □, 2-propanol; △, acetone; ●, 1-propanol.

mixture with 1-propanol as substrate was acidified, extracted with benzene, and then methylated with BF_3-methanol, the presence of the methyl ester of propionic acid was confirmed by GLC analysis. 2-Propanol was also oxidized by the viable cell suspensions during the 90-min incubation period. However, further degradation of acetone by these cell suspensions was negligible. Direct proof of the production of primary alcohol from propane at a rate equal to that for subterminal oxidation is described for our cell-free experiments.

Inhibition studies. The effect of metal-binding agents on the production of acetone from 2-propanol by cell suspensions of propane-grown *Brevibacterium* sp. strain CRL56 was examined. For comparison, data obtained from propane substrate are also listed (in Table 3). Metal-binding agents and other potential inhibitors such as potassium cyanide, 1,10-phenanthroline, α,α-dipyridyl, thiourea, imidazole, and 8-hydroxyquinoline inhibited the formation of acetone from propane, indicating the involvement of a metal ion(s) (Table 3). However, potassium cyanide and thiourea failed to inhibit the production of acetone from 2-propanol, suggesting that the metal complex(es) involved in the oxidation of secondary alcohol is not a heme type.

Cell-free system. The oxidation of alkanes, cyclohexane, and toluene by propane-grown *Brevibacterium* sp. strain CRL56 was studied at the cell-free level. Cell-free soluble fractions were prepared according to the procedure described above. Hydroxylation activity was found in the 80,000 × *g* soluble fraction. Both primary and secondary alcohols were produced at a similar rate (Table 4). These alcohols were further oxidized by alcohol dehydrogenase(s). To find out whether or not this alcohol dehydrogenase(s) activity is growth substrate dependent, the oxidations of 1-propanol and 2-propanol by cell-free extracts of bacteria grown on either propane, 1-propanol, or 2-propanol were studied. The results obtained are shown in Table 5. Secondary alcohol oxidation activity was found in 1-propanol-grown cells, and primary alcohol oxidation activity was also found in 2-propanol-grown cells, indicating that alcohol dehydrogenase(s) is a constitutive enzyme.

TABLE 3. Effect of metal-binding agents on the production of acetone from propane or 2-propanol by cell suspensions of propane-grown *Brevibacterium* sp. strain CRL56

Inhibitor (1 mM)	% Inhibition of acetone formation from:	
	Propane	2-Propanol
Potassium cyanide	95	0
1,10-Phenanthroline	100	85
α,α-Dipyridyl	85	40
Thiourea	90	0
8-Hydroxyquinoline	95	90
Imidazole	100	80

DISCUSSION

In *n*-alkane-grown cells, the *n*-alkane is metabolized via either terminal or subterminal oxidation. Vestal and Perry (26) indicate that subterminal oxidation of propane to acetone is the

TABLE 4. Oxidation of alkanes, cyclohexane, and toluene by soluble cell-free extract of propane-grown *Brevibacterium* sp. strain CRL56

Oxidation substrate	Product	Rate of product formation (μmol/10 min per mg of protein)
Ethane	Ethanol	0.090
Propane	1-Propanol	0.054
	2-Propanol	0.060
Butane	1-Butanol	0.062
	2-Butanol	0.065
Isobutane	Isobutanol	0.044
Pentane	1-Pentanol	0.035
	2-Pentanol	0.043
Hexane	1-Hexanol	0.048
	2-Hexanol	0.032
Cyclohexane	Cyclohexanol	0.065
Toluene	Benzyl alcohol	0.020

TABLE 5. Oxidation of 1-propanol and 2-propanol by cell-free extracts of bacteria grown on various substrates

Microbe	Oxidation rate (μmol/min per mg of protein)	
	1-Propanol	2-Propanol
Propane grown		
P. fluorescens NRRL B-1244	16	31
Brevibacterium sp. strain CRL56	19	23
1-Propanol grown		
P. fluorescens NRRL B-1244	11	20
Brevibacterium sp. strain CRL56	18	20
2-Propanol grown		
P. fluorescens NRRL B-1244	15	28
Brevibacterium sp. strain CRL56	14	25

major pathway for utilization of propane in *M. vaccae* JOB5. However, further studies by these same researchers suggest that some terminal oxidation of propane does occur in *M. vaccae* (27). In addition, *M. vaccae* can utilize *n*-butane as substrate, but apparently via terminal oxidation to butyric acid (24).

In this report, we describe the oxidation of 2-alcohols to their homologous methyl ketones by resting-cell suspensions of various C_2 to C_4 gaseous alkane-grown bacteria. The taxonomic characteristics of the newly isolated C_2 to C_4 alkane-utilizing microbes will be published elsewhere.

The effect of temperature on the production of methyl ketones is interesting. The optimum temperature for the production of acetone or 2-butanone from propane or *n*-butane was around 35 to 40°C for propane-grown cells of *Brevibacterium* sp. strain CRL56, *N. paraffinica* ATCC 21198, and *P. fluorescens* NRRL B-1244. However, the optimum temperature for the production of methyl ketones from 2-propanol or 2-butanol was around 60°C. The former reaction involves two enzymes, alkane monooxygenase and alcohol dehydrogenase. The latter case involves only alcohol dehydrogenase. These data suggest that 35 to 40°C is the optimum temperature for the production of methyl ketones from *n*-alkanes for the alkane monooxygenase reaction and that the alcohol dehydrogenase is a relatively thermally stable enzyme. We have indeed identified and purified a thermally stable secondary alcohol dehydrogenase from these microbes (10a). The optimum pH for the production of acetone or 2-butanone from their homologous 2-alcohols was 8.0 to 9.0, higher than that obtained for the hydroxylation of *n*-alkanes (pH 7.5). Similar phenomena were observed in cells of methane-grown methylotrophic bacteria (15). In inhibitor studies, metal-binding agents inhibited the methyl ketone formation from *n*-alkanes and from secondary alcohols, suggesting the possible involvement of metal(s) in both reactions. The purified secondary alcohol dehydrogenase mentioned above was also inhibited by these compounds. However, potassium cyanide and thiourea inhibited only the ketone formation from *n*-alkane and not ketone formation from secondary alcohol, suggesting the metal complex(es) involved in the oxidation of secondary alcohol is not a heme type. In methylotrophic microorganisms, secondary alcohols were oxidized to methyl ketones by a zinc-containing secondary alcohol-specific alcohol dehydrogenase (9, 11, 14, 21, 22).

Methane-grown cells of methylotrophs oxidize gaseous *n*-alkanes but not liquid *n*-alkanes (15, 23), whereas liquid *n*-alkane (*n*-octane)-grown cells oxidize liquid *n*-alkanes but not gaseous *n*-alkanes (1, 5). In propane-grown cells, propane and *n*-butane were oxidized at higher rates in comparison with other *n*-alkane substrates. Methane was not oxidized. Liquid *n*-alkanes had much lower rates of oxidation (Table 2). Ethane- and butane-grown cells also oxidized 2-alcohols to their homologous methyl ketones. The methyl ketone production rates with cells of both *Brevibacterium fuscum* ATCC 15993 and *N. paraffinica* ATCC 21198 grown on propane were found to be higher than those obtained with either ethane- or butane-grown cells (Table 1). It is not clear at this point whether this is due to differences in metabolic pathways operational during the utilization of odd-numbered or even-numbered alkanes.

Studies with the cell-free system provide direct evidence that *n*-alkanes were oxidized at both terminal and subterminal carbons. Both primary and secondary alcohols were produced at a similar rate (Table 4). Both alcohol dehydrogenase activities were found in cells grown on either 1-propanol or 2-propanol, indicating that these enzymes are constitutive (Table 5).

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