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Conversion of Mevalonic Acid to γ,γ-Dimethylallyl Pyrophosphate by *Mycoplasma*

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ABSTRACT

HENRIKSON, CARL V. (University of South Dakota, Vermillion), AND PAUL F. SMITH. Conversion of mevalonic acid to γ,γ-dimethylallyl pyrophosphate by *Mycoplasma*. J. Bacteriol. **92**:701–706. 1966.—Three representative strains of *Mycoplasma*, *M. laidlawii* strain B, *Mycoplasma* sp. avian strain J, and *M. hominis* type 2 strain O7, were examined for the presence or absence of enzymes associated with the biosynthetic pathway from mevalonic acid to γ,γ-dimethylallyl pyrophosphate. *M. laidlawii* served as a control organism, since it is capable of de novo biosynthesis of carotenoids. All four enzymes, namely, adenosine triphosphate (ATP)-mevalonate 5-phosphotransferase (EC 2.7.1.36), ATP-5-phosphomevalonate phosphotransferase (EC 2.7.4.2), ATP-5-pyrophosphomevalonate carboxy-lyase (EC 4.1.1.33), and isopentenylpyrophosphate Δ^3,Δ^2-isomerase (EC 5.3.3.2), were demonstrated in this organism. *Mycoplasma* sp. avian strain J, which contains all enzymes necessary for the biosynthesis of mevalonic acid, lacks the first three of the above enzymes but contains isopentenyl pyrophosphate Δ^3,Δ^2-isomerase. *M. hominis*, which lacks the enzymes necessary for the biosynthesis of mevalonic acid, also is deficient in the enzymes involved in its conversion to γ,γ-dimethylallyl pyrophosphate.

The *Mycoplasma* behave as two nutritional types on the basis of lipid requirements. *Mycoplasma* sp. avian strain J and *M. hominis* type 2 strain O7 require sterols for growth, whereas *M. laidlawii* strain B synthesizes carotenols (10). Carotenols appear to have a functional role similar to that of sterols, as evidenced by the sparing action of cholesterol on carotenoid synthesis (9) and the capacity of carotenoid intermediates or exogenous carotenols to substitute for sterol. Further evidence for analogous functions of sterol and carotenol in *Mycoplasma* is the reversal by cholesterol of growth inhibition due to inhibition of carotenoid synthesis (12). Biosynthesis of mevalonic acid in *Mycoplasma* proceeds by the condensation of 1 mole of acetyl coenzyme A (CoA) with 1 mole of acetoacetyl CoA to form β-hydroxyl-β-methylglutaryl CoA (HMG-CoA), followed by reduction by a pyridine nucleotide-linked reductase to form mevalonic acid (11). All the organisms are capable of acetate activation and acetoacetyl CoA formation (11, 15). *M. laidlawii* and *Mycoplasma* sp. strain J are capable of incorporation of acetate-2-C^{14} into mevalonic acid (11). No acetate-2-C^{14} incorporation occurs with *M. hominis*, owing to the lack of 3-hydroxy-3-methylglutaryl CoA acetoacetyl CoA-lyase (EC 4.1.3.5) and mevalonate-nicotinamide adenine dinucleotide phosphate-(NADP) oxidoreductase (EC 1.1.1.34), thus explaining its growth requirement for sterol (11). Nutritional experiments showed that none of the biosynthetic precursors of polyterpenes supported growth of *M. hominis*. Isopentenyl pyrophosphate partially replaced the sterol growth requirement of *Mycoplasma* sp. strain J, indicating an enzymatic block between mevalonic acid and isopentenyl pyrophosphate (Henrickson and Smith, J. Gen. Microbiol., *in press*).

The present study was undertaken to examine these representative strains of *Mycoplasma* for enzymes in the biosynthetic pathway from mevalonic acid to γ,γ-dimethylallyl pyrophosphate.

MATERIALS AND METHODS

Cultures. Three representative strains were examined, namely, *M. laidlawii* strain B, *Mycoplasma* sp. avian strain J, and *M. hominis* type 2 strain O7. *M. laidlawii* was grown in a medium of the following composition: 2% tryptose (Difco), 0.5% sodium chloride, 0.5% sodium acetate, and 0.5% glucose; the *p*H was 7.8. Sterol-requiring strains were grown in the medium described by Morton, Smith, and Leberman (7) supplemented with 1% PPLO Serum Fraction (Difco). The organisms were grown and harvested as reported previously (8). Thallium acetate

(1:4,000 w/v) was used as a selective inhibitor of bacterial growth.

Enzymatic methods. The organisms suspended in 0.2 M potassium phosphate buffer (*p*H 7.5) were disrupted in a 10-kc Raytheon sonic oscillator for 15 min. The protein concentration of the disrupted cells was determined by the trichloroacetic acid method of Stadtman, Novelli, and Lipmann (13), with bovine serum albumin as a standard.

Adenosine triphosphate (ATP)-mevalonate 5-phosphotransferase (EC 2.7.1.36) activity was determined by a modification of the procedure of Witting and Porter (16). Iodoacetamide (0.005 M) was employed as a selective inhibitor for isopentenylpyrophosphate Δ^3, Δ^2-isomerase (EC 5.3.3.2) to permit accumulation of the phosphorylated mevalonic acids (6). Controls containing heat-inactivated enzyme protein (10 min at 100 C) were employed with each experiment. At the termination of incubation, the enzyme proteins were inactivated by heating at 70 C for 3 to 5 min, coagulated protein was removed by centrifugation, and supernatant solutions were extracted four times with ethyl alcohol-ether (1:1, v/v). After solvent removal under a stream of nitrogen, the aqueous solutions were concentrated by lyophilization. Chromatographic separation by the ascending method was achieved on Whatman no. 1 filter paper with *t*-butanol–formic acid–water (40:10:16, v/v) as developing solvent (5). The dried chromatograms were cut into 0.5-cm strips and counted in a Packard TriCarb liquid scintillation counter with 2,5-diphenyloxazole (PPO) and 1,4-bis[2-(5-phenyloxazolyl)] benzene (POPOP) as scintillators.

Detection of ATP-5-phosphomevalonate phosphotransferase (EC 2.7.4.2) activity was carried out in an identical manner except for use of mevalonate-5′-phosphate (15 μmoles), doubling of enzyme proteins, and the addition of 10 μc of ATP^{32} labeled in the terminal position.

Both mevalonic acid-*1*-C^{14} and mevalonic acid-*2*-C^{14} served as substrates for determination of decarboxylation of mevalonic-5′-pyrophosphate. The reaction mixtures were incubated in screw-cap tubes to eliminate loss of carbon dioxide. After heat inactivation (70 C for 5 min), one-half of each reaction mixture was extracted and treated as described above. The remainder of the reaction mixtures, after acidification (*p*H 4), were bubbled with nitrogen for 30 min in a closed system, trapping the evolving carbon dioxide in saturated barium hydroxide. A small quantity of barium carbonate was added as a carrier, and the precipitates were washed, weighed, and counted in a Tracerlab automatic proportional counter. Counts were corrected for self-absorption by extrapolation to infinite thinness.

Determinations of ATP-5-pyrophosphomevalonate carboxylyase (EC 4.1.1.33) activities with either mevalonic-5′-pyrophosphate-*1*-C^{14} or mevalonic-5′-pyrophosphate-*2*-C^{14} as substrates were carried out in a similar way.

Isopentenylpyrophosphate Δ^3, Δ^2-isomerase was assayed by measuring the disappearance of substrate, i.e., isopentenyl pyrophosphate, according to a modification of the method described by Agranoff et al. (1). Samples taken at various time intervals were treated with 0.5 ml of trichloroacetic acid, which inactivated the enzyme and hydrolyzed γ,γ-dimethylallyl pyrophosphate. After removal of γ,γ-dimethylallyl alcohol by extraction with diethyl ether (three times), samples of the aqueous layer which still contained residual isopentenyl pyrophosphate were transferred to planchets and counted in a Tracerlab proportional counter. Isopentenylpyrophosphate Δ^3,Δ^2-isomerase also was assayed by measuring the disappearance of isopentenyl pyrophosphate by gas chromatography. After inactivation (70 C for 3 to 5 min), the residual isopentenyl pyrophosphate-*1*-C^{14} was extracted with ethyl alcohol-ether (1:1, v/v), the solvents were evaporated under a stream of nitrogen, and the aqueous solutions were concentrated by lyophilization. The phosphorylated compounds were converted to their alcoholic forms enzymatically by use of orthophosphoric monoester phosphohydrolase (EC 3.1.3.1) in a reaction mixture of (3): 0.02 M magnesium acetate; 0.2 ml of 0.04 M tris(hydroxymethyl)aminomethane-maleate buffer (Tris-maleate buffer), *p*H 10; and 8 mg of orthophosphoric monoester phosphohydrolase in a total volume of 1.5 ml. After the reaction mixtures were incubated for 30 min at 37 C, the enzyme protein was heat-inactivated (70 C for 3 to 5 min). The supernatant solutions were extracted four times with 1-ml quantities of diethyl ether, and ether extracts were dried under a stream of nitrogen. The residues were dissolved in 0.1 ml of tetrahydrofuran and 0.05 ml was subjected to gas-liquid chromatography in a Beckman model GC 2A instrument equipped with a Thermotrac temperature programmer, a thermistor detector, and matched 6-ft (1.8-meter) columns of 20% diethylene glycol succinate on Chromosorb W 42/60 (Beckman no. 70402) under the following conditions: column temperature, 50 C; gas, helium; flow rate, 85 ml/min; current, 300 ma; sensitivity, 50; chart speed, 0.5 inch/min. Concentrations of isopentenol were determined by measurement of areas under the peaks by planimetry relative to areas under peaks of known control amounts of isopentenol.

Materials. The following compounds were synthesized in our laboratory from mevalonic acid-*1*-C^{14} and mevalonic acid-*2*-C^{14} with enzymes isolated from dried yeast as described by Bloch et al. (3): mevalonic-5′-phosphate-*1*-C^{14}, mevalonic-5′-phosphate-*2*-C^{14}, mevalonic-5′-pyrophosphate-*1*-C^{14}, mevalonic-5′-pyrophosphate-*2*-C^{14}, and isopentenyl pyrophosphate-*1*-C^{14}. The yeast ATP-mevalonate 5-phosphotransferase reaction was carried out by the method of Tchen (14). All reagents were increased 10-fold, and 4 mg of enzyme protein was used per test. The reaction mixture was incubated at 30 C for 40 min in a total volume of 1.0 ml. The yeast ATP-5-phosphotransferase reaction was performed by the method of Bloch et al. (3). The reaction mixture contained the following components: mevalonic acid (carrier), 5 μmoles; mevalonic acid-*1*-C^{14} or mevalonic acid-*2*-C^{14}, 2 μmoles containing 0.5 μc/μmole; manganese chloride, 5 μmoles; phosphate buffer, 50 μmoles (*p*H 6.7); ATP, 10 μmoles; 0.03 M

potassium fluoride; ATP-mevalonate 5-phosphotransferase, 4 mg; ATP-5-phosphomevalonate phosphotransferase, 4 mg. The reaction mixture was incubated at 30 C for 40 min in a total volume of 1.0 ml. The ATP-5-pyrophosphomevalonate carboxy-lyase reaction also was conducted by the method of Bloch et al. (3). A control consisting of heat-inactivated enzyme protein was employed for each of the reactions. All reactions were stopped by heating at 70 C for 3 to 5 min. The phosphorylated intermediates were extracted with ethyl alcohol-ether (1:1, v/v). The solvents were removed under a stream of nitrogen, and the aqueous solutions were concentrated by lyophilization. Samples were chromatographed as described by Chaykin et al. (5), and detection of reaction products was accomplished by counting 0.5-cm strips in a Packard TriCarb liquid scintillation counter. For large quantities of substrate, the contents were increased 10-fold.

Adenine triphosphate labeled with P^{32} in the terminal phosphate position was obtained from Schwarz Bio Research, Inc., Orangeburg, N.Y. Mevalonic acid-*1*-C^{14} and mevalonic acid-*2*-C^{14} were obtained as the lactones from New England Nuclear Corp., Boston, Mass. After the benzene was evaporated, the residual lactones were hydrolyzed with potassium hydroxide, followed by adjustment of *p*H to 8.0 by the addition of acid (2).

DL-Mevalonic acid, DL-mevalonic-5'-phosphate (Tris cycloammonium salts), isopentenyl pyrophosphate (Tris cycloammonium salt), and DL-mevalonic acid-5'-pyrophosphate (dibrucine salt·$6H_2O$) were obtained from Mann Research Laboratories, Inc., New York, N.Y. Orthophosphoric monoester phosphohydrolase was obtained from Worthington Biochemical Corp., Freehold, N.J. Adenine-5'-triphosphate (disodium salt; ATP) was purchased from the Sigma Chemical Co., St. Louis, Mo. All other chemicals were commercial preparations of reagent grade.

RESULTS AND DISCUSSION

The biosynthetic pathway of terpenoids in *Mycoplasma* is believed to be identical to that of yeast. Mevalonic acid is converted to mevalonic-5'-phosphate by ATP-mevalonate 5-phosphotransferase. ATP-5-phosphomevalonate phosphotransferase catalyzes the next reaction in which mevalonic-5'-phosphate is phosphorylated to form mevalonic-5'-pyrophosphate. Mevalonic-5'-pyrophosphate is decarboxylated by ATP-5-pyrophosphomevalonate carboxy-lyase to form isopentenyl pyrophosphate. Isopentenyl pyrophosphate is converted to γ,γ-dimethylallyl pyrophosphate by isopentenylpyrophosphate Δ^3,Δ^2-isomerase.

ATP-mevalonate 5-phosphotransferase. Chromatographic data presented in Table 1 demonstrate the conversion of mevalonic acid-*2*-C^{14} to isopentenyl pyrophosphate-*1*-C^{14} by *M. laidlawii*. Hence, it would appear that ATP-mevalonate 5-phosphotransferase, ATP-5-phosphomevalonate phosphotransferase, and ATP-5-pyrophosphomevalonate carboxy-lyase are present in this organism. *Mycoplasma* sp. strain J and *M. hominis* exhibited no ATP-mevalonate 5-phosphotransferase activity, as evidenced by the nonappearance of any phosphorylated intermediates. In the presence of 0.005 M iodoacetamide, which is a specific inhibitor of isopentenylpyrophosphate Δ^3,Δ^2-isomerase (6), an accumulation of mevalonic-5'-phosphate and mevalonic-5'-pyrophosphate was noted in the *M. laidlawii* system (Table 2). No phosphorylated mevalonic acids accumulated with *Mycoplasma* sp. strain J or *M. hominis*.

ATP-5-phosphomevalonate phosphotransferase. Mevalonic-5'-pyrophosphate (R_F 0.35) and isopentenyl pyrophosphate (R_F 0.61) were detected only in *M. laidlawii* when cell-free extracts were incubated with mevalonic-5'-phosphate and ATP^{32}. The radioactive (P^{32}) spots on the chromatograms corresponded with the phosphate spots detected with a modified Hanes-Isherwood reagent developed under ultraviolet irradiation (4).

ATP-5-pyrophosphomevalonate carboxy-lyase. The formation of isopentenyl pyrophosphate from mevalonic acid results in the removal of carbon atom 1 of mevalonic acid by decarboxylation. Hence, mevalonic acid-*1*-C^{14} should give rise to carbon dioxide-C^{14}, whereas the mevalonic acid-*2*-C^{14} would produce unlabeled carbon dioxide. ATP-5-pyrophosphomevalonate carboxy-lyase activity was demonstrated only in *M. laidlawii* by employing mevalonic acid-*1*-C^{14} as the substrate, based upon liberation of labeled carbon dioxide. No activity was detected in *Mycoplasma* sp. strain J or *M. hominis*. When mevalonic acid-*2*-C^{14} was employed as a substrate, no label appeared in the trapped carbon dioxide (Table 3). Since the absence of ATP-mevalonate 5-phosphotransferase and ATP-phosphomevalonate phosphotransferase in *Mycoplasma* sp. strain J and *M. hominis* prevents the formation of mevalonic-5'-pyrophosphate-*1*-C^{14}, further experiments were performed with mevalonic-5'-pyrophosphate-*1*-C^{14} as the reaction substrate. With mevalonic-5'-pyrophosphate-*1*-C^{14} as the substrate, ATP-5-pyrophosphomevalonate carboxy-lyase activity was demonstrated in *M. laidlawii* by the liberation of labeled carbon dioxide. No labeled carbon dioxide was demonstrated with *Mycoplasma* sp. strain J and *M. hominis*. The use of mevalonic-5'-pyrophosphate-*2*-C^{14} as substrate failed to produce labeled carbon dioxide, as expected (Table 4).

Isopentenylpyrophosphate Δ^3,Δ^2-isomerase. Isopentenylpyrophosphate Δ^3,Δ^2-isomerase was measured as the rate of disappearance of isopentenyl pyrophosphate-*1*-C^{14}, as illustrated in

TABLE 1. *Incorporation of mevalonic acid-2-C^{14} into isopentenyl pyrophosphate by Mycoplasma*[a]

Compound	R_F values			
	Known	*M. laidlawii* strain B	*Mycoplasma* sp. strain J	*M. hominis* strain O7
Mevalonic acid	0.80	—	0.79–0.83	0.79–0.81
Mevalonic-5′-phosphate	0.66–0.68	—	—	—
Mevalonic-5′-pyrophosphate	0.37–0.40	—	—	—
Isopentenyl pyrophosphate	0.74	0.74	—	—

[a] Reaction conditions: each tube contained 20 μmoles of adenosine triphosphate, 30 μmoles of manganese chloride, 30 μmoles of glutathione, 500 μmoles of phosphate buffer (*p*H 7.5), 8 μmoles of mevalonic acid, 2 μmoles of mevalonic acid-2-C^{14} (0.5 μc/μmole), and enzyme protein from 2.5 liters of sonically treated cells (approximately 20 mg). Reactions were carried out at 37 C for 3 hr in a total volume of 5.0 ml.

TABLE 2. *Incorporation of mevalonic acid-2-C^{14} into mevalonic-5′-phosphate, mevalonic-5′-pyrophosphate, and isopentenyl pyrophosphate by Mycoplasma in the presence of iodoacetamide*[a]

Compound	R_F values			
	Known	*M. laidlawii* strain B	*Mycoplasma* sp. strain J	*M. hominis* strain O7
Mevalonic acid	0.80	—	0.77–0.83	0.80–0.81
Mevalonic-5′-phosphate	0.66–0.68	0.67	—	—
Mevalonic-5′-pyrophosphate	0.37–0.40	0.54	—	—
Isopentenyl pyrophosphate	0.74	0.75	—	—

[a] Reactions conditions: same as Table 1 except for the addition of 0.005 M iodoacetamide.

TABLE 3. *Liberation of $C^{14}O_2$ from mevalonic acid-1-C^{14} and -2-C^{14} by Mycoplasma*[a]

Organism	Substrate			
	Mevalonic acid-2-C^{14}		Mevalonic acid-1-C^{14}	
	Control	Test	Control	Test
	count/min	*count/min*	*count/min*	*count/min*
M. laidlawii strain B	1	0	7	108
Mycoplasma sp. strain J	0	0	3	3
M. hominis strain O7	0	0	3	3

[a] Reaction conditions: 20 μmoles of adenosine triphosphate, 30 μmoles of manganese chloride, 30 μmoles of magnesium chloride, 30 μmoles of glutathione, 500 μmoles of phosphate buffer (*p*H 7.5), 10 μmoles of mevalonic acid-2-C^{14} (0.5 μc/μmole) or 10 μmoles of mevalonic acid-1-C^{14} (0.5 μc/μmole), and enzyme protein from 2.5 liters of sonically treated cells (approximately 20 mg). Reaction was carried out at 37 C for 3 hr in a total volume of 5.0 ml.

TABLE 4. *Liberation of $C^{14}O_2$ from mevalonic-5′-pyrophosphate-1-C^{14} and -2-C^{14} by Mycoplasma*[a]

Organism	Substrate			
	Mevalonic-5′-pyrophosphate-2-C^{14}		Mevalonic-5′-pyrophosphate-2-C^{14}	
	Control	Test	Control	Test
	count/min	*count/min*	*count/min*	*count/min*
M. laidlawii strain B	0	33	77	6,338
Mycoplasma sp. strain J	4	0	0	0
M. hominis strain O7	0	0	0	0

[a] Reaction conditions: each tube contained 20 μmoles of adenosine triphosphate, 30 μmoles of manganese chloride, 30 μmoles of magnesium chloride, 30 μmoles of glutathione, 500 μmoles of phosphate buffer (*p*H 7.5), 0.03 M potassium fluoride, mevalonic-5′-pyrophosphate-1-C^{14} synthesized from 20 μmoles of mevalonic acid-1-C^{14} (0.5 μc/μmole) or mevalonic-5′-pyrophosphate-2-C^{14} synthesized from 20 μmoles of mevalonic acid-2-C^{14} (0.5 μc/μmole), and 30 mg of enzyme protein.

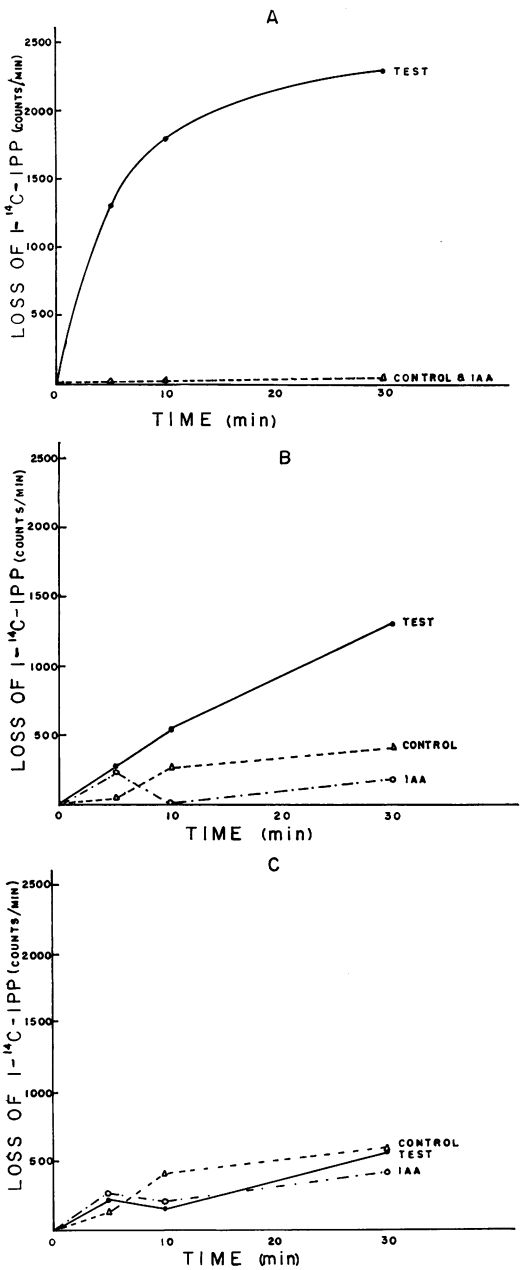

FIG. 1. *Isopentenyl pyrophosphate isomerase activity of Mycoplasma. (A) M. laidlawii strain B. (B) Mycoplasma sp. strain J. (C) M. hominis strain O7. Reaction mixtures: each tube contained 15 μmoles of glutathione, 2 μmoles of magnesium chloride, 2 μmoles of Tris buffer (pH 8.0), isopentenyl pyrophosphate-1-C^{14} (IPP) synthesized with yeast enzymes from 20 μmoles of mevalonic acid-2-C^{14} (0.5 μc/μmole); 0.03 M potassium fluoride, and 15 mg of enzyme protein. The tubes containing 0.005 M iodoacetamide (IAA) lacked glutathione. Control tubes contained heat-inactivated enzyme proteins. The reaction was carried out at 37 C in a total volume of 2.0 ml with samples being removed at zero-time and at 5, 10, and 30 min for assay purposes.*

Fig. 1. No disappearance was expected in the tubes which contained iodoacetamide, which is a specific inhibitor of isopentenylpyrophosphate Δ^3,Δ^2-isomerase. Figures 1a and 1b demonstrate that isopentenylpyrophosphate Δ^3,Δ^2-isomerase activity was present in *M. laidlawii* and *Mycoplasma* sp. strain J. No activity was demonstrated in *M. hominis* (Fig. 1c). By employing similar reaction mixtures, confirmation of the presence of isopentenylpyrophosphate Δ^3,Δ^2-isomerase was demonstrated in *M. laidlawii* and *Mycoplasma* sp. strain J by measurement of the disappearance of isopentenyl pyrophosphate gas chromatographically by quantitating residual isopentenol (Table 5). No γ,γ-dimethylallyl alcohol would be expected to accumulate, since the succeeding steps in the biosynthetic pathway were not inhibited. The lack of inhibition by iodoacetamide in this experiment can be attributed to the lower concentration (0.0025 M versus 0.005 M) of inhibitor and the higher concentration of enzyme proteins (20 mg versus 15 mg). Even so, some inhibition is noted with *Mycoplasma* sp. strain J.

These results indicate that all four enzymes required to transform mevalonic acid to γ,γ-dimethylallyl pyrophosphate are contained in *M. laidlawii* but are absent from *M. hominis*. Although *Mycoplasma* sp. strain J is capable of synthesis of mevalonic acid (11), it is deficient in the three enzymes involved in the formation of isopentenyl pyrophosphate: ATP-mevalonate 5-phosphotransferase, ATP-5-phosphomevalonate phosphotransferase, and ATP-5-pyrophosphomevalonate carboxy-lyase. However, this organism appears to possess isopentenylpyrophosphate Δ^3,Δ^2-isomerase. This evidence is compatible with the results of nutritional experiments in which growth was not supported by mevalonic acid, mevalonic-5'-phosphate, or mevalonic-5'-pyrophosphate when these compounds were substituted for the sterol requirement of *M. hominis* and *Mycoplasma* sp. strain J (Henrikson and Smith, *in press*). The presence of isopentenylpyrophosphate Δ^3,Δ^2-isomerase in *Mycoplasma* sp. strain J explains the significant growth response when isopentenyl pyrophosphate was substituted for sterol. The ability of isopentenyl pyrophosphate to support growth of this organism in lieu of sterol indicates that all other enzymes of the carotenoid biosynthetic pathway probably are present. This possibility is currently under investigation. On the other hand, the absence of isopentenylpyrophosphate Δ^3,Δ^2-isomerase in *M. hominis* explains the inability of

TABLE 5. *Isopentenyl pyrophosphate isomerase activity of Mycoplasma*[a]

Organism	Isopentenyl pyrophosphate-1-C^{14} utilized		
	Control	Test	Test plus iodoacetamide
	μmoles	*μmoles*	*μmoles*
M. laidlawii strain B	0	13.7	13.2
Mycoplasma sp. strain J	0	14.1	11 8
M. hominis strain O7	0	0	0

[a] Reaction conditions: each tube contained the same components as described in Fig. 1 except for use of 20 mg of enzyme protein and 0.0025 M iodoacetamide. The reaction mixtures were heat-inactivated (70 C for 3 to 5 min) after incubation at 37 C for 30 min.

isopentenyl pyrophosphate to support growth when substituted for sterol.

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