Conversion of Mevalonic Acid to $\gamma$, $\gamma$-Dimethylallyl Pyrophosphate by Mycoplasma

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Received for publication 2 May 1966

ABSTRACT

HENRIKSON, CARL V. (University of South Dakota, Vermillion), AND PAUL F. SMITH. Conversion of mevalonic acid to $\gamma$, $\gamma$-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 07, were examined for the presence or absence of enzymes associated with the biosynthetic pathway from mevalonic acid to $\gamma$, $\gamma$-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-phosphomevalonate carboxy-lyase (EC 4.1.1.33), and isopentenylpyrophosphate $\Delta^4,\Delta^2$-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 $\Delta^4,\Delta^2$-isomerase. M. hominis, which lacks the enzymes necessary for the biosynthesis of mevalonic acid, is also deficient in the enzymes involved in its conversion to $\gamma,\gamma$-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 07 require sterols for growth, whereas M. laidlawii strain B synthesizes carotenoids (10). Carotenoids 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 carotenoids 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 $\beta$-hydroxyl-$\beta$-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-2-14C into mevalonic acid (11). No acetate-2-14C 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 $\gamma,\gamma$-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 07. 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 pH was 7.8. Sterol-requiring strains were grown in the medium described by Morton, Smith, and Leberman (7) supplemented with 1% PPL0 Serum Fraction (Difco). The organisms were grown and harvested as reported previously (8). Thallium acetate
Enzymatic methods. The organisms suspended in 0.2 M potassium phosphate buffer (pH 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 method of Witting and Porter (16). Iodoacetamide (0.005 M) was employed as a selective inhibitor for isopentenylpyrophosphate Δ5, Δ5-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 i-butanol-formic acid-water (40:10:16, v/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 determined in an identical manner except for use of mevalonate-5'-phosphate (15 μmoles), doubling of enzyme proteins, and the addition of 10 μC of ATP32 labeled in the terminal position.

Both mevalonic acid-1-C14 and mevalonic acid-2-C14 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 (pH 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-phosphomevalonate carboxyhydrolase (EC 4.1.1.5) activity were determined with either mevalonate-5'-pyrophosphate-1-C14 or mevalonate-5'-pyrophosphate-2-C14 as substrates carried out in a similar way.

Isopentenylpyrophosphate Δ5, Δ5-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 Δ5,Δ5-isomerase also was assayed by measuring the disappearance of isopentenylpyrophosphate by gas chromatography. After inactivation (70 C for 3 to 5 min), the residual isopentenyl pyrophosphate-l-C14 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 alchoholic 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), pH 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) glass columns. Gas carriers were helium-1 atm P/C and 0.5 ml/min or argon-1 atm P/C and 0.1 atm N2; flow rate, 85 ml/min; column, 300 mm; 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-C14 and mevalonic acid-2-C14 with enzymes isolated from dried yeast as described by Bloch et al. (3): mevalonic-5'-phosphate-l-C14, mevalonic-5'-phosphate-2-C14, mevalonic-5'-pyrophosphate-l-C14, mevalonic-5'-pyrophosphate-2-C14, and isopentenyl pyrophosphate-l-C14. 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-l-C14 or mevalonic acid-2-C14, 2 μmoles containing 0.5 μC/μmole; manganese chloride, 5 μmoles; phosphate buffer, 50 μmoles (pH 6.7); ATP, 10 μmoles; 0.03 M
potassium fluoride; ATP-mevalonate 5-phospho-
transferase; 4 mg; ATP-5-phosphomevalonate phos-
photransferase; 4 mg. The reaction mixture was incu-
bated at 30 C for 40 min in a total volume of 1.0 ml. 
The ATP-5-phosphomevalonate carboxy-lyase reac-
tion 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 reac-
tions. 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 con-
tents were increased 10-fold.

Adenine triphosphate labeled with \( ^{32} \)P in the
terminal phosphate position was obtained from
Schwarz Bio Research, Inc., Orangeburg, N.Y. Mev-
alonic 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 evap-
orated, the residual lactones were hydrolyzed with
potassium hydroxide, followed by adjustment of pH
8.0 by the addition of acid (2).

DL-Mevalonic acid, DL-mevalonic-5'-phosphate
(Tris cycloammonium salts), isopentenyl pyrophos-
phate (Tris cycloammonium salt), and DL-mevalonic
acid-5'-pyrophosphate (dibrucine salt-6H\(_2\)O) were
obtained from Mann Research Laboratories, Inc.,
New York, N.Y. Orthophosphoric monoester phos-
phohydrolase was obtained from Worthington Bio-
chemical Corp., Freehold, N.J. Adenine-5'-triphos-
phate (disodium salt; ATP) was purchased from the
Sigma Chemical Co., St. Louis, Mo. All other chemi-
cals 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-phospho-
transferase. ATP-5-phosphomevalonate phos-
photransferase catalyzes the next reaction in
which mevalonic-5'-phosphate is phosphorylated
to form mevalonic-5'-pyrophosphate. Mevalonic-
5'-pyrophosphate is decarboxylated by ATP-5-
phosphomevalonate carboxy-lyase to form
isopentenyl pyrophosphate. Isopentenyl pyro-
phosphate is converted to \( \gamma, \gamma \)-dimethylallyl pyrophosphate by isopentenylpyrophosphate \( \Delta^2, \Delta^2 \)-
isoformase.

ATP-mevalonate 5-phosphotransferase. Chromo-
tographic 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-pyrophospho-
mevalonate carboxy-lyase are present in this or-
ganism. Mycoplasma sp. strain \( J \) and \( M. \) hominis
exhibited no ATP-mevalonate 5-phosphotransfer-
ase activity, as evidenced by the nonappearance of
any phosphorylated intermediates. In the presence
of 0.005 M iodoacetamide, which is a specific inhibi-
tor of isopentenylpyrophosphate \( \Delta^2, \Delta^2 \)-isoformase
(6), an accumulation of mevalonic-5'-phosphate
and mevalonic-5'-pyrophosphate was noted in the
\( M. \) laidlawii system (Table 2). No phospho-
rylated mevalonic acids accumulated with Myco-
plasma sp. strain \( J \) or \( M. \) hominis.

ATP-5-phosphomevalonate carboxy-lyase.
Mevalonic-5'-pyrophosphate (\( R_p 0.35 \)) and iso-
pentenyl pyrophosphate (\( R_p 0.61 \)) were detected
only in \( M. \) laidlawii when cell-free extracts were
incubated with mevalonic-5'-phosphate and ATP\(^{32} \). The radioactive (\( ^{32} \)P) spots on the chro-
matograms 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 car-
boxy-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 Myco-
plasma sp. strain \( J \) or \( M. \) hominis. When meva-
lonic 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-phosphomeva-
lonate phosphotransferase in Mycoplasma sp.
strain \( J \) and \( M. \) hominis prevents the formation of
mevalonic-5'-pyrophosphate-1-C\(^{14}\), further exper-
iments were performed with mevalonic-5'-pyro-
phosphate-1-C\(^{14}\) as the reaction substrate. With
mevalonic-5'-pyrophosphate-1-C\(^{14}\) as the sub-
strate, ATP-5-pyrophosphomevalonate carboxy-
lyase activity was demonstrated in \( M. \) laidlawii with
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 sub-
strate failed to produce labeled carbon dioxide,
as expected (Table 4).

Isopentenylpyrophosphate \( \Delta^2, \Delta^2 \)-isoformase. Iso-
pentenylpyrophosphate \( \Delta^2, \Delta^2 \)-isoformase was
measured as the rate of disappearance of iso-
pentenyl pyrophosphate-1-C\(^{14}\), as illustrated in
TABLE 1. Incorporation of mevalonic acid-2-C\textsuperscript{14} into isopentenyl pyrophosphate by Mycoplasma\textsuperscript{a}

<table>
<thead>
<tr>
<th>Compounds</th>
<th>R\textsubscript{P} values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Known</td>
</tr>
<tr>
<td>Mevalonic acid</td>
<td>0.80</td>
</tr>
<tr>
<td>Mevalonic-5'-phosphate</td>
<td>0.66-0.68</td>
</tr>
<tr>
<td>Mevalonic-5'-pyrophosphate</td>
<td>0.37-0.40</td>
</tr>
<tr>
<td>Isopentenyl pyrophosphate</td>
<td>0.74</td>
</tr>
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</table>

\textsuperscript{a} Reaction conditions: each tube contained 20 \textmu moles of adenosine triphosphate, 30 \textmu moles of manganese chloride, 30 \textmu moles of glutathione, 500 \textmu moles of phosphate buffer (pH 7.5), 8 \textmu moles of mevalonic acid, 2 \textmu moles of mevalonic acid-2-C\textsuperscript{14} (0.5 \textmu C/\textmu mole), 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\textsuperscript{14} into mevalonic-5'-phosphate, mevalonic-5'-pyrophosphate, and isopentenyl pyrophosphate by Mycoplasma in the presence of iodoacetamide\textsuperscript{a}

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<td>0.74</td>
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\textsuperscript{a} Reaction conditions: same as Table 1 except for the addition of 0.005 M iodoacetamide.

TABLE 3. Liberation of C\textsuperscript{14}O\textsubscript{2} from mevalonic acid-1-C\textsuperscript{14} and -2-C\textsuperscript{14} by Mycoplasma\textsuperscript{a}

<table>
<thead>
<tr>
<th>Organism</th>
<th>Substrate</th>
<th>Mevalonic acid-2-C\textsuperscript{14}</th>
<th>Mevalonic acid-1-C\textsuperscript{14}</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Test</td>
<td>Control</td>
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<tr>
<td></td>
<td>count/ min</td>
<td>count/ min</td>
<td>count/ min</td>
</tr>
<tr>
<td>M. laidlaw ii strain B</td>
<td>1</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Mycoplasma sp. strain J</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>M. hominis strain 07</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Reaction conditions: 20 \textmu moles of adenosine triphosphate, 30 \textmu moles of manganese chloride, 30 \textmu moles of glutathione, 500 \textmu moles of phosphate buffer (pH 7.5), 10 \textmu moles of mevalonic acid-2-C\textsuperscript{14} (0.5 \textmu C/\textmu mole) or 10 \textmu moles of mevalonic acid-1-C\textsuperscript{14} (0.5 \textmu C/\textmu mole), 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\textsuperscript{14}O\textsubscript{2} from mevalonic-5'-pyrophosphate-1-C\textsuperscript{14} and -2-C\textsuperscript{14} by Mycoplasma\textsuperscript{a}

<table>
<thead>
<tr>
<th>Organism</th>
<th>Substrate</th>
<th>Mevalonic-5'-pyrophosphate-2-C\textsuperscript{14}</th>
<th>Mevalonic-5'-pyrophosphate-1-C\textsuperscript{14}</th>
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<tbody>
<tr>
<td></td>
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<td>Test</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>count/ min</td>
<td>count/ min</td>
<td>count/ min</td>
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<tr>
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<td>77</td>
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<td>Mycoplasma sp. strain J</td>
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<td>0</td>
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<tr>
<td>M. hominis strain 07</td>
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<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Reaction conditions: each tube contained 20 \textmu moles of adenosine triphosphate, 30 \textmu moles of manganese chloride, 30 \textmu moles of glutathione, 500 \textmu moles of phosphate buffer (pH 7.5), 0.03 M potassium fluoride, mevalonic-5'-pyrophosphate-2-C\textsuperscript{14} synthesized from 20 \textmu moles of mevalonic acid-1-C\textsuperscript{14} (0.5 \textmu C/\textmu mole) or mevalonic-5'-pyrophosphate-2-C\textsuperscript{14} synthesized from 20 \textmu moles of mevalonic acid-2-C\textsuperscript{14} (0.5 \textmu C/\textmu mole), and 30 mg of enzyme protein.
Fig. 1. No disappearance was expected in the tubes which contained iodoacetamide, which is a specific inhibitor of isopentenylpyrophosphate $\Delta^2,\Delta^2$-isomerase. Figures 1a and 1b demonstrate that isopentenylpyrophosphate $\Delta^2,\Delta^2$-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 $\Delta^2,\Delta^2$-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 $\gamma,\gamma$-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 $\gamma,\gamma$-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-phosphomevalonate carboxy-lyase. However, this organism appears to possess isopentenylpyrophosphate $\Delta^2,\Delta^2$-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 $\Delta^2,\Delta^2$-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 $\Delta^2,\Delta^2$-isomerase in $M$. hominis explains the inability of strains of this organism to convert mevalonic acid to isopentenyl pyrophosphate. 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.
isopentenyl pyrophosphate to support growth when substituted for sterol.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service research grant AI 04410-05 and graduate training grant 5 T1 AI 232-03 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED