Synthesis of Lipids from Acetate Is Not Characteristic of *Acholeplasma* or *Ureaplasma* Species

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Previous studies of *Acholeplasma* species indicated that this genus could be separated from *Mycoplasma* on the basis of the ability to synthesize lipids from acetate. We report that recently described strains of *Acholeplasma* *florum* (strain L1 isolated from a lemon flower and strain GF-1 isolated from a grapefruit flower) and two unclassified *Acholeplasma* strains (strain J233 from coconut palm and *Acholeplasma* sp. strain PS-1 from an insect) convert little if any acetate carbon to lipid, in contrast to *Acholeplasma* sp. strain 0502-CL1 isolated from broccoli and six other established *Acholeplasma* species of animal origin. Thus, the ability to synthesize lipid from acetate in Edward test medium containing horse serum is not characteristic of all members of the genus *Acholeplasma* (class Mollicutes), but appears to be a property only of a subgroup in the genus containing the species that are most frequently isolated from animals (*Acholeplasma* *laidlawii*, *Acholeplasma* *granularum* BTS39, *Acholeplasma* *oculi* 19L, *Acholeplasma* *equifetale* N93, and probably *Acholeplasma* *axanthum* S743 and *Acholeplasma* *modicum* PG49). Like other *Mycoplasma* species which we studied, *Mycoplasma* *bovigenitalium* PG11, *Mycoplasma* *hominis* PG27, *Mycoplasma* *arginini* G230, and four sterol-requiring mycoplasmas of insect or plant origin (*Mycoplasma* sp. strain MQ3 from an insect, *Mycoplasma* sp. strain pommier from an apple, *Mycoplasma* sp. strain melaleuca from a *Melaleuca* flower, and *Mycoplasma* sp. strain 831-C4 from lettuce) convert little acetate to lipid. Similarly, eight serovars of *Ureaplasma* *urealyticum* convert little if any acetate to lipid.

The *Mycoplasmatales*, the only order in the class Mollicutes, contains three families, the *Mycoplasmataceae*, with two genera (*Mycoplasma* and *Ureaplasma*), and the *Acholeplasmataceae* and *Spiroplasmataceae*, each containing one genus (*Acholeplasma* and *Spiroplasma*, respectively) (3). These three families are distinguished by whether sterol is essential for growth, by their genome sizes, by the ability to hydrolyze urea, by the localization of reduced nicotinamide adenine dinucleotide oxidase activity, and by the presence of helical forms during some phase of growth (3). We have suggested that members of the genus *Acholeplasma*, the single genus in the *Acholeplasmataceae*, can be distinguished from *Mycoplasma* species by their ability to synthesize lipid from acetate (4, 6). Other workers have reported that a strain of *Ureaplasma* *urealyticum* also synthesizes lipid from acetate (10) and that *Spiroplasma* *citri* does not (2).

We examined a number of sterol-requiring and non-sterol-requiring plant and insect isolates, as well as ureaplasmas and other members of the Mollicutes, for their ability to synthesize lipids from acetate. Contrary to our expectation, lemon flower strain L1 and grapefruit flower strain GF-1 of *Acholeplasma* *florum*, two other *Acholeplasma* sp. strains, and eight serovars of *U. urealyticum* did not synthesize significant amounts of lipid from acetate.

MATERIALS AND METHODS

Organisms. *Mycoplasma* *bovigenitalium* PG11, *Mycoplasma* sp. strain MQ3 (insect), *Mycoplasma* sp. strain pommier (apple), *Mycoplasma* sp. strain melaleuca (flower), *Acholeplasma* *laidlawii* H3-10, *A. laidlawii* 643N, *Acholeplasma* *morum* S2, *Acholeplasma* *hippikon* C1, *A. florum* L1 (lemon flower) passage 3, *A. florum* GL-1 (grapefruit flower) pas-

sage 5, *Acholeplasma* sp. strain 0502-CL1 (broccoli) passage 12, *Acholeplasma* sp. strain J233 (coconut palm) passage 10, and *Acholeplasma* sp. strain PS-1 (insect) passage 1 were obtained from J. G. Tully, National Institutes of Health, Frederick, Md. *Mycoplasma* sp. strain 831-C4 (lettuce) was isolated by and obtained from N. L. Somerson, Ohio State University, Columbus. *A. laidlawii* B-PG9, *Mycoplasma* *hominis* PG27, *Mycoplasma* *gallisepticum* S6, *Mycoplasma* *pulmonis* ATCC 19612, *Mycoplasma* *arginini* G230, and serovar standard strains of *Ureaplasma* *urealyticum* (9) were obtained from our stock collections.

Media and growth conditions. All *Acholeplasma* and *Mycoplasma* species were grown in our modification of Edward medium (1). The media were supplemented with 2% (vol/vol) (*acholeplasmas*) or 4% (vol/vol) (*mycoplasmas*) heat-inactivated (56°C, 1 h) horse serum (control lots 268095 and 200011H; K. C. Biologicals, Lenexa, Kans.). Media for the growth of *M. arginini* were supplemented with L-arginine hydrochloride (Calbiochem, La Jolla, Calif.) at a final concentration of 0.1% (wt/vol). Ureaplasmas were grown in B-broth as described by Robertson (8). All growth media contained approximately 5×10^5 dpm of [U - 14 C]acetic acid (sodium salt; 52 to 58 mCi/mmol; ICN, Irvine, Calif., or Amersham Corp., Arlington Heights, Ill.) per ml. Temperature-equilibrated media (125 to 250 ml) were inoculated with 1 to 3-day-old cultures of *acholeplasmas* or *mycoplasmas* (2 to 10%, vol/vol) or ureaplasmas (0.01%, vol/vol) grown under the same conditions. Most organisms were incubated at 37°C; the exceptions were *Mycoplasma* sp. strain MQ3, *Mycoplasma* sp. strain melaleuca, and *Acholeplasma* sp. strain PS-1, which were incubated at 30°C. After 1 to 3 days of incubation, the cells were harvested, and in most instances the pellets were washed four times in 200 volumes of Kappa buffer by centrifugation at $16,000 \times g$ for 20 min at 4°C (7). *Ureaplasma* pellets were washed four times in 300 volumes of Kappa buffer by centrifugation at $29,000 \times g$ for 20 min at 4°C.

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FIG. 1. Effect of different amounts of horse serum in Edward medium (1) on the incorporation of radioactivity from [^{14}C]acetate into the lipids of two growing *Mollicutes* strains.

The cells of *Acholeplasma* sp. strain 0502-C1 and *Acholeplasma* sp. strain PS-1 were osmotically labile to Kappa buffer, even when it was used at 10 $\times$ concentration. We washed the cells of these organisms four times with fresh Edward medium without horse serum or radioactive acetate; this was followed by a final wash with 10X Kappa buffer.

The washed cells were freeze-dried until the lipids were extracted as described previously (4). The lipids were purified (4) and weighed to 10⁻⁶ g by using an electromicrobalance (model ME30; Mettler Instrument Corp., Hightstown, N.J.).

RESULTS

The effects of different concentrations of serum in the medium on the incorporation of acetate radioactivity into the lipids of *Mollicutes* strains were tested by using *M. gallisepticum* and *A. laidlawii* B-PG9. For *M. gallisepticum*, the horse serum concentration in the medium was varied from 0.5 to 10.0% (vol/vol); for *A. laidlawii* B-PG9 it was varied from 0 to 10% (vol/vol). From *M. gallisepticum* grown in the presence of 0.5% serum, we recovered 8.51×10^3 dpm of lipids per mg, whereas in the presence of 10.0% serum we recovered 1.44×10^2 dpm of lipids per mg. From *A. laidlawii* grown without serum, we recovered 1.55×10^8 dpm of lipids per mg, whereas in the presence of 10% serum we recovered 4.86×10^7 dpm of lipids per mg (Fig. 1). We concluded that although serum concentration inversely affected the incorporation of acetate radioactivity into the lipids of *A. laidlawii* B-PG9, over the same serum concentration range there was little effect on such incorporation into the lipids of *M. gallisepticum*. Furthermore, at any concentration of serum, there was about 500 times more radioactivity incorporated into *A. laidlawii* B-PG9 lipids than into the lipids of *M. gallisepticum*.

We tested the various *Mollicutes* strains to determine whether they could synthesize lipid from acetate (Table 1). We found that all *Mycoplasma* species, all *U. urealyticum* serovars, and *Acholeplasma* sp. strains L1, GF-1, J233, and PS-1 synthesized relatively little or no ^{14}C -labeled lipid from

[^{14}C]acetate; six other *Acholeplasma* isolates produced significantly more ^{14}C -labeled lipid from [^{14}C]acetate.

DISCUSSION

We found that the *Mycoplasma* species which we tested, like all other species that have been examined, convert relatively little acetate to lipids (4, 6). *Acholeplasma* species have been shown to synthesize lipids from acetate (4, 6). However, this ability can no longer be used to differentiate *Acholeplasma* from *Mycoplasma* species, because we found that recently described *A. florum* and *Acholeplasma* sp. strains from coconut flowers and an insect synthesize very little lipid from acetate.

A. florum, *A. morum* 72-043, and *Acholeplasma axanthum* S743 exhibit positive growth responses to additions of fatty acids and albumin when they are grown in sterol-free growth media (5, 11, 12; J. G. Tully, unpublished data). As stated above, we found that *A. florum* strains produce little lipid from acetate, and we have reported previously that *A. morum* S2 and *A. axanthum* S743 produce intermediate levels of acetate-derived lipids (4). All of these findings may be related, in that the inability or diminished capacity of the strains to convert acetate to lipids may reflect a need for, and hence a positive growth response to, longer-chain fatty acids or perhaps phospholipids or both. We consider phospholipid requirement a strong possibility, because in *acholeplasmas* phospholipids are the primary repository of acetate-derived radioactivity (4).

TABLE 1. Synthesis of ^{14}C -labeled lipids from [^{14}C]acetate by *Mollicutes* strains

Strain	Lipid synthesis (kdpm of lipid per mg)	No. of cell batches tested
<i>A. laidlawii</i> B-PG9	341.00 $\pm$ 25.11 ^a	5
<i>A. laidlawii</i> 643N	130.49 $\pm$ 1.26	3
<i>A. laidlawii</i> H3-10	136.27 $\pm$ 2.28	2
<i>A. morum</i> S2	4.24 $\pm$ 0.11	3
<i>A. hippikon</i> C1	3.37 $\pm$ 0.05	3
<i>A. florum</i> L1 (lemon)	0.36 $\pm$ 0.95	6
<i>A. florum</i> GF-1 (grapefruit)	0.04 $\pm$ 0.01	3
<i>Acholeplasma</i> sp. strain J233 (coconut palm)	0.28 $\pm$ 0.03	3
<i>Acholeplasma</i> sp. strain PS-1 (insect)	0.04 $\pm$ 0.01	3
<i>Acholeplasma</i> sp. strain 0502-CL1 (broccoli)	43.59 $\pm$ 2.26	3
<i>M. gallisepticum</i> S6	0.19 $\pm$ 0.02	5
<i>M. bovigenitalium</i> PG11	0.24 $\pm$ 0.19	3
<i>M. pulmonis</i> ATCC 19612	0.01 $\pm$ <0.00	2
<i>M. hominis</i> ATCC 14027	0.17 $\pm$ 0.11	3
<i>M. arginini</i> G230	0.05 $\pm$ 0.01	3
<i>Mycoplasma</i> sp. strain MQ3 (insect)	0.03 $\pm$ 0.02	3
<i>Mycoplasma</i> sp. strain pommier (apple)	0.19 $\pm$ 0.07	3
<i>Mycoplasma</i> sp. strain melaleuca (flower)	0.02 $\pm$ 0.01	3
<i>Mycoplasma</i> sp. strain 831-C4 (lettuce)	0.29 $\pm$ 0.03	3
<i>U. urealyticum</i> 1 (serovar 1)	0.01	1
<i>U. urealyticum</i> 27 (serovar 3)	0.01	1
<i>U. urealyticum</i> 58 (serovar 4)	0.01	1
<i>U. urealyticum</i> 354[NIH] (serovar 5)	0.01	1
<i>U. urealyticum</i> Pi (serovar 6)	0.02 $\pm$ <0.00	2
<i>U. urealyticum</i> Co (serovar 7)	<0.01	1
<i>U. urealyticum</i> T960(Cx8) (serovar 8)	0.03 $\pm$ <0.00	3
<i>U. urealyticum</i> U38 (serovar 13)	0.01	1

^a Mean $\pm$ standard deviation.

Although to us it is less likely, it is possible that high rates of fatty acid turnover result in apparently low incorporation. Preliminary studies only with *A. axanthum* S743 showed that this organism did not release significant amounts (<1%) of $^{14}\text{CO}_2$ during the first 22 h of growth in Edward medium containing [^{14}C]acetate (unpublished data). Also, other studies with different achleoplasma showed that the residual lipid-extracted pellets of whole cells grown in the presence of [^{14}C]acetate were not radioactive (unpublished data).

All eight serovars of *U. urealyticum* converted very little if any acetate to lipid. This finding is in conflict with an earlier study of *U. urealyticum* P108 (10). Although isolate P108 was unavailable, we studied the same serovar (serovar 6) and found no conversion. All eight *Ureaplasma* serovars that we studied had been subcultured many times, and perhaps they had lost their ability to convert acetate to lipid. Romano has reported that *U. urealyticum* P108 has lost its ability to synthesize lipid from acetate and that some freshly isolated ureaplasmas lose this property after laboratory passage (N. Romano, unpublished data).

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