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Role of Carotenoids and Cholesterol in the Growth of *Mycoplasma laidlawii*

SHMUEL RAZIN AND SHLOMO ROTTEM

Department of Clinical Microbiology, The Hebrew University-Hadassah Medical School, Jerusalem, Israel

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Requirement of cholesterol or related sterols for growth might serve as a property distinguishing *Mycoplasma* from other bacteria, were it not for a number of strains of the species *M. laidlawii* which form an exception in being able to grow in the absence of cholesterol (S. Razin and B. C. J. G. Knight, J. Gen. Microbiol. **22**:492, 1960). The sterol-nonrequiring strains also differ from the other mycoplasmas in being capable of carotenoid biosynthesis. P. F. Smith (p. 518, *in* N. E. Gibbons [ed.], *Recent progress in microbiology*, University of Toronto Press, Toronto, 1963) postulated that carotenoids in *M. laidlawii* fulfill functions analogous to those of cholesterol in the other mycoplasmas. The arguments in favor of this hypothesis have been recently summarized by P. F. Smith and C. V. Henrikson (J. Bacteriol. **91**:1854, 1966). Carotenoid biosynthesis would accordingly be essential for the growth of *M. laidlawii* in a cholesterol-free medium. The present report, however, demonstrates the ability of *M. laidlawii* strains to grow without cholesterol and without carotenoid synthesis.

TABLE 1. *Effect of coenzyme A (CoA) and cholesterol on growth, carotenogenesis, and acetate incorporation by Mycoplasma laidlawii*[a]

Organism	Growth medium	Cell yield (mg of protein)	Carotenoids[b]	^{14}C-acetate incorporation (counts/min)	
				Polar lipids	Neutral lipids
M. laidlawii strain A	−CoA −Cholesterol	13.5	0.0	0	0
	−CoA +Cholesterol (10 μg/ml)	12.6	0.0	0	0
	+CoA (2 μg/ml) −Cholesterol	30.2	0.9	2,560	57
	+CoA (2 μg/ml) +Cholesterol (10 μg/ml)	31.0	0.8	2,320	48
M. laidlawii strain B	−CoA −Cholesterol	8.4	0.0	0	0
	+CoA (2 μg/ml) −Cholesterol	17.4	28.2	1,620	798

[a] The organisms were grown in 500-ml volumes of the partially defined medium of S. Razin and A. Cohen (J. Gen. Microbiol. **30**:141, 1963) modified by replacement of the amino acid mixture by 1% Difco Vitamin Free Casamino Acids (acid-hydrolyzed) and the addition of Tween 80 to a final concentration of 0.005%. The albumin included in the medium was extracted with acetone (S. Razin and S. Rottem, J. Gen. Microbiol. **33**:459, 1963). A 2-μc amount of uniformly labeled ^{14}C-sodium acetate was added to each flask. Cholesterol was added dissolved in Tween 80. The organisms were harvested after 72 hr of incubation at 37 C and washed three times in 0.25 M NaCl. Methods for estimating cell protein and carotenoids and for the extraction of cell lipids and their fractionation were as described by S. Razin et al. (J. Bacteriol. **91**:609, 1966). Radioactivity was measured in a Packard Tri-Carb liquid scintillation spectrometer. The data represent the average results of three experiments.

[b] Optical density at 442 mμ × 1,000 per milligram of cell protein.

The experiments were performed in the sterol-free medium of S. Razin and A. Cohen (J. Gen. Microbiol. **30**:141, 1963). Cells of *M. laidlawii* grown in this medium without the addition of

coenzyme A (CoA) or cholesterol were completely devoid of carotenoid pigments and cholesterol. Furthermore, the inability of these cells to incorporate exogenous acetate into their lipids (Table 1) indicates the absence of nonpigmented polyterpenes, since acetate is the primary precursor for polyterpene biosynthesis (*see* P. F. Smith and C. V. Henrikson, J. Bacteriol. **91**:1854, 1966). The addition of CoA to the medium improved growth and enabled the cells to incorporate acetate into their lipids (Table 1). In *M. laidlawii* strain A, the acetate was almost exclusively incorporated into polar lipids, and very little, if any, was utilized for carotenoid synthesis. In *M. laidlawii* strain B, a significant portion of the acetate was consistently incorporated into neutral lipids, as shown by the high carotenoid content of the cells. The results indicate that stimulation of growth by CoA does not depend on stimulation of carotenoid biosynthesis, since in *M. laidlawii* strain A growth was markedly improved without significant carotenoid biosynthesis. Contrary to the observations of Smith and Henrikson, cholesterol did not improve growth of *M. laidlawii* in the absence of carotenoid biosynthesis (Table 1).

Propionate has been found to affect acetate incorporation and carotenoid synthesis in *M. laidlawii*, probably by inhibition of the acetokinase activity of this organism (S. Rottem and S. Razin, *to be published*). Table 2 shows that the very marked reduction in the carotenoid content of *M. laidlawii*, caused by propionate, did not affect the growth of the organisms. The level of cholesterol in the cells remained practically unchanged, supporting our previous study (S. Razin and R. C. Cleverdon, J. Gen. Microbiol. 41:409, 1965), which indicated that the level of cholesterol in *M. laidlawii* membranes does not depend on their carotenoid content.

TABLE 2. *Effect of sodium propionate on the carotenoid and cholesterol content of Mycoplasma laidlawii cells*[a]

Propionate in medium	Cell yield (mg of protein)	Carotenoids[b]	Cholesterol (μg/mg of cell protein)
M			
0	22.5	32.8	13.7
2×10^{-2}	25.0	16.2	13.6
1×10^{-1}	29.5	2.5	12.3
2×10^{-1}	22.0	1.1	12.0

[a] The oral strain of *M. laidlawii* was grown in 250-ml volumes of Edward Broth (S. Razin, J. Gen. Microbiol. **33**:471, 1963) containing 1% PPLO Serum Fraction (Difco), 2×10^{-2} M sodium acetate, and propionate as indicated. The organisms were harvested after 24 hr of incubation at 37 C and were washed three times in 0.25 M NaCl. Cell protein, carotenoids, and cholesterol were determined as described by Razin et al. (J. Bacteriol. **91**:609, 1966).

[b] Optical density at 442 mμ × 1,000 per milligram of cell protein.

In conclusion, the finding that carotenoids are not essential for growth of *M. laidlawii* in a sterol-free medium contradicts the hypothesis that they fulfill functions analogous to those of sterols in other mycoplasmas.

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