Mycoplasma Growth Inhibition by Arginine

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Arginine enhances the growth of nonfermenting mycoplasmas. However, arginine can restrict the growth of glucose-fermenting mycoplasmas and should not be added to media used to cultivate these species.

Nonfermenting mycoplasmas can obtain energy from amino acids, particularly from arginine (4, 9-11). These mycoplasmas metabolize arginine via the arginine desimidase pathway with end products of ammonia, carbon dioxide, and ornithine. Frequently, a single growth medium is used for the isolation and cultivation of both glucose-fermenting and nonfermenting mycoplasmas, and arginine is often added to culture media to facilitate detection and isolation of nonfermenting mycoplasmas (1-3, 8). Arginine is useful also as a medium supplement in metabolic inhibition tests, since its utilization causes an alkaline pH shift.

We attempted to grow *Mycoplasma pneumoniae* and *Mycoplasma arthritidis* in media to which both glucose and L-arginine monohydrochloride (two sources: Calbiochem, Los Angeles, Calif., and Nutritional Biochemicals Corp., Cleveland, Ohio) had been added. We used two broth media, one designated SSR2 (6, 7) and the second a formulation modified from Hayflick (5). SSR2 is a buffered medium prepared with mycoplasma broth base (Baltimore Biological Laboratory, Cockeysville, Md.) and supplemented with yeast extract, glucose, pleuropneumonia-like organism (PPLO) serum fraction (Difco Laboratories, Detroit, Mich.), phenol red, Eagle minimum essential medium with glutamine (Grand Island Biological Co., Grand Island, N.Y.), and N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) buffer (Calbiochem). The modified Hayflick broth is similar in composition but lacks buffer and Eagle minimum essential medium.

Uniform inocula for growth experiments were prepared by growing mycoplasmas as thick confluent layers in 6-ounce (174-ml) prescription bottles containing 25 ml of broth each. Glass-adherent mycoplasma (GAM) were removed from the culture bottles by either treatment with glass beads, as in the case of *M. arthritidis*, or trypsinization, as for *M. pneumoniae*, to prevent clumping of the organisms. The GAM were suspended in 2.5 ml of fresh

broth. Growth inhibition experiments were performed in 3-ounce (87-ml) prescription bottles containing 12 ml of broth medium each. Each test culture received 0.05 ml of one of these mycoplasma suspensions. Mycoplasma growth was assayed by determining the Lowry protein content of the GAM after 48 h of incubation for *M. arthritidis* and 72 h for *M. pneumoniae*. Results are expressed as micrograms of GAM protein.

In SSR2 broth, L-arginine monohydrochloride added to a final concentration of 0.5% (0.02 M) dramatically inhibited growth of *M. pneumoniae* strain C1-8, a glucose-fermenting organism (Table 1). At this same concentration, growth of an arginine-utilizing mycoplasma, *M. arthritidis*, was enhanced almost 10-fold.

We examined the effect of arginine concentration on growth of *M. pneumoniae* strain 65-2161 and *M. arthritidis* strain PG-27 in an attempt to devise one medium formulation suitable for cultivation of both species. A minimum concentration of 0.5% added arginine was required for optimal growth of *M. arthritidis* in modified Hayflick broth (Fig. 1A); however, levels added above 0.2% (0.009 M) significantly reduced growth of *M. pneumoniae* (Fig. 1B). Similar results for *M. pneumoniae* were obtained, regardless of whether the medium contained bovine serum fraction (BSF) or horse serum.

TABLE 1. Growth of *M. pneumoniae* strain C1-8 and *M. arthritidis* strain PG-27 in SSR2 broth with L-arginine monohydrochloride^a

Arginine addition	μg of GAM protein ± SEM	
	<i>M. pneumoniae</i>	<i>M. arthritidis</i>
None	712 ± 10	128 ± 4
0.5%	133 ± 28	1,260 ± 45

^a GAM protein and standard error of the mean (SEM) values were calculated from sets of four replicate cultures. Glucose was included in SSR2 broth at a final concentration of 0.5% (wt/vol).

FIG. 1. Effect of L-arginine HCl concentration on growth of *M. arthritis* strain PG-27 (A) and *M. pneumoniae* strain 65-2161 (B) in modified Hayflick broth. *M. pneumoniae* was tested in broth supplemented with either BSF (final concentration, 5% [solid line]) or horse serum (final concentration, 20% [broken line]). *M. arthritis* was tested in broth containing BSF. For *M. pneumoniae* grown with horse serum, each point represents the mean of duplicate cultures. For mycoplasmas grown with BSF, each point represents the mean of from four to ten replicate cultures; vertical lines represent one standard error to either side of the mean.

TABLE 2. Growth of *M. pulmonis* strain JB in modified Hayflick broth with L-arginine HCl^a

Arginine addition	pH of broth after 48 h of incubation			
	Horse serum		BSF	
	20%	5%	20%	5%
None	6.14; 6.15	6.16; 6.18	6.67; 6.64	6.69; 6.66
0.5%	7.43; 7.56	7.57; 7.56	7.17; 7.04	7.56; 7.58

^a Initial pH of the broth was 7.6 ± 0.1 ; pH values from duplicate cultures are shown. Modified Hayflick broth was prepared with glucose at a final concentration of 1.0%.

Preliminary experiments showed arginine to be inhibitory also to a second glucose-fermenting mycoplasma, *Mycoplasma pulmonis* strain JB. Inoculum for growth experiments was prepared as described for *M. arthritis*. Since this strain of *M. pulmonis* adhered poorly to glass under the test conditions, we estimated the amount of growth by noting the decline in the pH of the broth medium after 48 h of incubation (6). Our results (Table 2) showed that in all but one instance the pH of the cultures containing arginine did not change significantly from the initial pH of the broth, suggesting total inhibition of growth. The kind and quantity of serum supplement in the broth appeared to have some effect on the degree of inhibition, since when 20% (vol/vol) PPLO serum fraction (BSF) was used, light growth occurred in the presence of

TABLE 3. Growth of *M. fermentans* strain PG-18 in modified Hayflick broth with L-arginine HCl

Arginine addition	μ g of GAM protein ^a		
	Horse serum	BSF	
	(5%)	20%	5%
None	815; 800	1,080; 1,130	890; 940
1.0%	860; 690	1,150; 1,410	1,250; 1,230

^a Results from duplicate cultures. Cultures were grown in medium containing horse serum or BSF at the indicated percentage.

arginine, although the inhibitory effect was still marked.

Finally, we tested the effect of arginine on growth of *Mycoplasma fermentans* strain PG-18 (Table 3). This species not only ferments glucose but also possesses the arginine desiminase pathway (4). Inoculum was prepared as described for *M. arthritis*; GAM protein content was measured after 96 h of incubation. In contrast to the other fermenting species tested, growth of *M. fermentans* was not inhibited by added arginine and may have been slightly enhanced.

Additional experiments indicated that the monohydrochloride portion of the molecule was not responsible for growth inhibition, since the L-arginine-free base had the same effect on *M.*

pneumoniae growth as L-arginine HCl. The effect of the arginine-free base on the pH of modified Hayflick broth was counteracted by the addition of HEPES, so that alteration of pH by the added amino acid was also not an inhibitory factor. Although the mechanism of growth inhibition is not understood, we noted in preliminary experiments that other compounds with structures similar to L-arginine, such as D-arginine HCl and, to a lesser extent, L-homoarginine HCl and L-lysine HCl, also reduced *M. pneumoniae* growth. On the other hand, addition of L-histidine HCl or glycine HCl had no effect.

Addition of L-arginine HCl to culture medium is very useful for enhancing the growth of nonfermenting mycoplasmas. However, too much arginine can restrict the growth of glucose fermenters and should be avoided in media used to isolate and cultivate these species.

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