Arginine Catabolism by *Mycoplasma meleagris* and Its Role in Pathogenesis

ALI A. IBRAHIM AND RICHARD YAMAMOTO*

Department of Epidemiology and Preventive Medicine, University of California, Davis, California 95616

Received for publication 18 April 1977

A thin-layer chromatography technique was used to study the arginine metabolism of *Mycoplasma meleagris*. The technique reflected the enzyme activity of the dihydrolase pathway through detection of readily visible end products on X-ray film. Strains of *M. meleagris* differing in their pathogenicity for turkeys did not vary in arginine metabolism. In addition, no significant difference was observed in plasma arginine concentrations between *M. meleagris*-infected and uninfected poults. It was concluded that the pathogenesis of *M. meleagris* infection in turkeys was not based on its competition with the host for arginine.

Members of the genus *Mycoplasma* may be divided into two physiological groups: those that ferment glucose, and those that do not (8, 14). Certain species in the latter group catabolize arginine to ornithine with concomitant generation of adenosine 5'-triphosphate (dihydrolase pathway) (14). Arginine-utilizing *Mycoplasma* in cell cultures have been found to alter cell metabolism, morphology, and growth, to cause chromosomal changes, and to interfere with (or enhance) virus multiplication (7, 9, 18). Most of these changes were traced to depletion of arginine in the medium by the *Mycoplasma*. In other studies, suspensions or extracts of arginine-catabolizing *Mycoplasma* inhibited mitotic activity of lymphocytes stimulated by antigen or phytohemagglutinin (1, 4, 10); the active fractions in the extract were enzymes of the dihydrolase system (16). Thus, these and other studies (15, 17) suggest that arginine depletion by *Mycoplasma* may play a role in pathogenesis. In a previous study from this laboratory, strains of *Mycoplasma meleagris* pathogenic and nonpathogenic for turkeys were characterized (6). *M. meleagris* is a nonfermenter that catabolizes arginine (6). In the present study, a sensitive procedure to detect the products of arginine metabolism by *Mycoplasma* was developed to determine if a pathogenic strain of *M. meleagris* varied in its arginine metabolism from nonpathogenic strains. An experiment was also conducted to ascertain if plasma arginine levels in *M. meleagris*-infected poults would differ from those of uninfected poults.

(This report is taken from a thesis submitted by A. A. Ibrahim in partial fulfillment of the requirements for the Ph.D. degree at the University of California, Davis.)

**MATERIALS AND METHODS**

**Cultures.** One pathogenic (RY-39A) and two nonpathogenic (MC-25B and RY-39C) strains of *M. meleagris* (6) were used in the study. In addition, with respect to studies on arginine metabolism, *Mycoplasma gallinarum* strain ATCC 19708 and *Mycoplasma hominis* strains PG21 (L. Hayflick, Stanford University) and ATCC 14027 were included as positive controls (2).

**Media and chemicals.** A serum broth was prepared from PPLO broth (Difco) supplemented with 1% yeast autolysate (Albimi) and 15% horse serum (heat inactivated at 56°C for 30 min). The pH of the broth was adjusted to 7.6. A serum agar was prepared by adding 1.2% agar (Difco) to the serum broth. Penicillin (1,000 U/ml) and thallium acetate (1:4,000) were added to both media as bacterial inhibitors. Five millimolar l-arginine (Sigma) was added to the broth. The serum broth was used to propagate and the serum agar was used to enumerate the organisms.

1-[U-14C]arginine monohydrochloride, 1-[guanido-14C]arginine monohydrochloride (Amersharm/Searle), and 1-[U-14C]ornithine monohydrochloride (New England Nuclear) were incorporated separately into the serum broth, each at a concentration of 5 μCi/ml of medium.

**Kinetics of arginine depletion.** Arginine depletion by pathogenic (RY-39A) and nonpathogenic (RY-39C) strains of *M. meleagris* was determined in serum broth. Each organism was inoculated into 50 ml of broth and incubated at 37°C. At various intervals, 10 ml of culture was removed for viable count and arginine assay. For the latter, the culture was centrifuged at 27,000 × g for 15 min, and the supernatant was assayed for arginine by the Sakaguchi procedure (13) as modified by Van Pilsum et al. (19).

**Thin-layer chromatography.** Three strains of *M. meleagris*, two of *M. hominis*, and one of *M. gallinarum* were used in this study. The organisms were grown in 1-ml amounts of broth containing the radioactive arginine or ornithine. After 3 days of incubation at 37°C, the cultures were checked for contamination.
by Giemsa stain or by blood agar plating, and 0.5 ml of each was precipitated with 4.5 ml of 2% perchloric acid. The extract was collected after centrifugation at 3,020 $\times$ g for 10 min, and its pH was adjusted to 7.0 with potassium carbonate-tris(hydroxymethyl)aminomethane buffer. Five microliters of each extract was dropped on one end of a silica gel glass plate (20 by 20 cm) (Mann Research) previously activated at 110°C for 10 min and allowed to cool for 0.5 h. The plates were developed in phenol-water (75:25, wt/wt) to nearly 15 cm. The chromatogram was then dried by hot air and exposed to X-ray film for 24 h. The film was processed according to Randerath (12).

Silica gel plates were also used to determine whether polyamines were products of arginine metabolism. The procedure used was similar to that of Cohen et al. (3).

**Plasma arginine.** *M. meleagridis* (strain RY-39A) was inoculated into 9-day-old turkey embryos via the yolk sac. The dosage was 10$^7$ colony-forming units in 0.1 ml per embryo. After hatching, the poults (n = 10) were raised for 19 days of age, at which time they were bled (for plasma and for serum) and necropsied. The experiment included 15 uninfected poults of the same source as the infected group. The plasma (taken in heparin) was harvested immediately and stored at $-70^\circ$C until tested for arginine by the Sakaguchi procedure (13) as modified by Van Pilsen et al. (19). The air sacs of each poult were examined for lesions and cultured for *Mycoplasma*, and the sera were tested for antibodies by the tube agglutination test (20).

![Fig. 1](https://iai.asm.org/) Growth and utilization of arginine by pathogenic (RY-39A) and nonpathogenic (RY-39C) strains of *M. meleagridis* in broth. Symbols: Solid lines, arginine concentration; broken lines, growth curve; ▲, RY39A; ●, RY39C.

**RESULTS**

Utilization of arginine in broth cultures. Both strains of *M. meleagridis* utilized the arginine in the medium. The pathogenic strain (RY-39A) reduced the level of arginine from an initial concentration of 12.7 mM to 2.4 mM, whereas the nonpathogenic strain (RY-39C) reduced it to 4.5 mM in cultures tested after 6 days of incubation. The growth curves of both strains were similar (Fig. 1).

When L-[U-$^{14}$C]arginine was used, five strains (*M. meleagridis* RY-39A, RY-39C, and MC25B; *M. hominis* ATCC 14027; and *M. gallinarum*) completely catabolized arginine with subsequent formation of radioactive ornithine and citrulline. When L-[guanido-$^{14}$C]arginine was used, only radioactive citrulline was detected. In the case of *M. hominis* PG21, L-[U-$^{14}$C]- and L-[guanido-$^{14}$C]arginine were not catabolized. Figure 2 shows an example of an autoradiogram of an organism that catabolized arginine through the dihydrolase pathway (*M. meleagridis* RY-39A) and an organism that did not have the enzymes for this pathway (*M. hominis* PG21).

Labeled polyamines were not detected in the perchloric acid extract of organisms grown in L-[U-$^{14}$C]arginine; however, cold polyamines were detected in the same extracts.

**Plasma arginine and pathogenicity.** All poults inoculated with *M. meleagridis* (RY-39A) showed air sac lesions and high-antibody responses. Leg abnormalities were observed in 60% (6 of 10). The organism was recovered from air sacs, sinuses, and bursa of Fabricius of all poults. This response pattern was considered typical of the disease caused by *M. meleagridis* (6). The poults in the control group did not show any signs or lesions or develop antibody, and their tissues did not yield *Mycoplasma*. The plasma arginine levels (in $\mu$M/100 ml) in the infected group was 40.75 ± 3.05 and that of the uninfected group was 37.93 ± 1.83. These values were not significantly different (α = 0.05; Student's t test).

**DISCUSSION**

The role of arginine-utilizing *Mycoplasma* in disease production or tissue alteration has been attributed to their ability to compete with the host for arginine (1, 4, 7, 9, 10, 16). In the present experiment, a pathogenic and a nonpathogenic strain of *M. meleagridis* were compared with respect to arginine utilization. Both strains used arginine from the growth medium (Fig. 1). Furthermore, the technique of the thin-layer chromatography with radiolabeled substrates showed that both organisms utilized arginine via the dihydrolase pathway. The thin-layer chroma-
 Autoradiogram of a resulting X-ray film showing arginine metabolism by *M. meleagridis* (RY-39A) and *M. hominis* (PG-21). (A) *M. hominis* grown in L-[U-14C]arginine; the spots (triplicate) identify radiolabeled arginine. (B) *M. hominis* grown in L-[guanido-14C]arginine, and the spots (triplicate) identify radiolabeled arginine. (C) *M. meleagridis* grown in L-[U-14C]arginine; spot (triplicate) C1 identifies radiolabeled ornithine, and C2 identifies radiolabeled citrulline. (D) *M. meleagridis* grown in L-[guanido-14C]-arginine; spots (triplicate) identify radiolabeled citrulline. The relative positions of amino acids (arginine, ornithine, and citrulline) on silica gel plates were determined by both radiolabeled and cold amino acids.

tography technique used has advantages over other biochemical methods: it is a visual test that is easily read, and it has the added advantage of determining if the enzymes of the pathway are active or not, since the test is based on the detection of products of the pathway and not on the presence of enzymes per se.

All of the organisms tested by thin-layer chromatography, with the exception of *M. hominis* PG21, converted all of the [U-14C]arginine to ornithine. Since arginase is not present in *Mycoplasma* (2, 14), the most plausible explanation of the results is that the organism utilized the dihydrolase pathway. The detection of radiolabeled citrulline and not ornithine from L-[guanido-14C]arginine (Fig. 2) indicates both the presence of arginine deaminase and the sequential nature of the enzymes involved in the dihydrolase pathway.

Since the pathogenic (RY-39A) and nonpathogenic (RY-39C and MC-25B) strains of *M. meleagridis* did not differ in arginine metabolism, arginine does not appear to play a role in the pathogenesis of disease caused by this organism. Recently, Fenske and Kenny (5) presented evidence indicating that the dihydrolase pathway may not be the essential energy-yielding pathway for nonglycolytic *Mycoplasma*. It is possible, however, that such strains differ quantitatively in arginine catabolism. Nevertheless, that no significant difference was observed in arginine levels in plasma of *M. meleagridis*-infected versus uninfected poults further negates the importance of arginine. Since the loss of virulence of *M. meleagridis* correlates with loss of antigenicity and invasiveness (6), the pathogenetic mechanism may be related to surface characteristics of the organism rather than with metabolic factors.

The presence of an arginine dihydrolase pathway was confirmed for *M. hominis* (ATCC 14027) and *M. gallinarum* (ATCC 19708) (2). However, our finding that the dihydrolase pathway was absent in *M. hominis* PG21 is contrary to the findings of Barile et al. (2) and requires further studies to clarify the classification of this particular strain.

Polyamines, which are products of arginine metabolism by many organisms, were investigated because they play a role in osmoregulation (11) and thus could be important to membrane integrity of *Mycoplasma*. Failure to detect radiolabeled polyamines from extracts of organisms grown in L-[U-14C]arginine indicates that such compounds are not products of arginine metabolism by *Mycoplasma*. The detection of
“cold” polyamines in the cell extracts suggests that such compounds were supplied exogenously from the complex medium.

ACKNOWLEDGMENTS

We express our sincere appreciation to J. L. Ingraham for his helpful suggestions during various parts of this study. The technical assistance of H. B. Ortmayer is also acknowledged. This investigation was supported by a Public Health Service grant no. AI-07805-08 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED