

# MYCOPLASMA INOCUUM SP. N., A SAPROPHYTE FROM CHICKENS

H. E. ADLER, M. SHIFRINE, AND HERRAD ORTMAYER

*School of Veterinary Medicine, University of California, Davis, California*

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## ABSTRACT

ADLER, H. E. (University of California, Davis), M. SHIFRINE, AND HERRAD ORTMAYER. *Mycoplasma inocuum* sp. n., a saprophyte from chickens. *J. Bacteriol.* **82**:239-240. 1961.—A new species of *Mycoplasma* was isolated from the infraorbital sinuses of chickens. The organism, a saprophyte, differs from all known mycoplasmas of avian origin. A description of this isolate is given. It has been named *Mycoplasma inocuum* sp. n.

Fifteen species of *Mycoplasma* (so-called pleuropneumonia-like organisms or PPLO) are listed in *Bergey's Manual of Determinative Bacteriology* (Breed, Murray, and Smith, 1957); of these, fourteen are classified as "parasitic to pathogenic" and one, *Mycoplasma laidlawii*, as saprophytic. Saprophytic strains of *M. laidlawii* have been isolated from sewage, manure, humus, and soil.

We have isolated a saprophytic strain from the infraorbital sinuses of chickens, a description of which is given in this paper.

## MATERIALS AND METHODS

**Culture media.** The basal medium employed was PPLO broth (Difco), enriched with 1% yeast autolysate (Albimi), and 0.05% glucose. For a solid medium, agar was added at the level of 1.5%. To study hemolysis, 5% of horse or bovine blood was added. Incubation was aerobic at 37 C and a moist atmosphere was provided by taping the plates.

To determine fermentation reactions, phenol red and 1% carbohydrate were added to the basal growth.

**Antigens and antibodies.** Antigens were prepared by the procedure of Adler and Yamamoto (1956) omitting crystal violet. Specific antibodies were produced in rabbits. Antigens were concentrated by centrifugation, brought to the density of McFarland no. 10 tube, and precipitated with 0.1% aluminum-alum. Two rabbits

for each strain were injected intravenously five times with 1.0 ml of the precipitated organisms, at 2-day intervals. Ten days after the last inoculation, the blood was collected by cardiac puncture. Agglutination tests were made as described by Adler, Yamamoto, and Berg (1957).

**Pathogenicity studies.** Seven- to eight-day-old embryonating eggs were inoculated in the yolk sac. To study pathogenicity, 6 to 7-week-old chickens and turkeys were inoculated into the infraorbital sinuses and abdominal air sac with 0.5 ml of 3-day-old cultures. Birds were subjected to necropsy 2 weeks after exposure, and observed for lesions.

## RESULTS

To isolate the *Mycoplasma*, exudates from the sinuses were seeded into enrichment broth (basal broth with 10% horse serum). After 3 days of incubation, the broth was streaked onto basal agar enriched with 10% horse serum. Three cultures of *Mycoplasma* were isolated from the infraorbital sinuses of chickens with coryza. All three isolates were found to be morphologically and physiologically identical, and are considered the same strain. The organism has not been previously described.

Following is a description of the new isolate:

***Mycoplasma inocuum* sp. n.** (in-nocuum L. adj. *innocuus* harmless). A *Mycoplasma* isolated from the infraorbital sinus of a chicken.

**Horse serum agar.** Colonies were smooth with large central spot.

**Beef heart infusion agar.** Granular growth at 37 C and at 20 C.

**Horse or bovine blood agar.** Good growth with hemolysis.

**Growth.** Smooth growth throughout in PPLO broth with yeast autolysate (0.5%) and glucose (0.05%) (not inhibited by 4,000 units of penicillin per ml). Good growth at 20 C.

**Tinctorial properties.** From broth, coccoid rods stained readily with Giesma and poorly by the Gram's method. Gram-negative.

Colonies readily stained by the procedure of Dienes (1953, *personal communication*). Colonies

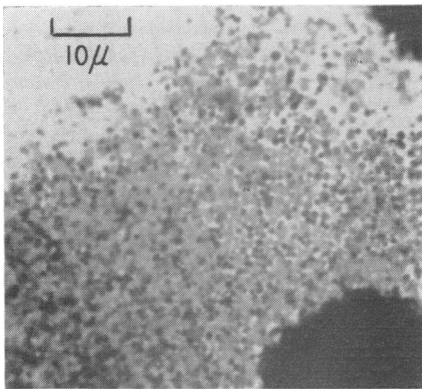


FIG. 1. Edge of a colony of *Mycoplasma inocuum* stained by the method of Klieneberger-Nobel ( $\times 900$ ).

TABLE 1. Results of cross-agglutination among strains of *Mycoplasma* of poultry origin

Antisera	Antigen		
	<i>M. inocuum</i>	<i>M. gallisepticum</i>	<i>M. gallinarum</i>
<i>M. inocuum</i> .....	160*	1	1
<i>M. gallisepticum</i> .....	1	320	1
<i>M. gallinarum</i> .....	1	1	40

\* Final dilution at which agglutination occurred.

TABLE 2. Characteristics of strains\* of *Mycoplasma* from chickens and turkeys

Strain	Pathogenicity for			Hemolytic	Hemagglutination	Ferment sugars
	Turkeys	Chickens	Chicken embryos			
<i>M. gallisepticum</i> .....	+	+	+	-	+	+
<i>M. gallinarum</i> .....	-	-	+	+	-	-
<i>M. iners</i> .....	-	-	+	+	-	-
C.....	-	-	+	-	-	+
SA.....	-	-	-	-	-	-
N.....	+	-	-	-	-	-
<i>M. inocuum</i> .....	-	-	-	+	-	+

\* All the cultures in this table were isolated in our laboratory. They are unrelated antigenically.

stained by the method of Klieneberger-Nobel (1950) were composed of large coccoid bodies (Fig. 1).

*Acid produced.* From glucose, maltose, sucrose,

and galactose. No acid from mannose, lactose, trehalose, salicin, and mannitol.

*Satellitism.* No stimulation by staphylococci on 5% bovine blood agar.

*Hemagglutination.* Does not agglutinate chicken red blood cells.

*Agglutination.* See Table 1.

Certain characteristics of mycoplasmas used to differentiate species of avian origin are presented in Table 2. It can be seen that *M. inocuum* is not pathogenic to chickens, turkeys, or embryonated eggs. Thus it differs from all other strains but strain SA, from which it differs by its ability to hemolyze blood and ferment sugars.

#### DISCUSSION

The isolation of a saprophytic *Mycoplasma* from a chicken leads one to wonder whether saprophytic strains are not more prevalent in nature than heretofore assumed. No search has been made as yet for mycoplasmas in nature: soil, flowers, insects, etc. It would be of great interest to determine whether saprophytic strains reside in sources other than animals and birds, and whether they are related to known pathogens. Studies of the nutrition and physiology of saprophytic strains, which can be accomplished with greater facility than with pathogenic strains, may throw some light on the relations between this group of microbes and the true bacteria.

#### ACKNOWLEDGMENT

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