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A Review of Mycoplasma in Laboratory Mice

Zahra Masoumalinejad [1], Mohammad Reza Zinatizadeh*[2], Najmeh Tahmasebiabdar [1]

1. Dept. of Microbiology, Sirjan Branch, Islamic Azad University, Sirjan, Iran

2. Dept. of Genetics, Faculty of Science, Tonekabon Branch, Islamic Azad University, Mazandaran, Iran

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ABSTRACT

Mycoplasma are small, cell-free bacteria enclosed by a membrane. These bacteria belong to the class of Mollicutes, the order of Mycoplasma tales, and the genus of Mycoplasma. There are more than 100 identified species of mycoplasma. The ratio of cytosine to guanine in its DNA is 23–40% and its genome size is 1350–600 kb. Mycoplasma require cholesterol to grow, and the temperature suitable for the growth of this bacteria is 37°C. Mycoplasma cause contamination and infections in humans and animals. Some mycoplasma species are seen only in animals. In general, mycoplasma are colonized at the surface of the mucus, and most species are non-invasive. Five main species of mycoplasma have been identified in laboratory mice, including: *M.arthritidis*, *M.collis*, *M.muris*, *M.neurolyticum*, and *M.pulmonis*. These species generally require protein-rich environments that contain 10–15% of the animal's serum, and their growth requires nicotinamide adenine nucleotide (NAD), which is commonly used to cultivate mycoplasma in mice. Laboratory research has found that mycoplasmas contamination has an adverse effect on animals. Therefore, it is important that health monitoring programs are implemented as a quality control for animals used in laboratory research.

Corresponding Information: Mohammad Reza Zinatizadeh, Dept. of Genetics, Faculty of Science, Tonekabon Branch, Islamic Azad University, Mazandaran, Iran, E-mail: zinati3333@gmail.com

Introduction

In 1898, Nocard and Roux separated certain bacteria from cattle with pneumonia (1). In 1929, Novak proposed the name of mycoplasma for these bacteria without cell walls, despite the split strands in production and reproduction. The name derives from, two words, the Greek word myco, meaning fungus, and the French word "plasma", meaning formed (2). By analyzing the sequence of the 16S rRNA gene, mycoplasma are thought to have been derived from gram-positive bacteria and clostridia around 600 million years ago, with a loss of the unnecessary parts of its genome (3).

Mycoplasma are small, Gram-negative, lack cell walls, and are enclosed by a membrane. These microorganisms grow relatively slowly and generally prefer the environment to be about 37-38° C. They are almost resistant to thallous acetate and penicillin, which are often used in culture environments to postpone the growth of bacterial and fungal infections (4).

Five main species of mycoplasma have been identified in laboratory mice namely, *M. arthritidis*, *M. collis*, *M. muris*, *M. neurolyticum*, and *M. pulmonis*. Among these mycoplasma, *M. pulmonis* is responsible for one of the most common mycoplasma contaminations in mice and rats (5).

Sanchez et al. reported *M. pulmonis* to be an etiologic agent. A high count of these bacteria is often found in the ovaries, uterus, and respiratory systems in mice and rats (6).

Classification

Mycoplasma, as a member of the class Mollicutes, the order Mycoplasma tales, the genus of Mycoplasma, include more than 100 identified species (Fig 1). The ratio of cytosine to guanine in its DNA is 23-40% and its genome size is 1350–600 kb. It requires cholesterol to grow, and the

temperature suitable for the growth of these bacteria is 37°C (7).

The class Mollicutes covers more than 100 species of mycoplasma of plants and vertebrates, as well as insects. The order Mycoplasmatales is divided into three families of Mycoplasmataceae, Acholeplasmatacea, and Spiroplasmatacea. The Mycoplasmataceae family consists of two genus Mycoplasma, of which more than 70 species have been identified, and many of which are pathogenic to humans and animals, and Ureaplasma, differentiated by urea hydrolysis (3,7,8).

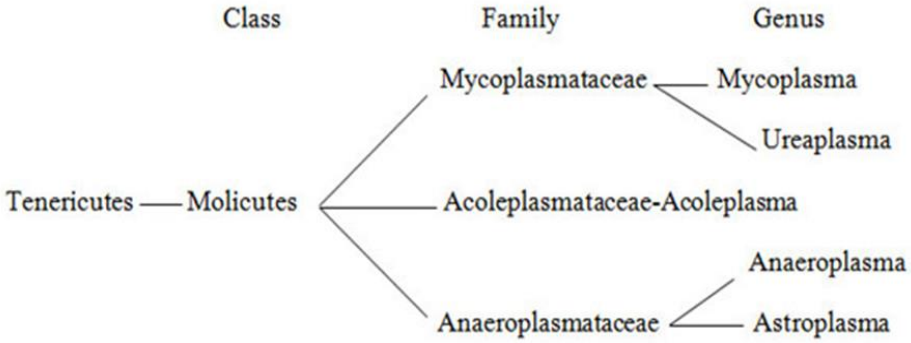

Fig1. Mycoplasma classification

Features of Mycoplasma in Laboratory Mice

In general, the mycoplasma species in mice require protein-rich environments that contain 10-15% of the serum. Other elements are derivatives of yeasts. The fermented carbohydrates include: 1. Those that ferment glucose 2. Those that do not ferment glucose (9).

Glucose is often added to the fluid medium for the growth of species that ferment glucose. Glucose is also a sign of growth. When glucose is fermented, it produces acid. So, in order to identify fermentation, phenol is added to the environment (8,3).

Phosphatase activity is often used in mycoplasma species that do not ferment glucose from arginine as an energy source, as shown in **Table 1**.

Table 1. Fermentation of Glucose and Arginine Hydrolysis in Mycoplasma in Mice

Species	Usual host	Fermentation of Glucose	Arginine Hydrolysis
M.arthritidis	Mice	-	+
M.collis	Mice/Dog	+	-
M.muris	Mice	-	+
M.neurolyticum	Mice	+	-
M.pulmonis	Mice	+	-

Mycoplasma Contamination in Mice

Mycoplasma pulmonis

This contamination is very common in laboratory mice and affects the middle ear space. Infection in the middle ear causes middle ear otitis media, which causes the neck to twist and deviate (10). This mycoplasma contamination causes severe respiratory problems in mice and laboratory rats, and it causes infection of the reproductive system in mice (11). The prevalence of this disease among the experimental animals is between 20-60%. It also colonizes the trachea and throat, causing pneumonia and genital diseases, thus reducing reproductive rates (12). The transmission of the mycoplasma during fetal development occurs in two ways: by transmission through amniotic fluid or invasion of a pair and by transmission due to intrauterine infection or during implantation (13). In 1984, Furr

and Robinson found that, *the M. pulmonis* infection in the vaginal tract of TO and CBA mice was greatly cured with the help of hormonal therapy (progesterone), which was used to treat 33% of TO and 50% of RBC mice (14). In 2002, Barto et al. isolated bacteria from M. pulmonis from Mus musculus mice with symptoms of respiratory disease (15) In 2014, Shafaati et al., isolated the molecular identification of *M. pulmonis* from rat respiratory tract using PCR on the 16s rRNA gene, this being the first study in Iran to isolate *M. pulmonis* from the respiratory tract of rats (16).

Mycoplasma neurolyticum

In 1938, the bacteria were first isolated from the brain of the mice (17). In 1965, *M. neurolyticum* was isolated from the nasal mucous membranes and lungs of carrier animals, which showed no clinical symptoms (18). In 1979, Hill, studied *M. neurolyticum* in mice and rats, and the results showed that 78% of rats and 58% of mice with *M. neurolyticum* were infected (19). Taley, in 1981, described the bacteria as a mammalian brain organism that remains stressed and causes nerve disorders (20).

Mycoplasma collis

In 1983, these bacteria were isolated from the nasal cavity and conjunctiva of mice and rats for the first time (21). This species of mycoplasma grows in an environment of pH=7.8 at an optimal temperature of 35°C in anaerobic conditions. Some researchers describe this species of mycoplasma as mycoplasma in dogs, but *M. collis* was originally identified in rodents (22). So far, no accurate and complete report has been made on this mycoplasma species.

Mycoplasma muris

In 1983, McGarrity et al. a study based on the immune response mice led to the identification of the mycoplasma (23). In this study, all mice were pregnant and had tumors. The age group of the mice was three to 10 months old, which based on the morphological similarity with the mycoplasma, a new species of mycoplasma called Muris came into existence (23).

M. muris are small pathogenic bacteria that lives in the genital tract of female mice (23). Infection by *M. muris* may have harmful effects on the reproductive health of female mice (23). Weisburg et al., based on the 16s rRNA gene, identified M. muris as the ancestors of the group of pneumonia, which consists of three distinct clusters of M. pneumonia, *M. muris*, and *Ureaplasma urealyticum* (24). Van Kuppeveld et al. designed specific primers for nine species of mycoplasma for humans and rodents (including the five species mentioned in this article) from 16s rRNA and evaluated them with the PCR test (25). In 2017, Zinatizadeh et al. identified this rare mycoplasma in NIH mice from Razi Vaccine and Serum Research Institute, Alborz, Iran. A total of 18% of the NIH rats were infected with M. muris in the Department of Animal Breeding, Razi Vaccine and Research Institute, and by using a phylogenetic analysis, a new species of M. muris was recorded in the gene bank (26).

Mycoplasma arthritidis

This mycoplasma infection is not common and is usually found in large laboratory mice. *M. arthritidis* causes arthritis of the joints in the mice (27). Some researchers believe that the microorganism enters the body through the mouth and mucous membranes, and there may be a latent infection (27). The clinical signs include swelling of the fingers and legs (28). This species of mycoplasma grows in a neutral pH medium (7.0) at an ideal temperature of 37°C, and is able to grow in the presence or absence of oxygen (29). The growth of *M. arthritidis* depends on the culture medium. In fiber tissue, it expands as a dense mass in the center, which requires sugar, proteins, amino acids, vitamins, and nucleic acids for growth (29).

Conclusion

Mycoplasma contamination has an adverse effect on laboratory animals, which interferes with the results of the researches conducted in laboratories. The presence of mycoplasma should be monitored for this reason. It is significant that many of the contaminated laboratory animals show no clinical symptoms. Therefore, it is important that health monitoring programs are implemented as a quality control for animals used in laboratory research.

Conflict of Interest

Authors declared no conflict of interest.

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