Isolation and Identification of Mycoplasma Strains from Various Species of Wild Rodents

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ABSTRACT. Attempts were made to isolate mycoplasmas from respiratory and urogenital tracts of 35 apparently healthy wild rodents comprising 7 species under 4 genera. Mycoplasmas were isolated from nasal and oral cavities, tracheas, vaginas and penises of the wild rats: ricefield rats (*Rattus argentiventer*), roof rats (*R. rattus*) and Polynesian rats (*R. exulans*), but none was isolated from brown rats (*R. norvegicus*), house mice (*Mus musculus*), smithi's voles (*Eothenomys smithi*) and soft-furred field rats (*Millardia meltada*). These mycoplasma strains were identified as *Mycoplasma pulmonis* and *M. arthritidis* on the basis of their biological and serological properties. —KEY WORDS: mycoplasma, wild rodent.

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Although mycoplasmas have been isolated from various domestic and experimental animals, few reports are available concerning isolations of these organisms from wild rodents [3, 4, 9]. This report concerns some results of the isolation of mycoplasmas from various wild rodents, and the biological and serological characteristics of these isolates.

A total of 35 apparently healthy wild rodents comprising 7 species under 4 genera were examined for mycoplasmas. They were captured in various places of Japan and other countries including Indonesia and India (Table 1). Some of them were delivered to the Experimental Animal Center of Miyazaki Medical College, Miyazaki, Japan and were kept for some periods of time at the isolated rooms. The rest were directly sent to the Laboratory of Veterinary Microbiology, Faculty of Agriculture of Kagoshima University as soon as possible after their capture.

The reference mycoplasma strains, *Mycoplasma pulmonis* m53 and *M. arthritidis* PG6, were supplied by the Department of Veterinary Microbiology, Faculty of Agriculture, the University of Tokyo.

Media and procedures for isolation of mycoplasmas were described previously [5, 6]. For the isolation of ureaplasmas, the media contained 0.01% of urea and 0.004% of phenol red, with pH adjusted to 6.0±0.2. To discriminate mycoplasmas from L-form of bacteria, the isolates were subcultured 3 times in liquid medium without antibacterial agents. Serum requirement of the isolates was determined after 3 passages in serum-free liquid and solid media. Fermentation of glucose, hydrolysis of urea and arginine, and phosphatase activity were tested by the methods of Aluotto *et al.* [1].

For serological characteristics, mycoplasma strains were subjected to the growth inhibition test [2] as well as the metabolism inhibition test [8]. The rabbit antisera against mycoplasma strains were prepared by the method described previously [7].

Mycoplasmas were isolated from nasal cavities (4/4), oral cavities (4/4), tracheas (4/4), vaginas (2/2) and penises (2/2) of *Rattus argentiventer*, from nasal cavities (4/7), oral cavities (3/7), and tracheas (3/7) of *R. rattus*, and from nasal cavities (6/9), oral cavities (5/9) and tracheas (4/9) of *R. exulans*. No mycoplasma was detected from any site of *R. norvegicus, Eothenomys smithi, Millardia meltada* and *Mus musculus* (Table 1).

A total of 47 cloned strains comprising 19 derived from *R. argentiventer,* 10 from *R. rattus* and 18 from *R. exulans* did not convert to bacterial form. They were proved to require serum for growth and not to hydrolyze urea, and were differentiated into two groups by their biological characteristics. One comprising 38 strains fermented glucose within 48 hours but did not hydrolyse arginine, and another comprising 9 strains decomposed agrginine but not glucose (Table 2). Neither ureaplasma nor acholeplasma species was isolated.

In serological tests, it was shown that the hyperimmune serum against *M. pulmonis* m53 inhibited the growth and the metabolism of the 38 mycoplasma strains which fermented glucose. On the other hand, antiserum against *M. arthritidis* PG6 inhibited the growth and the metabolism of the 9 strains which hydrolyzed arginine (Table 2).

On the basis of the biological and serological properties, it was concluded that the 38 strains which were derived from *R. argentiventer, R. rattus* and *R. exulans* were identified as *M. pulmonis*, and the 9 strains from *R. argentiventer* as *M. arthritidis*.

This survey revealed the existence of both *M. pulmonis* and *M. arthritidis* in wild rats captured at different places of field. It might be possible that the prevailing contamination of these mycoplasmas took place among some captured wild rats when they were kept in the rooms of the Experimental Animal Center of Miyazaki Medical College. Therefore, the findings of the survey may not present the natural ecological features of mycoplasmas in wild rodents, but the fact that pathogenic mycoplasmas harbor in wild rodents is important enough to appeal for attention, especially when the application of wild rodents for experimental purposes in the field of biomedical science becomes popular at present.

Contrary to *M. pulmonis* and *M. arthritidis* in laboratory mice and rats, the knowledge concerning the ecological and pathogenic situations of these organisms in wild rodents is limited. The pathogenicity of these mycoplasma isolates to wild rodents and laboratory mice and rats are subjected to further study.

Table 1. Isolation of mycoplasmas from various sites of wild rodents

Animal examined			Positive for mycoplasma				
Species	Origin	Head	Nasal cavity	Oral cavity	Trachea	Vagina	Penis
Ricefield rat[a] (*Rattus argentiventer*)	Indonesia: Jatisari	4	4	4	4	2	2
Roof rat (*R. rattus*)	Japan: Miyazaki	1	1	1	1	0	ND[b]
	Tokyo	3	2	2	2	0	0
	Kagoshima	3	1	0	0	0	0
Polynesian rat[a] (*R. exulans*)	Indonesia: Sulawesi	9	6	5	4	0	0
Brown rat (*R. norvegicus*)	Japan: Shizuoka	3	0	0	0	0	0
House mouse (*Mus musculus*)	Japan: Chiba	5	0	0	0	ND	0
Smithi's vole[a] (*Eothenomys smithi*)	Japan: Shikoku & Kyushu	4	0	0	0	0	0
Soft-furred field rat[a] (*Millardia meltada*)	India: Bangalore	3	0	0	0	0	ND
Total		35	14	12	11	2	2

a) These animals had been maintained at the Experimental Animal Center of Miyazaki Medical College before being received for isolation of mycoplasmas. b) Not done.

Table 2. Biological and serological properties of mycoplasmas isolated from wild rodents

Origin of mycoplasma	No. of strain tested	Biological test			Serological test				
		Glucose fermentation	Arginine hydrolysis	Phosphatase activity	Antiserum against *M. pulmonis* GI[a]	*M. pulmonis* m53 MI[b]	*M. arthritidis* GI	*M. arthritidis* MI	PG6
R. argentiventer	10	+	−	−	+	+	−	−	
R. rattus	10	+	−	−	+	+	−	−	
R. exulans	18	+	−	−	+	+	−	−	
R. argentiventer	9	−	+	+	−	−	+	+	

a) Growth inhibition test, b) Metabolism inhibition test.
Growth inhibitory zones were 1–3 mm and metabolism inhibition titers were ×2,500~×5,120.

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