Isolation of Mycoplasmas from Experimental Ferrets (*Mustela putorius*)

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A total of 21 apparently healthy experimental ferrets, 8 males and 13 females, comprizing 1, 2 and 3 year-old animals were examined for Mycoplasmatales. Mycoplasmas were isolated from 17 samples of 21 oral cavities (81.0%), and from 2 of 21 nasal cavities (9.5%), but not from the prepuce or vagina of the animals. Neither ureaplasma nor acholeplasma was demonstrated in any of the locations of the ferrets examined. These mycoplasma strains proved to metabolize glucose but not arginine and urea. The growth inhibition test revealed that all the strains had similar antigenicity but were not related antigenically to any reference strains from dogs, cats, sheep, cattle, mice, raccoon dogs and a Japanese badger. They are the first mycoplasmas to be isolated from ferrets.

It is well known that among carnivorous animals, dogs and cats harbor mycoplasmas as well as ureaplasmas[3,5,7-12]. However, there has been no study on Mycoplasmatales in ferrets (*Mustela putorius*).

An attempt has recently been made at our laboratories to isolate mycoplasmas and ureaplasmas from ferrets, with the result of the demonstration of mycoplasmas from the respiratory and urogenital tracts of these animals. This report presents the results including the findings concerning the biological and serological characteristics of these isolates.

A total of 21 apparently healthy ferrets, 8 males and 13 females, comprizing 1, 2 and 3 year-old animals were examined. They had been kept at the Hikari Plant, Takeda Chemical Industries to be used as laboratory animals.

Both liquid and solid media were used for the isolation of mycoplasmas and ureaplasmas as described previously by the present authors[8,9]. Sterile cotton swabs were passed over the oral and nasal cavities or prepuce or into the vagina of the animals to collect mucus specimens to make culture. The inoculated liquid cultures were incubated at 37°C for a week, and tubes showing color changes were subcultured. The solid media were incubated at 37°C for 5 days in a mixture of 95% N₂ and 5% CO₂. Antisera used for the growth inhibition test[2] were prepared by immunizing rabbits with cloned strains by the procedures described previously[7].

As can be seen from Table 1, regardless of the age and sex of the ferrets, mycoplasmas were isolated from 17 samples of 21 oral cavities (81.0%), and from 2 of 21 nasal cavities (9.5%), but not from the

prepuce or vagina. The biological and serological properties of 28 strains cloned from these colonies were investigated and two type strains were designated as FR 5-O-2 and FR 10-O-5, respectively. None of these strains were converted into bacterial form after 3 passages in liquid medium devoid of bacterial inhibitors. As shown in Table 2, they failed to grow in serum-free medium, and were sensitive to 2.5% digitonin[4]. They also proved to metabolize glucose but not arginine and urea[1]. The reduction of tetrazolium chloride and phosphatase activity were found to be negative, but they produced film and spots on solid media. The growth inhibition test revealed that all the mycoplasma strains isolated from the ferrets had similar

antigenicity but were not related antigenically to any reference strains derived from dogs, cats, sheep, cattle, mice as shown in Table 3. Besides, they had no antigenic relations to those recently isolated from raccoon dogs (*Nyctereutes procyonoides viverrinus*) and a Japanese badger (*Meles meles anakuma*) by the present authors[6].

In this survey, neither ureaplasma nor acholeplasma was demonstrated in any of the locations of the ferrets examined. The ecological or etiological significance of these mycoplasmas isolated from ferrets is unknown. Although further studies will be necessary to determine whether these mycoplasmas constitute new species or not, they are the first mycoplasmas to be isolated from ferrets.

Table 1. Isolation of mycoplasmas from ferrets

Site tested	No. of examined	No. of positive (%)
Oral cavity	21	17 (81.0)
Nasal cavity	21	2 (9.5)
Prepuce	8	0
Vagina	13	0

Table 2. Biochemical reactions of mycoplasma strains isolated from ferrets

Characteristic	Reaction of strains	
	FR 5-O-2	Others (27 strains)
Serum requirement	+	+
Sensitivity to digitonin	+	+
Fermentation of glucose	+	+
Hydrolysis of arginine	-	-
Hydrolysis of urea	-	-
Reduction of tetrazolium chloride (aerobic/anaerobic)	-/-	-/-
Production of phosphatase (aerobic/anaerobic)	-/-	-/-
Production of "film and spots"	+	+

Table 3. Serological comparison between mycoplasma strains from ferrets and other reference strains determined by growth inhibition test

Species and strain	Origin	Antiserum against													
		FR 5-O-2	FR 10-O-5	PG 14	PG 24	H 542	H 831	PG 13	PG 15	MH 5408	CO	Ben	Mart	G 230	PG 11
<i>Mycoplasma</i> sp.	FR 5-O-2	4*	5	-**	-	-	-	-	-	-	-	-	-	-	-
<i>Mycoplasma</i> sp.	FR 10-O-5	4	5	-	-	-	-	-	-	-	-	-	-	-	-
<i>M. canis</i>	PG 14	-	-	4	-	-	-	-	-	-	-	-	-	-	-
<i>M. edwardii</i>	PG 24	-	-	-	4	-	-	-	-	-	-	-	-	-	-
<i>M. molare</i>	H 542	-	-	-	-	3	-	-	-	-	-	-	-	-	-
<i>M. cynos</i>	H 831	-	-	-	-	-	3	-	-	-	-	-	-	-	-
<i>M. spumans</i>	PG 13	-	-	-	-	-	-	4	-	-	-	-	-	-	-
<i>M. maculosum</i>	PG 15	-	-	-	-	-	-	-	5	-	-	-	-	-	-
<i>M. opalescens</i>	MH 5408	-	-	-	-	-	-	-	-	3	-	-	-	-	-
<i>M. felis</i>	CO	-	-	-	-	-	-	-	-	-	5	-	-	-	-
<i>M. feliminutum</i>	Ben	-	-	-	-	-	-	-	-	-	-	3	-	-	-
<i>M. gateae</i>	Mart	-	-	-	-	-	-	-	-	-	-	-	5	-	-
<i>M. arginini</i>	G 230	-	-	-	-	-	-	-	-	-	-	-	-	4	-
<i>M. bovis genitalium</i>	PG 11	-	-	-	-	-	-	-	-	-	-	-	-	3	-
<i>M. pulmonis</i>	m 53	-	-	-	-	-	-	-	-	-	-	-	-	-	8
<i>M. neurolyticum</i>	PG 28	-	-	-	-	-	-	-	-	-	-	-	-	-	7
<i>Mycoplasma</i> sp.	LM 2	-	-	-	-	-	-	-	-	-	-	-	-	-	4
<i>Mycoplasma</i> sp.	AGM 1	-	-	-	-	-	-	-	-	-	-	-	-	-	2

* The width (mm) of a growth inhibitory zone.

** No growth inhibition.

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実験用フェレットからのマイコプラズマ分離

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1, 2 年および 3 年齢を含む雄 8 匹, 雌 13 匹, 計 21 匹の外見的に健康な実験用フェレットからマイコプラズマの分離をこころみた。

マイコプラズマは口腔 21 検体中 17 (81.0%) および鼻腔 21 検体中 2 (9.5%) から分離されたが, 包皮あるいは膣からは分離されなかった。これらのマイコプラズマ株はブドウ糖を分解したがアルギニンおよび尿素は分解

しなかった。発育阻止試験により血清学的性状を調べたところ, 分離株はすべて類似の抗原性を示したが, イヌ, ネコ, ヒツジ, ウシ, マウス, タヌキおよびアナグマ由来の参照株とは抗原的に関係が認められなかった。

フェレットからのマイコプラズマ分離報告はこれが最初である。