Atypical Mycobacteria with Reference to Porcine Mycobacteriose in Japan

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In veterinary medicine the increasing attentions have been paid to the potential role of atypical mycobacteria as opportunist pathogens in animal health as a result of the successful control of bovine tuberculosis, as reviewed by Kazda[1].

This tendency will be partially explained from the fact that one is tempted to interpret the occurrence of the so-called no visible lesion reactors, a very perplexing problem in the tuberculin testing of cattle, being due to tuberculin hypersensitivity resulting from the infection or sensitization of cattle with atypical mycobacteria. On the other hand, irrespective of the successful control of bovine tuberculosis in advanced countries, the frequent occurrence of tuberculous lesions in lymph nodes of pigs still exist and the isolates from these lesions proved to be frequently mycobacteria other than the authentic pathogenic tubercle bacilli.

In human medicine the infection of atypical mycobacteria is a current problem and many knowledges concerning them are now available.

In the following a berief description of atypical mycobacteria will be made, with the reference to our experiences concerning porcine mycobacteriose.

Bacteriology of atypical mycobacteria

Runyon[2] classified many strains of atypical mycobacteria into 4 groups, based on the colonial pigmentation and the growth speed as shown in Table 1.

Table 1. Classification of atypical mycobacteria

Photochromogen	*M. kansasii*
	M. marinum (*M. balnei*)
Scotochromogen	*M. scrofulaceum* (*M. marianum*)
	M. gordonae
	M. flavescens
Nonphotochromogen	*M. avium*
	M. intracellulare
	M. xenopi
	M. terrae
	M. gastri
	M. triviale
Rapid grower	*M. fortuitum*
	M. chelonei (*M. abscessus*. *M. borstelense*)
	M. smegatis (*M. butyricum*. *M. lacticola*)
	M. phlei
	M. vaccae
	M. diernhoferi

The name in parenthesis is synonym.

The colonies of photochromogens are non-pigmented when cultivated in the darkness, but become yellow pigmented when incubated further after the exposure to the light. *Mycobacterium marinum* and *M. kansasii* are potential pathogens, and *M. marinum* must be incubated at 30°C because of its inability to grow at 37°C for the isolation.

In contrast to photochromogens the colonies of scotochromogens are yellow pigmented even when cultivated in the darkness, and only *M. scrofulaceum* among them is potential pathogen in human medicine and sometimes isolated from animals. The other two species

are saprophytes and *M. gordonae* is alike to tap water scotochromogens in the cultural characteristics. *M. flavescens* showing rough colonial appearance is often included into rapid growers due to its rather speedy growth.

The colonies of nonphotochromogens are usually nonpigmented, and *M. intracellulare, M. xenopi, M. terrae, M. gastri* and *M. triviale* are included in this group and sometimes *M. avium* is also included from its close resemblance with *M. intracellulare* in its biochemical characters. *M. intracellulare* and *M. xenopi* are potential pathogens and the former is frequently isolated from human and animal materials and consists of many serotypes as demonstrated by Schaefer[9]. *M. xenopi* is yellow pigmented, but usually included into nonphotochromogen for convenience. The other nonphotochromogens are saprophytes.

Many species of mycobacteria found in the surroundings which usually grow within 10 days are named rapid growers and among them *M. fortuitum* and *M. chelonei* are potential pathogens.

Many identification methods and techniques are available at present, and the methods employed for clinical microbiology must be no elaborate and time-consuming ones because the differentiation of potential pathogens from saprophytic mycobacteria is important as pointed out by Runyon[8].

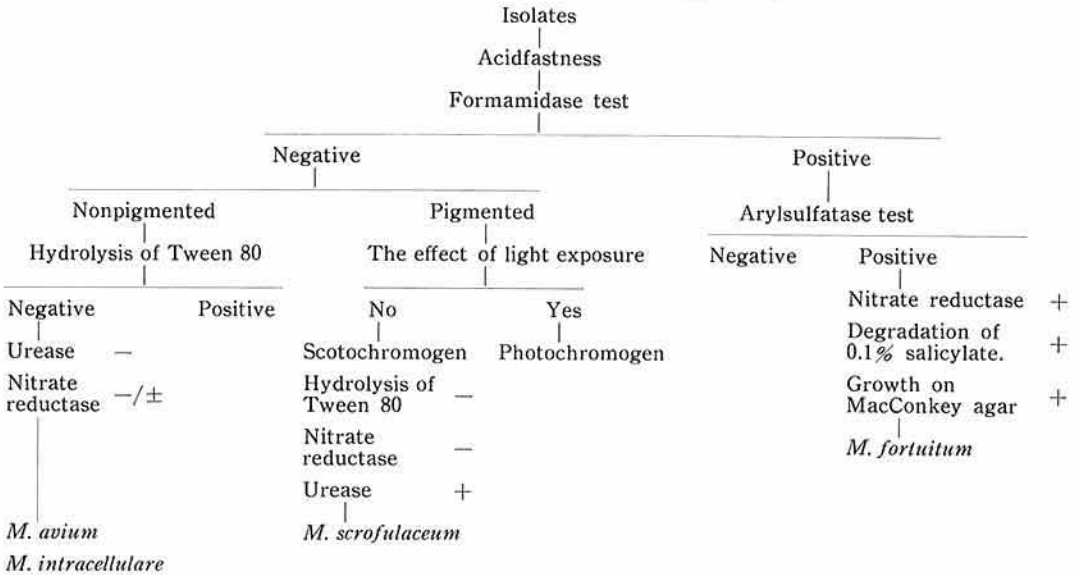
For these reasons the identification schema shown in Table 2 is employed for the presumptive identification of mycobacterial isolates in the author's laboratory.

Usually rapid growers are positive for formamidase test[16] and further differentiation of *M. fortuitum* and *M. chelonei* from the others is made employing arylsulfatase test[5]. The arylsulfatase negative strains may be saprophytes. For the confirmation of *M. fortuitum* nitrate reductase test[3], degradation of 0.1% salicylate[13] and the growth on MacConkey agar are examined, because *M. fortuitum* is usually positive to these tests.

Formamidase negative isolates are differentiated based on the colonical pigmentation into photo-, scoto- and nonphotochromogens respectively. The difference in the cultural temperature is useful for the identification of *M. marinum* as stated above. *M. scrofulaceum* is positive for urease test[12] and negative for Tween 80 hydrolysis[14] and nitrate reductase tests.

For the differentiation of nonphotochromogens the Tween 80 hydrolysis test is indis-

Table 2. The presumptive identification of atypical mycobactria



pensable and the test-positive mycobacterial isolates are saprophytes. *M. avium* and *M. intracellulare* are negative for urease and nitrate reductase tests and serological identification and chicken inoculation test can be carried out for the further identification.

In practise *M. avium-intracellulare-scrofulaceum* complex and *M. fortuitum* complex are frequently isolated from clinical materials.

Porcine mycobacteriose in Japan

Many papers concerning porcine mycobacteriose[2,6,11] were published in foreign countries. In the following, studies in Japan are presented.

The identification of mycobacterial isolates from tuberculoid lesions in the mandibular lymph nodes of pigs was attempted in the author's laboratry. From the first survey at Tachikawa slaughter-house in 1970, 4 nonchromogenic rapid growers, 4 scotochromogens and 21 nonphotochromogens were obtained, and 3 rapid growers proved to be *M. fortuitum* and 4 scotochromogens to be *M. scrofulaceum* respectively. Among 21 nonphotochromogens 6 urease-negative and Tween 80 hydrolysis test-positive strains, and 3 urease and nitrate reductase-positive and Tween 80 hydrolysis-negative strains were detected. The remaining 12 ones proved to be *M. intracellulare*. From the second survey in 1971, 10 nonchromogenic and 2 chromogenic rapid growers, 1 scotochromogen and 25 nonphotochromogens were obtained. Ten nonchromogenic rapid growers proved to be *M. fortuitum* and the other chromogenic strains to be saprophytes respectively. Among 25 nonchromogens 13 urease-negative and Tween 80 hydrolysis-positive strains and 1 urease and nitrate reductase-positive and Tween 80 hydrolysis-negative strain were detected respectively. The remaining 11 nonphotochromogens proved to be *M. intracellulare*. The serotypes of these isolates of *M. intracellulare* according to Schaefers classification are shown in Table 3.

Nine mycobacterial isolates from pigs at Hiroshima were made available and 8 strains proved to be *M. intracellulare* and the other one to be *M. fortuitum* respectively.

Yachida et al. proved many mycobacterial isolates from tuberculoid lesions in mesentric lymph nodes of pigs in Hokkaido to be *M. intracellulare* as their serotypes were those shown in Table 3.

Table 3. Serotypes of *M. avium-intracellulare* complex of porcine origin in Japan

Serotypes	Origin of isolates			Total
	Tachikawa	Hiroshima	Hokkaido	
III a	0	0	2	2
III c	1	0	0	1
IV	5	3	1	9
Davis	7	1	62	70
Watson	4(1)	3	0	7
New type (T 96)	2	0	1	3
New type (116)*	3	1	0	4
Dent	1	0	0	1
Altman	2(1)	0	0	2
Avium 3	1(1)	0	0	1
Unidentified	0	0	4	4
Total	26	8	70	104

* This strain was identified to be a new serotype, but later found to be M. avium serotype 2 by Dr. Schaefer.

From these findings it seems true that *M. intracellulare* is an important causal agent of porcine mycobacteriose in Japan. This situation is different from those in foreign countries[4,10] where *M. avium* is frequently isolated from pigs.

In our study the above mentioned urease and nitrate reductase-positive and Tween 80 hydrolysis-negative nonphotochromogens reacted positive in Schaefers agglutination tests as their serotypes shown in parenthesis in Table 3. Urease-negative and Tween 80 hydrolysis-positive nonphotochromogens were subdivided by nitrate reductase test or the tolerance to 5% NaCl in Ogawa egg media because nitrate reductase negative strains were tolerant to 5% NaCl.

The virulence of an isolate of *M. intracellulare* serotype Davis for pigs was examined. The infecting dose of 8×10^6 and 8×10^7 viable units were inoculated into the submucosa of mouth angle of each 4 pigs respectively and each two infected pigs were killed for examination at 1 month and 1 and half month later respectively.

The macroscopic tuberculoid lesions in the mandibular lymph nodes and the inoculated sites were detected in all 4 pigs infected with a larger infecting dose. When the infecting dose was reduced the lesions were detected in th pigs killed one month later. The recovery of the infecting bacilli failed in one pig having no lesion.

The response to avian PPD tuberculin was about twice more stronger than those to mammalian PPD tuberculin when tested one month later.

Further studies will be needed for the elucidation of pathogenesis and epidemiology of atypical mycobacterial infection in domestic animals.

The studies concerning porcine mycobacteriose were carried out in association with Drs. Yugi and Watanabe.

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