Mycobacteriocin Classification of Rapidly Growing Mycobacteria

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Since bacteriocins of mycobacteria could be demonstrated by our technique, bacteriocin classification of rapidly growing mycobacteria was attempted. The results coincide well with those obtained by other methods of classifying and differentiating these organisms.

Bacteriocinogeny is known to be a stable genetic character, and typing of bacteria within a species using bacteriocin production as a marker has been successfully applied to *Shigella sonnei* (1) and *Pseudomonas aeruginosa* (2). However, there have been difficulties in demonstrating mycobacteriocins, and the bacteriocin typing of mycobacteria has not been possible.

Recently we have succeeded in developing a technique for detecting bacteriocins produced by mycobacteria and, based on this technique, mycobacteriocin classification of rapidly growing mycobacteria was attempted.

A total of 79 strains of rapidly growing mycobacteria were studied for production of mycobacteriocin: 1 strain of *Mycobacterium abscessus*, 12 strains of *M. runyonii*, 5 strains of *M. parafortuitum*, 5 strains of *M. chitae*, 17 strains of *M. fortuitum*, 1 strain of *M. minetti*, 1 strain of *M. peregrinum*, 4 strains of *M. smegmatis*, 1 strain of *M. lacticola*, 2 strains of *M. butyricum*, 5 strains of *M. vaccae*, 6 strains of *M. aurum*, 3 strains of *M. phlei*, 3 strains of *M. diernhoferi*, and 5 strains of *M. flavescens*. Most of them were received from the American Type Culture Collection. The set of indicator strains used to detect bacteriocin consisted of *M. diernhoferi* ATCC 19340-S and 19340-R, *M. smegmatis* (*M. lacticola*) ATCC 19980, *M. phlei* ATCC 11758, *M. phlei* 14003 (obtained from Dr. Tsukamura), *M. fortuitum* (*M. minetti*) ATCC 19542, and *M. acapulcensis* ATCC 14473. Of these, *M. diernhoferi* ATCC 19340 showed the widest sensitivity spectrum and was sensitive even to the bacteriocin produced by itself.

The "streak-plate" method (1) on heart infusion-agar medium containing 4% glycerin and 0.05% Tween 80 was used for demonstrating mycobacteriocin. After a test organism was grown as a streak for 48 or 72 hr at 37 C on the

typing medium, the growth was killed (with chloroform vapor for 1 hr) and then scraped off. Thereafter, seven indicator strains were inoculated as parallel lines at right angles to the position of the original inoculum. Reading of the results was done after 48 or 72 hr of

FIG. 1. Inhibition pattern of mycobacteriocin 1A. Order of indicator strains from the top is: *M. diernhoferi* ATCC 19340-S, *M. diernhoferi* ATCC 19340-R, *M. smegmatis* (*M. lacticola*) ATCC 19980, *M. phlei* ATCC 11758, *M. phlei* 14003, *M. fortuitum* (*M. minetti*) ATCC 19542, and *M. acapulcensis* ATCC 14473.

incubation at 37 C. By using seven indicator strains as stated above, rapidly growing mycobacteria could be grouped into 11 bacteriocin subdivisions (Table 1).

The preliminary results of mycobacteriocin classification are shown in Table 2. The present

TABLE 1. Tentative mycobacteriocin classification scheme

Indicator strains	Bacteriocin patterns ^a										
	1A	1B	2	2A	3	4	5	6	7	8	9
<i>M. diernhoferi</i> (19340S) . . .	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
<i>M. diernhoferi</i> (19340R) . .	+++	+++	+++	+++	+++	+++	+++	+++	—	+	+++
<i>M. smegmatis</i> (19980) (<i>M. lacticola</i>)	—	+++	—	++/+	—	+++	—	—	—	+	+++
<i>M. phlei</i> (11758)	+++	+++	+++	—	—	—	—	—	—	—	+++
<i>M. phlei</i> (14003)	+++	+++	+++	+++	+++	—	—	+++	—	++	—
<i>M. fortuitum</i> (19542) (<i>M. minetti</i>)	—	—	—	—	—	—	—	—	—	—	+++
<i>M. acapulcensis</i> (14473) . . .	—	—	+/-	—	++	—	—	—	—	+++	+++

^a Symbols: + to +++, width of inhibition zones measured 5 to 10, 11 to 20, and 21 mm or greater; ±, thinning out of growth in the central area; —, resistant; +/-, ++/+, different strains giving either of the reactions.

TABLE 2. Bacteriocin classification of rapid growers (preliminary results)

Species	Bacteriocin patterns ^a								
	1A	1B	2	2A	3	4	5	6	7
<i>M. chelonae</i> (<i>M. abscessus</i>) (<i>M. runyonii</i>)	1 } 13 12 } 5	5	17 } 1 } 1 } 4 } 23						
<i>M. chitae</i>									
<i>M. parafortuitum</i>									
<i>M. fortuitum</i>									
<i>M. giae</i>									
<i>M. minetti</i>									
<i>M. peregrinum</i>									
<i>M. aurum</i>				6					
<i>M. vaccae</i>				5					
<i>M. smegmatis</i>							4 } 1 } 7 2 }		
<i>M. lacticola</i>									
<i>M. butyricum</i>									
<i>M. phlei</i>									
<i>M. diernhoferi</i>								3	
<i>M. flavescens</i>									5

^a Since only 1 or 2 strains belonging to types 6, 8, and 9 have been tested so far, they will be reported on in the future after more strains have been examined.

data reveal that, instead of typing within a species, grouping or differentiation of species was made possible by the pattern of mycobacteriocin activity. *M. runyonii* is considered to be synonymous with *M. abscessus* (3), which has recently been displaced by *M. chelonae* (Kubica et al., submitted for publication). All strains designated as either *M. abscessus* or *M. runyonii* fell into bacteriocin pattern 1A. All strains of *M. chitae* also belonged to this pattern. According to recent investigations (3; Kubica et al., submitted for publication; Takeya and Muraoka, Unpublished data), *M. giae*, *M. Minetti*, and *M. peregrinum* are considered synonymous with *M. fortuitum*. All of these organisms showed bacteriocin pattern

2. Bacteriocin pattern 2A characterizes strains of *M. aurum* and *M. vaccae*. All strains of *M. smegmatis*, including those labeled *M. lacticola* and *M. butyricum*, which are known to be synonymous with *M. smegmatis* (3), exhibited bacteriocin pattern 3. Strains of *M. phlei*, *M. diernhoferi*, and *M. flavescens* showed bacteriocin patterns 4, 5, and 7, respectively.

The results given here are only preliminary, and some of the bacteriocin classification may undergo further subdivisions even within a species when more indicator strains are found to be available. However, the present data indicate that bacteriocin production provides an important genetic character useful for both taxonomy and species identification.

LITERATURE CITED

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