Differentiation of Known Strains of BCG from Isolates of *Mycobacterium bovis* and *Mycobacterium tuberculosis* by Using Mycobacteriophage 33D

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The use of mycobacteriophage 33D to differentiate strains of BCG from isolates of *Mycobacterium bovis* was investigated. The procedure was found to be reproducible and, using the commercially available media described, can be recommended for use in mycobacterial reference laboratories.

The current use of BCG in the treatment of tumors (2, 10) has stimulated investigations with the different strains of BCG. A recent report of local and disseminated infection in cancer patients undergoing BCG therapy (2) and the reinvestigation of the variation in the degree of virulence among different strains of BCG (8) raises the question of the identification of BCG isolates submitted to the diagnostic laboratories. At the present time the only means of differentiating strains of BCG from virulent strains of *Mycobacterium bovis* is animal pathogenicity tests (6, 9), which are both time consuming and expensive. Mycobacteriophage 33D has been reported to differentiate strains of BCG from *M. bovis* (3, 4). The present study was undertaken to evaluate the use of mycobacteriophage 33D for the separation of recently isolated strains of *M. bovis* from known strains of BCG and to modify the procedure so that it could be used in reference laboratories performing tests for the identification of mycobacterial isolates.

A total of 133 mycobacterial cultures was used in this study: 80 strains of *M. tuberculosis*; 45 strains of *M. bovis*; and 8 strains of BCG. The source of these cultures is listed in Table 1. The niacin and nitrate reductase tests (9) were performed on all cultures before the phage testing. Since susceptibility of strains of *M. bovis* to 10 μ g of thiopen-2-carboxylic acid hydrazide per ml is used to separate *M. bovis* from *M. tuberculosis* (9), this test was also performed on all 133 test cultures. The results of the niacin, nitrate reductase, and thiopen-2-carboxylic acid hydrazide tests are listed in Table 2.

Mycobacteriophage DS6A (indicator host strain *M. tuberculosis* H₃₇R_v) and phage 33D (indicator host strain *M. smegmatis* ATCC 607)

were obtained from W. B. Redmond. High-titered phage lysates were prepared by the methods described earlier (5, 7). All cultures were grown in 7H9 broth, 7H9 broth base (Difco), and 0.25% Tween 80 (Atlas Powder Co.). Before phage testing, the cultures were centrifuged at 5,000 rpm for 15 min and the pelleted cells were resuspended in heart infusion base broth (heart infusion base [Difco] supplemented with 0.02 M calcium chloride). Phage tests were performed by using the soft-agar overlay method of Adams (1). The hard basal medium was oleic acid-albumin agar base (BBL) supplemented with Dubos oleic acid-albumin complex (Difco). The soft agar was Dubos broth base, without Tween 80 (Difco), supplemented with 0.7% agar. These media were prepared and used as described earlier (5). The routine test dilution of each phage was determined on the respective phage host strain (7).

For phage testing, 0.5 ml of the heart infusion base bacterial suspension (approximately 10⁷ bacteria/ml) was placed in the soft-agar plate. The soft agar was allowed to harden at room temperature. The inoculated plates were placed in a 37 C incubator and dried overnight. The next day fresh routine test dilutions were made for each of the two phages and the inoculated plates were spotted with the phages. The plates were then returned to the 37 C incubator. The plates were examined for lysis from the 4th through 7th days of incubation.

The results of the phage tests are listed in Table 1. All of the 133 mycobacterial strains tested were lysed by mycobacteriophage DS6A. The strains of *M. tuberculosis* and *M. bovis* were also lysed by phage 33D whereas the strains of BCG were not. The results of the phage susceptibility tests are listed in Table 1.

TABLE 1. Susceptibility of strains of *M. tuberculosis*, *M. bovis* and BCG to mycobacteriophages DS6A and 33D

Mycobacterial strain	Phage ^a	
	DS6A	33D
BCG ^b		
Galaxo	+	0
Tice	+	0
Russian	+	0
Danish	+	0
Canadian	+	0
Berkhaug	+	0
Japanese	+	0
French	+	0
<i>M. bovis</i>		
Type (TMC 410) ^c	+	+
CDC (14 strains) ^b	+	+
Iowa (30 strains) ^d	+	+
<i>M. tuberculosis</i>		
Type culture (TMC 102) H37Rv ^c	+	+
79 wild strains	+	+

^a Phages DS6A and 33D were used at routine test dilutions of 10⁶ and 10⁴ plaque-forming units per ml, respectively. +, Lysis; 0, no lysis.

^b Strains from the stock culture collection of the Center for Disease Control.

^c Provided by the U.S.-Japan Cooperative Medical Science Program of the National Institute of Allergy and Infectious Diseases.

^d Animal isolates received from C. O. Thoen, Veterinary Services Diagnostic Laboratory, Ames, Iowa.

Each of 133 strains of mycobacteria was phage tested three separate times to test the reproducibility of the phage testing, and the results were the same as in Table 1. Also, both phage DS6A and 33D are quite stable in the undiluted lysates and can be stored in the refrigerator for 12 months without a significant loss of titer.

The results of this study confirm the report of Buraczewska et al. (3, 4) on the use of mycobacteriophage 33D as a rapid and simple method to distinguish wild strains of *M. bovis* from strains of BCG. The stability of the two mycobacteriophages and the use of commercially available media described here makes the phage test

TABLE 2. Niacin, nitrate reductase, and thiophen-2-carboxylic acid hydrazide (TCH) tests results of the 133 mycobacterial strains used^a

Strain	Niacin test	Nitrate reductase test	Growth on TCH (10 µg/ml)
<i>M. tuberculosis</i>	80/80	80/80	80/80
<i>M. bovis</i>	0/45	0/45	0/45
BCG	0/8	0/8	0/8

^a Results are expressed as number of strains positive/number of strains tested.

easily available for use in mycobacterial reference laboratories.

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