β -Glucosidase Activity in Mycobacteria

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A procedure to test β -glucosidase was developed for identification of the mycobacteria. One hundred and thirty-three strains representing 17 species were assayed in a buffered system containing whole cells and the substrate *p*-nitrophenyl- β -D-glucoside. Qualitative differences between species indicated that the test was useful in discriminating among the mycobacteria.

Most mycobacteria isolated from clinical specimens can now be differentiated using a few key tests (6). Some of these tests are read the same day they are performed; however, others essential for identification require days or weeks before results can be evaluated. In mycobacteriology there are, therefore, continuing efforts to develop simple and rapid diagnostic procedures. It is the purpose of this report to describe a simple and rapid test based on the enzymatic hydrolysis of *p*-nitrophenyl- β -D-glucoside (PNPG), to show that the mycobacteria differ in their ability to hydrolyze the substrate, and to present data indicating that the test is useful for the differential identification of these bacteria.

The 133 strains of mycobacteria assembled for this study were distributed as follows: 17 strains were representative of the 17 species most commonly isolated from clinical specimens and were kindly supplied by H. Boisvert, Institut Pasteur, Paris; 8 strains of *Mycobacterium bovis* BCG were seed lots from various producers of the BCG vaccine and were received through the courtesy of M. Gheorghiu, Service du BCG, Institut Pasteur, Paris; the remaining 108 strains were obtained from the repository culture collection of the Service de la Tuberculose et des Mycobactéries, Institut Pasteur, and were identified by current methods by H. Boisvert.

β -glucosidase was assayed in reaction mixtures containing 0.5 ml of a colorless 10^{-2} M solution of PNPG (Sigma Chemical Co., St. Louis, Mo.) in 0.05 M tris(hydroxymethyl)-aminomethane buffer, pH 7.0 (Sigma), and one spade of bacterial cells (approximately 2 to 5 mg [wet weight] scraped from the surface of 3-week-old Lowenstein-Jensen culture slants. The reaction mixtures were incubated for 3 h at 37°C and the development of a distinct yellow color caused by the enzymatic hydrolysis of the substrate and release of *p*-nitrophenol indicated

a positive reaction. The reaction mixtures containing chromogenic mycobacteria, which give yellow suspensions, were centrifuged before the results of the test could be determined. The liberation of *p*-nitrophenol was found to be a function of the time of incubation and was generally completed within 1 to 2 h. For practical purposes, a 3-h reaction time was thus established, and longer incubations were not useful. On overnight incubation, often there was spontaneous hydrolysis of the substrate in controls without added cells.

Preliminary studies using whole cells of *Mycobacterium fortuitum*, strain HB 1792, showed that PNPG was hydrolyzed without a lag. The enzyme was found in cell-free extracts of the bacteria grown in liquid Proskauer and Beck medium containing either glycerol or glucose.

The β -glucosidases are enzymes widely distributed in the bacteria and the fungi. In the bacteria the enzymes are phospho- β -D-glucosidases, and the substrates are phosphorylated during active transport by specific permease systems (3, 4); in the fungi, previous phosphorylation of the substrates is not required for enzyme action (1, 3, 5). To determine whether phosphorylation was required, *M. fortuitum* cells were incubated in reaction mixtures containing 10^{-5} μ mol of potassium cyanide and sodium azide. Because no significant differences were found in respect to the controls (data not shown), we concluded that these enzymes in the mycobacteria were not phospho- β -D-glucosidases.

The distribution of β -glucosidase activity among the 17 species of mycobacteria is depicted in Table 1. Most strains of *Mycobacterium tuberculosis* (93.7%) gave a positive test result, whereas most strains of *M. bovis*, including the strains of BCG, gave negative test results. Among Runyon's group II mycobacteria, 96.7% of *Mycobacterium scrofulaceum* strains were found negative, whereas the

TABLE 1. Distribution of β -glucosidase among 17 mycobacterial species

Species	Representative strains ^a		Other strains					No. + or ++/no. tested (% positive)
	Strain identifica- tion	β -Glucosi- dase activ- ity ^b	No. tested	No. giving the indicated re- sult				
				0	(+)	+	++	
<i>Mycobacterium tuber- culosis</i>	H 37 Rv	++	16	0	1	1	14	15/16 (97.3%)
<i>M. bovis</i>	Barth (H.B.)	0	8	4	3	1	0	1/32 (3.1%)
<i>M. bovis</i>	BCG (Pasteur)	0	24 ^c	22	2	0	0	
<i>M. marinum</i>	ATCC 927	+	3	0	0	3	0	3/3 (100%) ^d
<i>M. kansasii</i>	HB 4962	+	2	1	0	1	0	1/2 (—) ^d
<i>M. scrofulaceum</i>	HB 1595	0	33	30	2	0	1	1/33 (3.3%)
<i>M. szulgai</i>	NCTC 10831	++						— ^d
<i>M. gordonae</i>	ATCC 14470	++	3	0	0	0	3	3/3 (100%) (4)
<i>M. flavescens</i>	ATCC 14474	+						— ^d
<i>M. avium</i>	HB 4061	0	15	3	2	8	2	10/15 (66.6%)
<i>M. intracellulare</i>	P-44	+						
<i>M. gastri</i>	W-471	0						— ^d
<i>M. terrae</i>	HB 2045	+	1	0	0	0	1	1/1 (100%) ^d
<i>M. triviale</i>	T-254-3	0	1	1	0	0	0	0/1 (0)
<i>M. xenopi</i>	HB 1895	+	1	0	0	1	0	1/1 (100%) ^d
<i>M. fortuitum</i>	HB 1792	++	5	0	0	0	5	5/5 (100%)
<i>M. chelonae</i>	HB 4902	0	3	0	3	0	0	0/3 (0) ^d (4)
<i>M. segmatis</i>	HB 4495	+						— ^d

^a Representative strains: ATCC, American Type Culture Collection; NCTC, National Collection of Type Cultures (England); P-44, from E. Runyon; W-471, from L. Wayne; T-254-3, from G. Kubica; HB, from H. Boisvert.

^b β -glucosidase activity: 0 and $\pm$, intensity of yellow color of a solution of *p*-nitrophenol giving an absorbancy at 410 nm of less than 0.5; +, intensity of yellow color of a solution of *p*-nitrophenol giving an absorbancy ranging from 0.5 to 1.0; and ++, intensity of yellow color of a solution of *p*-nitrophenol giving an absorbancy higher than 1.0. Because the substrate is rather instable, it was prepared weekly and was stored at 4°C.

^c The 24 strains of BCG include the seed lots from BCG Pasteur, BCG-Copenhagen; BCG-Japan; BCG-USSR; BCG-England; BCG-Canada; BCG-Sweden and BCG-Czechoslovakia; and 16 strains isolated from the tissues from patients that developed complications from BCG vaccination.

^d These data must be regarded with caution because too few strains were studied to provide meaningful information.

strains from other species within the group yielded positive test results. Thus, the β -glucosidase test is useful in discriminating *M. scrofulaceum*, and we suggest that it may advantageously substitute for the Tween 80 degradation test that requires at least 5 days (6).

The results obtained in *Mycobacterium avium*-*Mycobacterium intracellulare* were deceptively variable in this test. Indeed, although results with the representative strains HB 4061 and P 44 suggested a possible separation of these two species, further study of 15 other strains showed variable reactions that may or may not be related to species identification. The usefulness of the test as an aid to separate *Mycobacterium gastri* and *Mycobacterium triviale* from *Mycobacterium terrae* and *Mycobacterium xenopi* and *M. fortuitum* from *Mycobacterium chelonae* requires further evaluations.

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strains of BCG. We gratefully acknowledge the technical assistance to Jeanine Grandry.

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