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Nitrate Reductase Activity of *Mycobacterium tuberculosis* and *Mycobacterium bovis* in the Presence of Electron Donors

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The activities of the nitrate reductase enzyme of *Mycobacterium tuberculosis*, *M. bovis*, and of BCG were assayed with and without addition of electron donors. *M. tuberculosis* always reduced nitrate; *M. bovis* did so only in the presence of electron donors, and BCG did not show enzymatic activity.

One of the most frequently used methods for differentiating *Mycobacterium bovis* and *M. tuberculosis* is enzymatic reduction of nitrate to nitrite; this test is negative for *M. bovis* and positive for *M. tuberculosis*. Bonicke et al. (1) assayed this technique in the presence of various electron donors in two standard strains of *M. tuberculosis* and one of BCG.

The purpose of this study was to establish the value of the above enzymatic test when applied to wild species of *M. bovis* and *M. tuberculosis* in the presence of fatty acids and other related compounds. The behavior of the Pasteur strain of BCG was also studied under identical conditions.

Twenty-seven strains of *M. tuberculosis* from secretions of patients, BCG (Pasteur strain), all cultivated in Lowenstein-Jensen medium, and 20 strains of *M. bovis* from bovine lymph nodes, cultivated in Stonebrink medium, were used. From well-developed cultures, colonies were collected with loops; suspensions were then prepared in 0.0667 M phosphate buffer at pH 7.0 and agitated in flasks with glass balls. Concentration was controlled with a standard suspension containing 1 mg of mycobacteria per ml.

The technique was developed as described by Bonicke et al. (1). The concentration of the nitrite formed was determined colorimetrically by using a spectrophotometer (Coleman Instrument Corp., model 6A) and a standard absorbance concentration curve. Reaction times were 3 and 24 h. The electron donor compounds used were: acetic acid, propionic acid, pyruvic acid, DL-alanine, DL-serine, and Tween 80. The pH of all solutions was adjusted at 7.0. Lactic acid was not included in the study in view of the limited increase in the enzymatic activity it produces on *M. tuberculosis* GRW, as stated before (1).

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Table 1 shows the average values of nitrite formed from nitrate by *M. tuberculosis* (27 strains) and by *M. bovis* (20 strains), in the presence and absence of the various electron donor compounds used.

The BCG strain failed to reduce nitrate under all the conditions of the experiment and time periods.

All the *M. tuberculosis* strains tested showed some nitrate reductase activity after 3 h of incubation, and all showed an increase in this activity in the reading of the reaction 24 h after incubation, in the presence or absence of reducing compounds. None of the *M. bovis* strains assayed reduced nitrate enzymatically without adding such compounds, even after 24 h of incubation. On the other hand, all these strains showed measurable enzymatic activity in the presence of some of the compounds. Of these, pyruvic acid had the most marked effect on the nitrate reductase activity of *M. bovis* and *M. tuberculosis*.

We observed that, although the amount of nitrite formed in the 20 wild strains of *M. bovis* varied greatly from one strain to another, at the 24-h reading the percentages of the strains that produced measurable quantities of nitrite in the presence of acetic acid, pyruvic acid, DL-alanine, and Tween 80 were 90, 100, 95, and 70%, respectively. Bonicke et al. have stated in their paper (1) that bovine and BCG strains remain incapable of reducing nitrate, even in the presence of such compounds. Apparently, Argentine wild bovine strains would have a different behavior from that of the collection strains used by the above-mentioned authors.

Our results differ quantitatively from those obtained by Bonicke et al. (1). These differences may be accounted for by our use in this study of a bacillary suspension of 1 mg/ml and wild strains, as compared with a suspension of 10 mg/ml and collection strains used in the original study.

TABLE 1. *Enzymatic reduction of nitrate to nitrite (average values) by M. tuberculosis and M. bovis in the presence of various electron donors*

Electron donor	Nitrite formed (μg/ml)[a]					
	M. tuberculosis[b]		*M. bovis*[c]		BCG (Pasteur strain)	
	3 h	24 h	3 h	24 h	3 h	24 h
Acetic acid	1.4 (<0.1–4.2)	5.6 (0.8–16.0)	<0.1	3.9 (<0.1–>20)	<0.1	<0.1
Propionic acid	0.9 (<0.1–3.2)	4.1 (0.9–>20)	<0.1	1.4 (<0.1–>20)	<0.1	<0.1
Pyruvic acid	1.2 (<0.1–3.7)	20.0 (0.7–>20)	<0.1	9.2 (0.48–>20)	<0.1	<0.1
DL-Alanine	1.0 (<0.1–4.1)	4.6 (1.5–8.5)	<0.4	4.2 (<0.1–>20)	<0.1	<0.1
DL-Serine	0.9 (<0.1–3.8)	3.2 (0.2–8.0)	<0.1	0.7 (<0.1–17.0)	<0.1	<0.1
Tween 80	0.8 (<0.1–4.0)	1.9 (0.1–5.5)	<0.1	1.8 (<0.1–16.0)	<0.1	<0.1
Control without electron donor	1.2 (<0.1–4.2)	3.2 (0.7–7.2)	<0.1	<0.1	<0.1	<0.1

[a] Degree of color change (from no color to red, corresponding to change in nitrite concentration from <0.1 μg/ml to >20 μg/ml).
[b] Average value of 27 strains. Range is expressed in parentheses.
[c] Average value of 20 strains. Range is expressed in parentheses.

The results of our experiments showed that *M. tuberculosis, M. bovis,* and the BCG strains behave differently, a fact which can be useful for their identification in the laboratory: *M. tuberculosis* reduces nitrate even in the absence of compounds that can act as electron donors; *M. bovis* does so only in the presence of such compounds, and BCG (Pasteur strain) does not show this enzymatic activity in any conditions.

A more complete set of related mycobacterial species (*M. tuberculosis, M. bovis, M. africanum,* and different BCG strains) has been included in a comparison of nitrate reductase activity which is at present under way in our laboratory.

LITERATURE CITED

1. **Bonicke, R., S. E. Juahaz, and V. Diemer.** 1970. Studies on the nitrate reductase activity of mycobacteria in the presence of fatty acids and related compounds. Am. Rev. Resp. Dis. **102**:507–515.