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H R L P A C E P *Mycobacterium tuberculosis* S M

I R ,[1]* R A. S ,[2] J C ,[3] C A ,[1]
C E. B III,[2] P A [1]

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The events involved in the establishment of a latent infection with *Mycobacterium tuberculosis* are not fully understood, but hypoxic conditions are generally believed to be the environment encountered by the pathogen in the central part of the granuloma. The present study was undertaken to provide insight into *M. tuberculosis* protein expression in in vitro latency models where oxygen is depleted. The response of *M. tuberculosis* to low-oxygen conditions was investigated in both cellular and extracellular proteins by metabolic labeling, two-dimensional electrophoresis, and protein signature peptide analysis by liquid chromatography-mass spectrometry. By peptide mass fingerprinting and immunodetection, five proteins more abundant under low-oxygen conditions were identified from several lysates of *M. tuberculosis*: Rv0569, Rv2031c (HspX), Rv2623, Rv2626c, and Rv3841 (BfrB). In *M. tuberculosis* culture filtrates, two additional proteins, Rv0363c (Fba) and Rv2780 (Ald), were found in increased amounts under oxygen limitation. These results extend our understanding of the hypoxic response in *M. tuberculosis* and potentially provide important insights into the physiology of the latent bacilli.

Mycobacterium tuberculosis (27). A . I . T (28). T O . T . I H, (9). I . T (29). I . B . M *M. tuberculosis* *M. bovis* BCG (*M. bovis*) . A 16- D α- (H X, R 2031) (7, 30), *M. tuberculosis* H X (31). R *M. bovis* BCG (4, 10). U W (29) (2-DE), A *M. tuberculosis* *M. bovis* BCG 100 BCG (3, 16), . I , *M. tuberculosis* . W W [35S]

* C . M : D TB I , S S I , 5 A , DK-2300 C S, D . P 45-3268-3721. F : 45-3268-3035. E- : @ . .
P : D M , C S U , F . C , C .

[35S] *M. tuberculosis* I (LC-MS) 2-DE . Q LC-MS (13). T . LC-MS / . *M. tuberculosis*, 3,924 . W *M. tuberculosis* LC-MS

MATERIALS AND METHODS

Metabolic labeling. A *M. tuberculosis* H37Rv 37 C S 580 (OD_{580}) . 1.0 (6). S , 10- (N , R , D) 125- E (C , A , M .) 6.7 20 S 8×10^6 . E 37 C ( ), 37 C ~100 ( ) . M L-[35S] (R P , A P B , B , U K)/ . A 20 , T (PBS; H 7.4) 300 PBS 0.1- 0.1% (SDS) 1 M T 5 MS2 (IKA W , I ., W , N.C.). T ( ) . 20 C -3 (A , D , M).

Growth under defined oxygen tensions. *M. tuberculosis* H37Rv M 1, 5, 20% (31). C OD_{650} . 0.5 . B 0.1- .

2-DE analysis. D , 40 13- IPG H 4-7L (A P B , U , S) . F 500 V (1), 1,000 V (1), 8,000 V (2) IPG (A P B). T SDS 10, 20% P II (B -R , R , C .). T (26) C (G B ; P , R , I .). MS, (PVDF) B MR (K , R , N.Y.) 3 42 .

Image analysis of 2-DE gels. A U P III (U S G H, W , G) P 2D (5.01; N L D , N T , U K). S *M. tuberculosis* . S . T

Antibodies. M HYB 76-5 HBT10 *M. tuberculosis* H X A (1, 15). A K2050 *M. tuberculosis* Rv 0569 , H (22).

Identification of 2-DE spots. A PVDF . T (23). F MS, C 2-DE (26). B (MALDI-TOF) MS REFLEX III (B D , B , G). F P R2 1 μ 2,5- MALDI . F MALDI-TOF MS H37Rv NRDB (E M B L). T H37Rv 3,924 *M. tuberculosis* H37Rv (5).

LC-MS. C 16 37 C 0.2 M / 1:20. U 5% LC-MS F LCQ (T F , S J , C .). T M C18 (5μ 200A, 0.2 50 ; M B , I ., A , C .) 5 48% 30 48 64% 10 . T MS . R L (Rv L7/L12; Rv 0652) . T . C 2-DE LC-MS 1 5% 20% . I P P MS-D (:// /). S . W . T . W 96 97.5% 99.29 99.88% >99.9% . T

RESULTS AND DISCUSSION

Steady-state levels of *M. tuberculosis* proteins under defined oxygen tensions. I Y . (31), 2-DE *M. tuberculosis* 1.3 20% H X . A . T *M. tuberculosis* ( ), 1, 5, 20% 2-DE (Fe . 1). T 2-DE

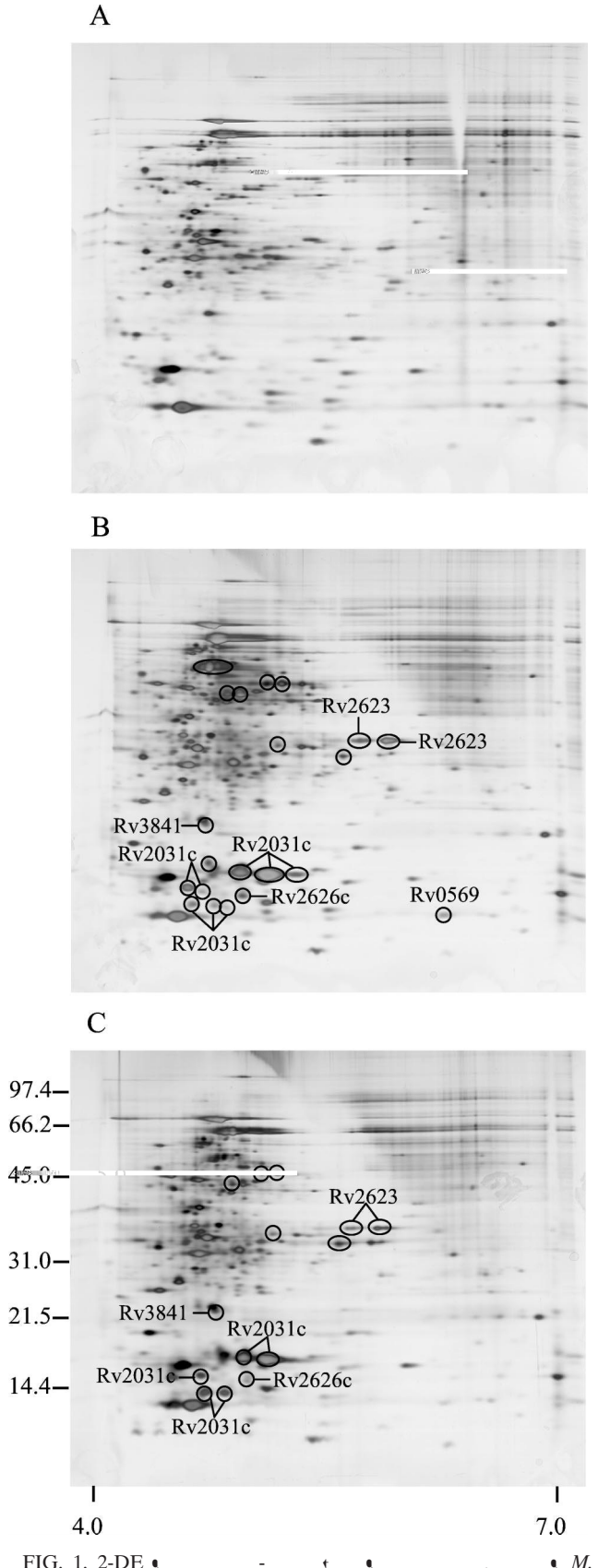

FIG. 1. 2-DE *M. tuberculosis* . T 20% (A), 5% (B), 1%

. A 5% , 21 ( ) 20% . T 1 20% 14 1% . T 2-DE 1% 2-DE *M. tuberculosis* 1.3% (31).

Metabolic labeling of *M. tuberculosis* proteins under oxygen limitation. T *M. tuberculosis* [^{35}S] [^{35}S] . A (W [29]). T . B ( ) ( ) 2-DE, PVDF (F . 2 3). T 10 . 2-DE 14 . I

Identification of *M. tuberculosis* proteins more abundant under low-oxygen conditions. P C MALDI-TOF MS (T 1).

(i) HspX. I 5% ( ) 1% ( ) HYB 76-5 MALDI-TOF MS (F . 1). B *M. tuberculosis* H X (F . 2), (, F . 3). H X H X 5% . T H X (18). U H X *M. tuberculosis* *M. bovis* BCG (4, 7, 8, 10, 25, 31). I H X (19, 31). A *M. tuberculosis* S . (25) R 3133

(C) . L 2-DE . T H S

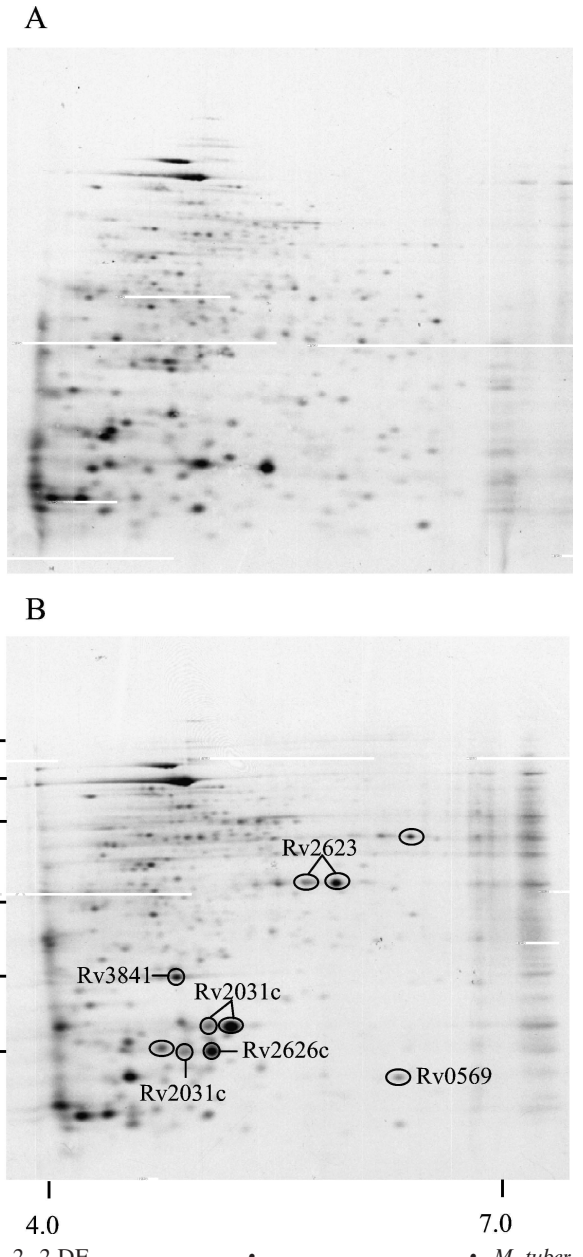

FIG. 2. 2-DE *M. tuberculosis* [^{35}S] *M. tuberculosis* 37°C (C, A) (B). T H S

T Rv3133 H X (25). I Rv3133 *M. bovis* BCG (4). I , Rv3133 . A

(ii) Rv2623. I Rv2623 5% ( ) 1% ( ) MALDI-TOF MS (Fig. 1). B *M. tuberculosis* Rv2623 (, Fig. 2), (, Fig. 3). E Rv2623 *M. bovis* BCG (4, 10)

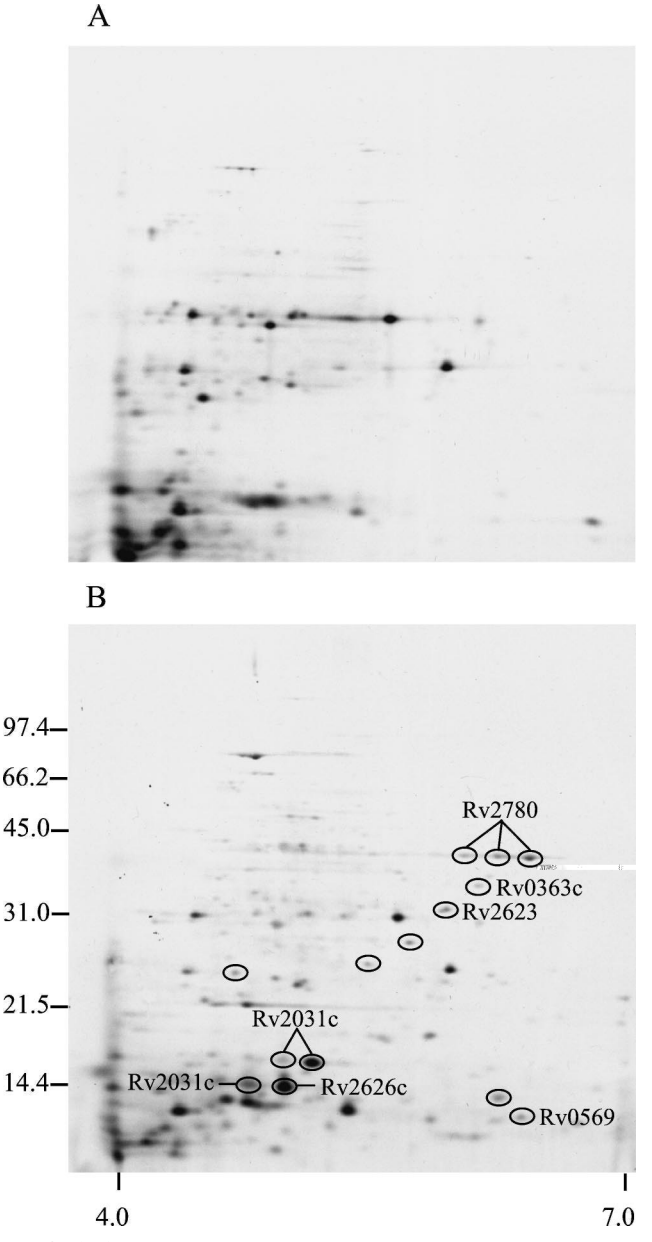

FIG. 3. 2-DE *M. tuberculosis* [^{35}S] *M. tuberculosis* 37°C (C, A) (B). T H S

TABLE 1. *M. tuberculosis* 2-DE

P	G	T (D)[a]	I[a]	I	S		D W	
					L (5%)[c]	L (1%)[d]	L [e]	C [f]
R 0363	*fba*	36.6	5.59	MS	NI	NI	NI	↑
R 0569[c]		9.5	6.08	MS,	↑	–	↑	↑
R 2031	*hspX*	16.2	4.75	MS,	↑	↑	↑	↑
R 2623[c]		31.6	5.56	MS	↑	↑	↑	↑
R 2626		15.5	4.77	MS	↑	↑	↑	↑
R 2780[c]	*ald*	38.7	6.18	I	–	–	–	↑
R 3841	*bfrB*	18.3	4.23	MS	↑	↑	↑	NI

[a] A T L (:// /T L /).
[b] ↑, M NI,
[c] S F . 1B.
[d] S F . 1C.
[e] S F . 2.
[f] S F . 3.

THP-1 *M. bovis* BCG (19), R 2623 . I , R 2623 . I U A (A) *Escherichia coli* (20), R 2623 *M. tuberculosis*.

(iii) Rv2626c. I R 2626 1 5% MALDI-TOF MS (F . 1). T *M. tuberculosis* (, F . 2) (, F . 3) . T R 2626 (25) *M. bovis* BCG W (4). R 2626 *B. subtilis* Y B IMP (5).

(iv) BfrB (Rv3841, bacterioferritin). B B MALDI-TOF MS (F . 1 3). B *bfrB* (25).

(v) Rv0569 (a conserved hypothetical protein). R 0569 MALDI-TOF MS (F . 2 3); 5% 1% . T R 0569 (25), *M. bovis* BCG (4, 10). T *M. tuberculosis* *M. bovis* BCG (<15 D) *M. bovis* BCG 10% (10) 12.5% (4) SDS-

(vi) Fba. T F (W), (, F . 3). I , B . (2) EDTA *M. tuberculosis* H37R . I *Bacillus subtilis* *Lactobacillus brevis*, (17, 24). F *M. bovis* BCG (4, 10, 25). T , (14, 21).

(vii) Ald. B HBT10, L- (40-D) (, F . 3). I *M. smegmatis*, W (11). I A (12). H , A *M. bovis* *M. bovis* BCG (1, 12). I (: // / /) (25) A

Differences in steady-state levels of *M. tuberculosis* proteins under defined oxygen tensions by the signature peptide analysis. LC- MS 2-DE . O LC MS . T . B R L . W 2-DE (T 1) . T *M. tuberculosis* . T LC-MS 1, 5, 20%

TABLE 2. S LC-MS *M. tuberculosis*

P	G	*m/z* [a]	R	
			1%	5%
Rv 0363c	*fba*	947.0 (1), 474.0 (2) 1,493.8 (1), 747.3 (2) 1,703.0 (1), 852.0 (2)	0.98	1.72
Rv 0569		1,164.2 (1), 582.6 (2) 1,270.4 (1), 635.7 (2) 1,617.7 (2), 1,078.4 (3)	1.33	7.31
Rv 2031c	*hspX*	1,163.2 (1), 582.1 (2) 1,459.6 (1), 730.3 (2) 1,715.9 (1), 858.4 (2)	4.06	6.87
Rv 2623		1,102.3 (1), 551.6 (2) 1,387.6 (1), 694.3 (2) 1,937.2 (1), 969.1 (2)	4.76	5.28
Rv 2626c		943.1 (1), 472.0 (2) 1,698.8 (1), 849.9 (2) 1,257.5 (1), 629.2 (2)	2.81	4.88
Rv 2780	*ald*	1,298.5 (1), 649.7 (2) 1,317.4 (1), 659.2 (2) 1,712.0 (1), 856.5 (2)	1.28	2.29
Rv 3841	*bfrB*	1,266.3 (1), 633.6 (2) 1,578.9 (1), 789.9 (2) 1,633.7 (1), 817.3 (2)	4.27	5.19

[a] *m/z* P MS-D (:// /). T P

[b] R 20% .

. Q R L

T . T 2. S 2-DE. T LC-MS F (Rv 0363c) A (Rv 2780), T *M. tuberculosis* I B LC-MS 2-DE T I LC-MS 1% 5% ;

Rv 0569 5% 1% (7.31 1.33 ,). T 1 5% I 2-DE , MALDI-TOF MS LC-MS *M. tuberculosis* I (4, 10, 25). T *M. tuberculosis*, *M. tuberculosis* H

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W J B B E LC-MS . W T S S , C S A , V A

REFERENCES

1. **Andersen, Å. B., P. Andersen, and L. Ljungqvist.** 1992. S 40,000- *Mycobacterium tuberculosis*. I . I . **60:**2317 2323.
2. **Bai, N. J., M. R. Pai, P. S. Murthy, and T. A. Venkitasubramanian.** 1974. I *Mycobacterium tuberculosis* H37R . FEBS L . **45:**68 70.
3. **Behr, M. A., M. A. Wilson, W. P. Gill, H. Salamon, G. K. Schoolnik, S. Rane, and P. M. Small.** 1999. C BCG DNA . S **284:**1520 1523.
4. **Boon, C., R. Li, R. Qi, and T. Dick.** 2001. P *Mycobacterium bovis* BCG W . J. B . **183:**2672 2676.
5. **Cole, S. T., R. Brosch, J. Parkhill, T. Garnier, C. Churcher, D. Harris, S. V. Gordon, K. Eiglmeier, S. Gas, C. E. Barry III, F. Tekaia, K. Badcock, D. Basham, D. Brown, T. Chillingworth, R. Connor, R. Davies, K. Devlin, T. Feltwell, S. Gentles, N. Hamlin, S. Holroyd, T. Hornsby, K. Jagels, and B. G. Barrell.** 1998. D *Mycobacterium tuberculosis*. N **393:**537 544.
6. **Collins, F. M., J. R. Lamb, and D. B. Young.** 1988. B *Mycobacterium tuberculosis* I . I . **56:**1260 1266.
7. **Cunningham, A. F., and C. L. Spreadbury.** 1998. M 16- α- J. B . **180:**801 808.
8. **Desjardin, L. E., L. G. Hayes, C. D. Sohaskey, L. G. Wayne, and K. D. Eisenach.** 2001. M (H X) RNA *Mycobacterium tuberculosis*. J. B . **183:**5311 5316.
9. **Fenton, M. J., and M. W. Vermeulen.** 1996. I . I . I . **64:**683 690.
10. **Florczyk, M. A., L. A. McCue, R. F. Stack, C. R. Hauer, and K. A. McDonough.** 2001. I Rv 2623 ATP- . I I . **69:**5777 5785.
11. **Hutter, B., and T. Dick.** 1998. I *Mycobacterium smegmatis*. FEMS M . L . **167:** 7 11.
12. **Hutter, B., and M. Singh.** 1999. P 40- D *Mycobacterium tuberculosis*, L- . B . J. 343 P **3:**669 672.
13. **Ji, J., A. Chakraborty, M. Geng, X. Zhang, A. Amini, M. Bina, and F. Regnier.** 2000. S . J. C B **745:**197 210.
14. **Jungblut, P. R., U. E. Schaible, H. Mollenkopf, U. Zimny-Arndt, B. Raupach, J. Mattow, P. Halada, S. Lamer, K. Hagens, and S. H. Kaufmann.** 1999. C *Mycobacterium tuberculosis Mycobacterium bovis* BCG : . M . M . **33:**1103 1117.

15. **Klausen, J., M. Magnusson, A. B. Andersen, and C. Koch.** 1994. C
M. tuberculosis . S . J. I . **40:**345 349.
16. **Mahairas, G. G., P. J. Sabo, M. J. Hickey, D. C. Singh, and C. K. Stover.** 1996. M *Mycobacterium bovis* BCG *M. bovis*. J. B . **178:**1274 1282.
17. **Marino, M., T. Hoffmann, R. Schmid, H. Mobitz, and D. Jahn.** 2000. C *Bacillus subtilis* . M **146:**97 105.
18. **Mattow, J., P. R. Jungblut, E. C. Muller, and S. H. Kaufmann.** 2001. I *Mycobacterium tuberculosis* H37R / P **1:**494 507.
19. **Monahan, I. M., J. Betts, D. K. Banerjee, and P. D. Butcher.** 2001. D . M **147:**459 471.
20. **Nystrom, T., and F. C. Neidhardt.** 1994. E , U A, *Escherichia coli* . M . M . **11:**537 544.
21. **Rosenkrands, I., A. King, K. Weldingh, M. Moniatte, E. Moertz, and P. Andersen.** 2000. T *Mycobacterium tuberculosis*. E **21:**3740 3756.
22. **Rosenkrands, I., P. B. Rasmussen, M. Carnio, S. Jacobsen, M. Theisen, and P. Andersen.** 1998. I 29- *Mycobacterium tuberculosis* . I . I . **66:**2728 2735.
23. **Rosenkrands, I., K. Weldingh, S. Jacobsen, C. V. Hansen, W. Florio, I. Gianetri, and P. Andersen.** 2000. M *Mycobacterium tuberculosis* . E **21:**935 948.
24. **Saier, M. H., Jr., J. J. Ye, S. Klinke, and E. Nino.** 1996. I E -M *Lactobacillus brevis*. J. B . **178:**314 316.
25. **Sherman, D. R., M. Voskuil, D. Schnappinger, R. Liao, M. I. Harrell, and G. K. Schoolnik.** 2001. R *Mycobacterium tuberculosis* . P . N . A . S . USA **98:** 7534 7539.
26. **Shevchenko, A., M. Wilm, O. Vorm, and M. Mann.** 1996. M . A . C . **68:**850 858.
27. **Sudre, P., G. ten Dam, and A. Kochi.** 1992. T . B . W. H. O. **70:**149 159.
28. **Wayne, L. G.** 1960. T . II. O . A . R . R . D . **82:**370 377.
29. **Wayne, L. G., and L. G. Hayes.** 1996. A *Mycobacterium tuberculosis* . I . I . **64:**2062 2069.
30. **Yuan, Y., D. D. Crane, and C. E. Barry III.** 1996. S *Mycobacterium tuberculosis* α- . J. B . **178:**4484 4492.
31. **Yuan, Y., D. D. Crane, R. M. Simpson, Y. Q. Zhu, M. J. Hickey, D. R. Sherman, and C. E. Barry III.** 1998. T 16- D (A) *Mycobacterium tuberculosis* P . N . A . S . USA **95:**9578 9583.