INFECTION AND IMMUNITY, Aug. 2000, p. 4518–4522 Vol. 68, No. 8
0019-9567/00/$04.00+0

Growth of *Mycobacterium tuberculosis* in a Defined Medium Is Very Restricted by Acid pH and Mg^{2+} Levels

DEBRA L. PIDDINGTON, ALI KASHKOULI, AND NANCY A. BUCHMEIER*

Department of Pathology, University of California, San Diego, La Jolla, California 92093-0640

Received 1 February 2000/Accepted 17 May 2000

Mycobacterium tuberculosis **grows within the phagocytic vacuoles of macrophages, where it encounters a moderately acidic and possibly nutrient-restricted environment. Other mycobacterial species encounter acidic conditions in soil and aquatic environments. We have evaluated the influence of pH and divalent cation levels on the growth of *M. tuberculosis* and seven other mycobacterial species. In a defined medium, the growth of *M. tuberculosis* was very restricted by acidic pH. Higher levels of Mg^{2+} were required for growth of *M. tuberculosis* in mildly acidic media (pH 6.0 to 6.5) compared to pH 7.0 medium. The divalent cations Ca^{2+}, Zn^{2+}, or Mn^{2+} could not replace Mg^{2+} during growth at pH 6.25, but Ca^{2+} could at least partially substitute for Mg^{2+} during growth at pH 7.0. Among eight species of mycobacteria tested, there was a diversity of growth rates in media with acidic pH and low Mg^{2+} levels. *M. tuberculosis* was the most restricted in growth at pH 6.0, and all of this growth required elevated levels of Mg^{2+}. *M. kansasii* and *M. smegmatis* also grew very poorly in acidic media with limiting Mg^{2+}. *M. fortuitum*, *M. marinum*, *M. scrofulaceum*, *M. avium*, and *M. chelonae* grew at pH 6.0 in an unrestricted manner. These results demonstrate that *M. tuberculosis* is unique among the mycobacteria in its extreme sensitivity to acid and indicate that *M. tuberculosis* must acquire sufficient Mg^{2+} in order to grow in a mildly acidic environment such as within the phagosome of macrophages.**

M. tuberculosis grows within the phagocytic vacuoles of macrophages (2), where it is exposed to a relatively hostile environment. The interior of the phagosome may be limited in nutrients (11, 12) as well as acidic in pH. Although *M. tuberculosis* limits acidification of phagosomes by exclusion of the vesicular proton ATPase (19, 20), the vacuole is moderately acidic with the pH measured at values between 6.1 and 6.5 (14, 17, 19, 20).

An acidic environment is one of the most stressful conditions encountered by living cells. In order for enzymes and proteins to function normally, bacteria must maintain an internal pH close to pH 7 (4). The importance of maintaining a constant internal pH is underscored by the multiple systems utilized by bacteria during growth under acidic conditions. These include transport systems which exchange protons for cations, systems which transport protons out of cells in association with ATP hydrolysis, and the production of cytoplasmic macromolecules which function as internal buffers (5, 10). Although mycobacterial species have been reported to grow under acidic conditions in a complex media (7, 18), their response to pH stress in a defined medium has not been examined. The stress induced by acidic pH is known to be more severe in a nutrient-limited environment (10). In addition, nothing is known about specific factors which may facilitate the growth of mycobacteria under acidic conditions.

During our study of *M. tuberculosis* genes that are required for growth within macrophages, we noticed an association between bacterial growth in acidic medium and the requirement for Mg^{2+}. Our experiments demonstrated that an *M. tuberculosis* mutant in the *mgtC* gene was attenuated for growth in media with reduced Mg^{2+} levels but only when the pH of the media was mildly acidic (6). The *mgtC* mutant of *M. tuberculosis* was also attenuated for growth in human macrophages and for growth in vivo in the lungs and spleens of mice. This suggested that during infection of the host *M. tuberculosis* grows in a moderately acidic environment with limiting levels of Mg^{2+}. We therefore wanted to study the relationship between the growth of mycobacteria, including virulent *M. tuberculosis*, in acidic media and relate this to the requirement for Mg^{2+} or other divalent cations. In this report we demonstrate that *M. tuberculosis* is very restricted for growth by acid pH by using a defined medium and that any growth in moderately acidic media requires elevated levels of Mg^{2+}. The requirement for Mg^{2+} during growth in acidic medium could not be replaced with the divalent cations Ca^{2+}, Mn^{2+}, or Zn^{2+}. We also observed a great diversity among eight mycobacterial species in their ability to grow in media with acidic pH and low levels of Mg^{2+}, with *M. tuberculosis* being among the most restricted for growth under these conditions.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used in this study include *M. tuberculosis* Erdman (ATCC 35801), *M. smegmatis* (ATCC 607), *M. chelonae* subsp. *chelonae* (ATCC 35752), *M. fortuitum* subsp. *peregrinum* (ATCC 14467), *M. kansasii* (clinical isolate), *M. scrofulaceum* (clinical isolate), *M. marinum* (ATCC 927), and *M. avium* (clinical isolate). The bacteria were routinely grown in Middlebrook 7H9 supplemented with ADC and 0.05% Tween 80 (15).

Growth assays under variable pH levels and divalent cation concentrations. Growth of mycobacteria under conditions of variable pH and divalent cation levels was studied using a defined medium (Sauton medium) that was modified so that the pH and divalent cation concentrations could be varied. Sauton medium (1) was modified by omitting the magnesium sulfate, which was the sole Mg^{2+} source, and replacing the sulfate with 28 mM potassium sulfate. To limit clumping during growth, 0.05% Tween 80 was added along with 10% ADC (15). Media were buffered with either 100 mM morpholineethanesulfonic acid (MES) or 100 mM morpholinepropanesulfonic acid (MOPS) (16), adjusted to the appropriate pH, filter sterilized, and then supplemented with various levels of magnesium chloride, calcium chloride, manganese chloride, or zinc chloride. For each assay, bacteria were grown to an optical density at 580 nm (OD_{580}) of 0.3 to 0.5 in Middlebrook 7H9, washed once in saline, and diluted 1/4,000 in 10 ml of the appropriate modified Sauton medium. Cultures were incubated at 37°C with 5% CO_2 for 3 to 4 weeks. The assays were terminated before bacterial clumping was evident. Growth was monitored by measuring the OD_{580}. Data are expressed as the mean and standard error of the mean from triplicate flasks.

* Corresponding author. Mailing address: University of California, San Diego, 9500 Gilman Dr., La Jolla, CA 92093-0640. Phone: (858) 534-6024. Fax: (858) 534-6020. E-mail: nbuchmeier@ucsd.edu

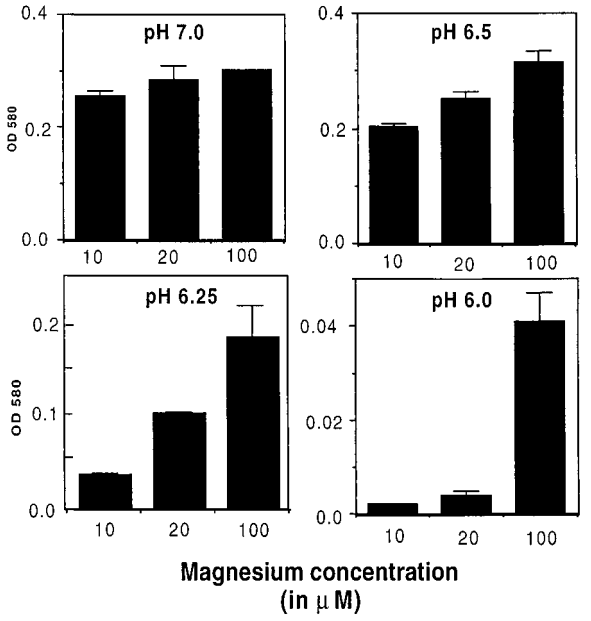

FIG. 1. *M. tuberculosis* requires elevated levels of Mg^{2+} for growth in acidic media. Growth of *M. tuberculosis* in modified Sauton media buffered to the indicated pH and containing various levels of Mg^{2+} is shown. Growth was measured at day 24 by measuring the OD_{580}. Data are expressed as the mean and standard error of the mean from three cultures.

RESULTS

The growth of *M. tuberculosis* is very restricted by acidic pH. A modified Sauton medium was used to evaluate the influence of pH and divalent cation concentration on the growth of mycobacteria. The Sauton medium was chosen because it is a defined medium in which divalent cation levels can be readily manipulated. To modify the Sauton medium, the sole divalent cation source (magnesium sulfate) was omitted and was replaced by potassium sulfate (as the sulfate source). Various levels of magnesium chloride, calcium chloride, zinc chloride, or manganese chloride were then added. In addition, all media were buffered with either 100 mM MES or 100 mM MOPS (16) to prevent the pH from changing during the course of the experiment.

The growth of *M. tuberculosis* in media buffered to pH values between pH 7.0 and 6.0 were examined. This pH range was chosen because it falls within the pH range measured in macrophage phagosomes which contain *M. tuberculosis* (14, 17, 19, 20). The extent of growth at day 24 is shown in Fig. 1. In media with moderate (100 μM) levels of Mg^{2+}, *M. tuberculosis* grew well at pH 7.0 and 6.5. As the pH dropped below 6.5, the amount of bacterial growth declined. At pH 6.25, there was a moderate but significant decrease in growth. At pH 6.0, growth of *M. tuberculosis* was almost completely absent in the 24-day culture.

***M. tuberculosis* requires elevated levels of Mg^{2+} to grow in acidic media.** We went on to examine the requirement for Mg^{2+} during growth of *M. tuberculosis* in both neutral and acidic media. At neutral pH (pH 7.0), the growth rate of *M. tuberculosis* was similar in cultures with low (10 and 20 μM) and moderate (100 μM) levels of Mg^{2+}. In mildly acidic media (pH 6.5), *M. tuberculosis* grew significantly better in the cultures containing higher levels (100 μM) of Mg^{2+} (OD_{580} 0.315) compared to medium with 10 μM Mg^{2+} (OD_{580} 0.203). In the more acidic media the requirement for Mg^{2+} became more pronounced. At pH 6.25, there was fourfold more growth in the culture with 100 μM Mg^{2+} compared to the culture with 10 μM Mg^{2+}. The culture with 20 μM Mg^{2+} produced intermediate levels of growth. At pH 6.0, the only measurable growth of *M. tuberculosis* was in the culture containing 100 μM Mg^{2+}. Thus, *M. tuberculosis* requires higher levels of Mg^{2+} for growth at acidic pH compared to growth at neutral pH.

Other divalent cations cannot replace Mg^{2+} for growth at acid pH. We next examined whether divalent cations other Mg^{2+} could facilitate the growth of *M. tuberculosis* in acidic media. Modified Sauton media containing 10 μM Mg^{2+} and buffered to either pH 6.25 or 7.0 served as the base media for these assays. We had previously determined that 10 μM Mg^{2+} was sufficient Mg^{2+} for growth of *M. tuberculosis* at pH 7.0 (Fig. 1). Aliquots of the base media were supplemented with 100 μM concentrations of the divalent cations Mg^{2+}, Ca^{2+}, Zn^{2+}, or Mn^{2+}. Growth of *M. tuberculosis* in the base medium at pH 6.25 or 7.0 was compared to growth in media supplemented with each of the divalent cations. The amount of growth attained at day 19 is presented in Fig. 2. At pH 6.25, the addition of Mg^{2+} (100 μM) increased the growth from an OD_{580} of 0.126 in the base medium to an OD_{580} of 0.200 with the Mg^{2+}. The addition of Ca^{2+} (100 μM) to the culture produced a slight increase in growth compared to the base medium from OD_{580} 0.126 to 0.137. However, growth in the Ca^{2+} culture was significantly less than the level attained with the addition of Mg^{2+} ($P < 0.05$). Zn^{2+} had no effect on growth, while 100 μM Mn^{2+} was inhibitory. At pH 7.0, the addition of Ca^{2+} enhanced the growth of *M. tuberculosis* to a level close to that with Mg^{2+}. The addition of Zn^{2+} or Mn^{2+} was inhibitory at pH 7.0. Higher levels (1,000 μM) of Ca^{2+}, Zn^{2+}, or Mn^{2+} or lower (50 μM) levels of Zn^{2+} or Mn^{2+} were unable to replace Mg^{2+} during growth at pH 6.25 (data not shown). Thus, *M. tuberculosis* specifically requires Mg^{2+} for maximum growth at pH 6.25.

The limited growth of *M. tuberculosis* at pH 6.0 is unique among eight species of mycobacteria. We went on to compare the growth rate of *M. tuberculosis* with mycobacteria that may

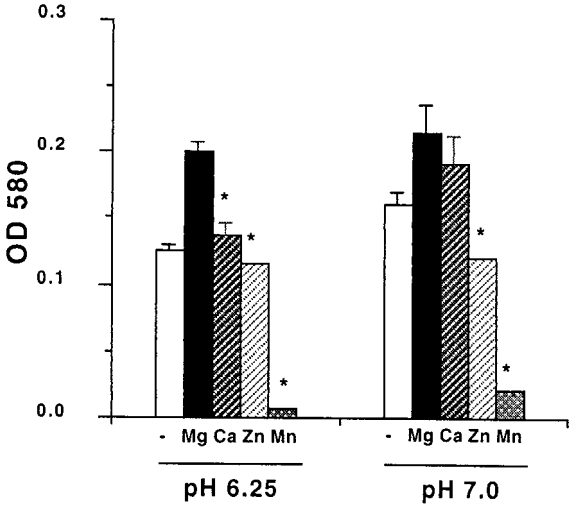

FIG. 2. Other divalent cations cannot replace Mg^{2+} during growth of *M. tuberculosis* at pH 6.25. Growth of *M. tuberculosis* in modified Sauton media buffered to the indicated pH and supplemented with 100 μM Mg^{2+}, Ca^{2+}, Mn^{2+}, or Zn^{2+} is shown. Growth was measured at day 19. Data are expressed as the mean and standard error of the mean from three cultures. *, $P < 0.05$ compared to growth in the Mg^{2+} supplemented culture.

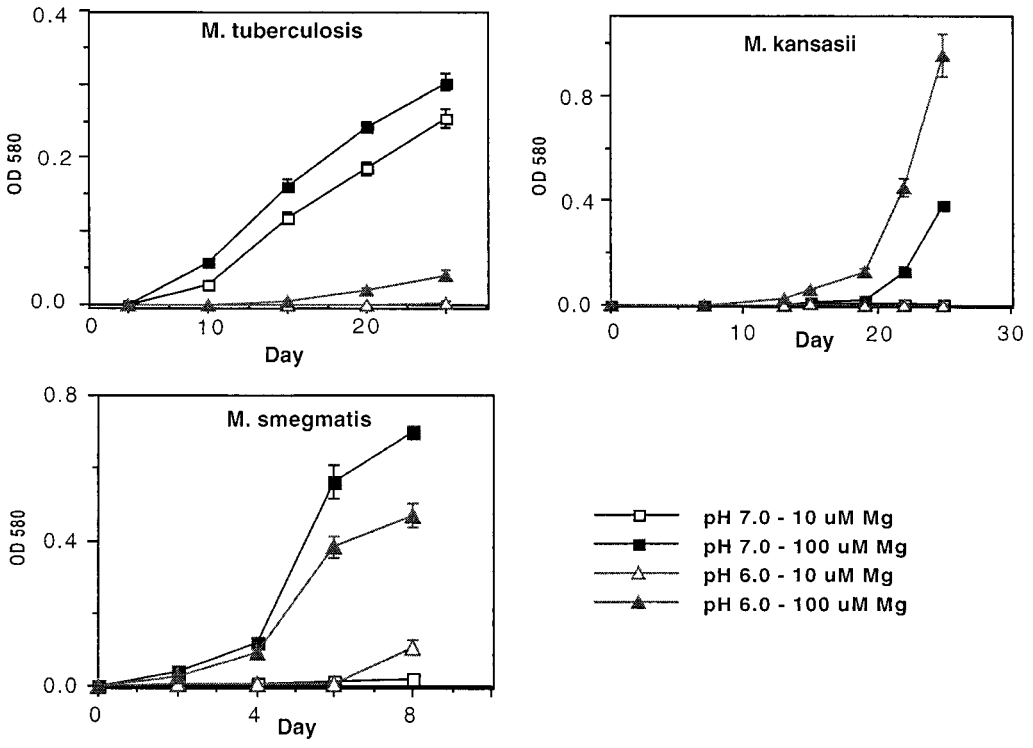

FIG. 3. *M. tuberculosis*, *M. smegmatis*, and *M. kansasii* require higher concentrations of Mg^{2+} for growth in acidic medium. Growth of mycobacterial species in modified Sauton media buffered to the indicated pH and containing 10 or 100 μM Mg^{2+} is shown. Data are expressed as the mean and standard error of the mean from three cultures.

encounter acidic conditions in soil or aquatic environments. The mycobacteria were grown in media buffered to pH 6.0 or 7.0 and containing either 10 or 100 μM Mg^{2+}. As we had previously observed, *M. tuberculosis* grew very poorly at pH 6.0 and required the higher level of Mg^{2+} for the limited growth that was observed (Fig. 3). Among eight mycobacterial species, *M. tuberculosis* was the most restricted for growth at pH 6.0 (Fig. 3 and 4). *M. smegmatis* and *M. kansasii* were also limited in growth in the pH 6.0 medium with low Mg^{2+} (10 μM) (Fig. 3). However, *M. smegmatis* and *M. kansasii* also failed to grow in the pH 7.0 medium with low Mg^{2+} (10 μM), indicating that they require higher concentrations of Mg^{2+} for maximum growth at neutral as well as acid pH.

The five additional mycobacterial species exhibited a diversity of growth patterns in acidic media, but none required the higher level of Mg^{2+} in order to grow at pH 6.0 (Fig. 4). *M. marinum* and *M. fortuitum* grew equally well at pH 7.0 and 6.0, with no restriction on growth by low Mg^{2+}. *M. avium* and *M. scrofulaceum* grew better in the acidic medium (pH 6.0) than at pH 7.0 and were not dependent upon increased Mg^{2+} for growth at pH 6.0. *M. chelonae* grew best at pH 6.0, and this growth was partially enhanced by higher levels of Mg^{2+}. A summary of these results is presented in Table 1. *M. tuberculosis* was the most limited for growth at pH 6.0, while *M. tuberculosis*, *M. kansasii*, and *M. smegmatis* required higher levels of Mg^{2+} for growth at pH 6.0.

DISCUSSION

This study has demonstrated that in an environment which contains only simple nutrients, *M. tuberculosis* is very restricted in growth by acid pH and requires higher concentrations of Mg^{2+} for growth at a pH of 6.5 or lower. Using a defined medium (Sauton) containing moderate levels of Mg^{2+} (100 μM), the growth of *M. tuberculosis* was reduced by a pH of 6.25 and was almost completely absent at pH 6.0. The sensitivity of *M. tuberculosis* to a moderate acidic pH correlates with observations made with *M. tuberculosis*-infected macrophages. Mycobacteria containing phagosomes exhibit limited fusion with late endosomes and lysosomes. This results in the exclusion of the vacuolar ATPase from the phagosome membrane which limits acidification of the vacuole (8, 19). Nevertheless, the pH of mycobacteria containing vacuoles is mildly acidic and has been measured at pH values between 6.1 and 6.5 (14, 17, 19). Thus, the pH within the phagosome is slightly above the pH level (pH 6.0) which excluded growth of *M. tuberculosis* in the modified Sauton medium and is within the pH range that required higher levels of Mg^{2+} for growth. Gomes et al. recently suggested that the sensitivity of *M. tuberculosis* to acidic pH contributes to the control of infection (13). Using a coinfection with *Coxiella burnetii*, these authors demonstrated that *M. tuberculosis* could not grow in acidified vacuoles. On the other hand, *M. avium* grew in the acidified macrophage vacuoles, which coincides with our observation that *M. avium* grew well in the pH 6.0 defined medium. Thus, the limited growth of *M. tuberculosis* in defined medium with acid pH parallels the observation that *M. tuberculosis* fails to grow in acidified vacuoles of macrophages.

Growth of *M. tuberculosis* in acidic media was even more restricted when the level of Mg^{2+} was low. The requirement for Mg^{2+} during growth in acidic media was specific for Mg^{2+} and could not be replaced by the divalent cations Ca^{2+}, Mn^{2+}, or Zn^{2+}. The need for Mg^{2+} was observed in the presence of millimolar levels of K^+ and Na^+ which are normal components

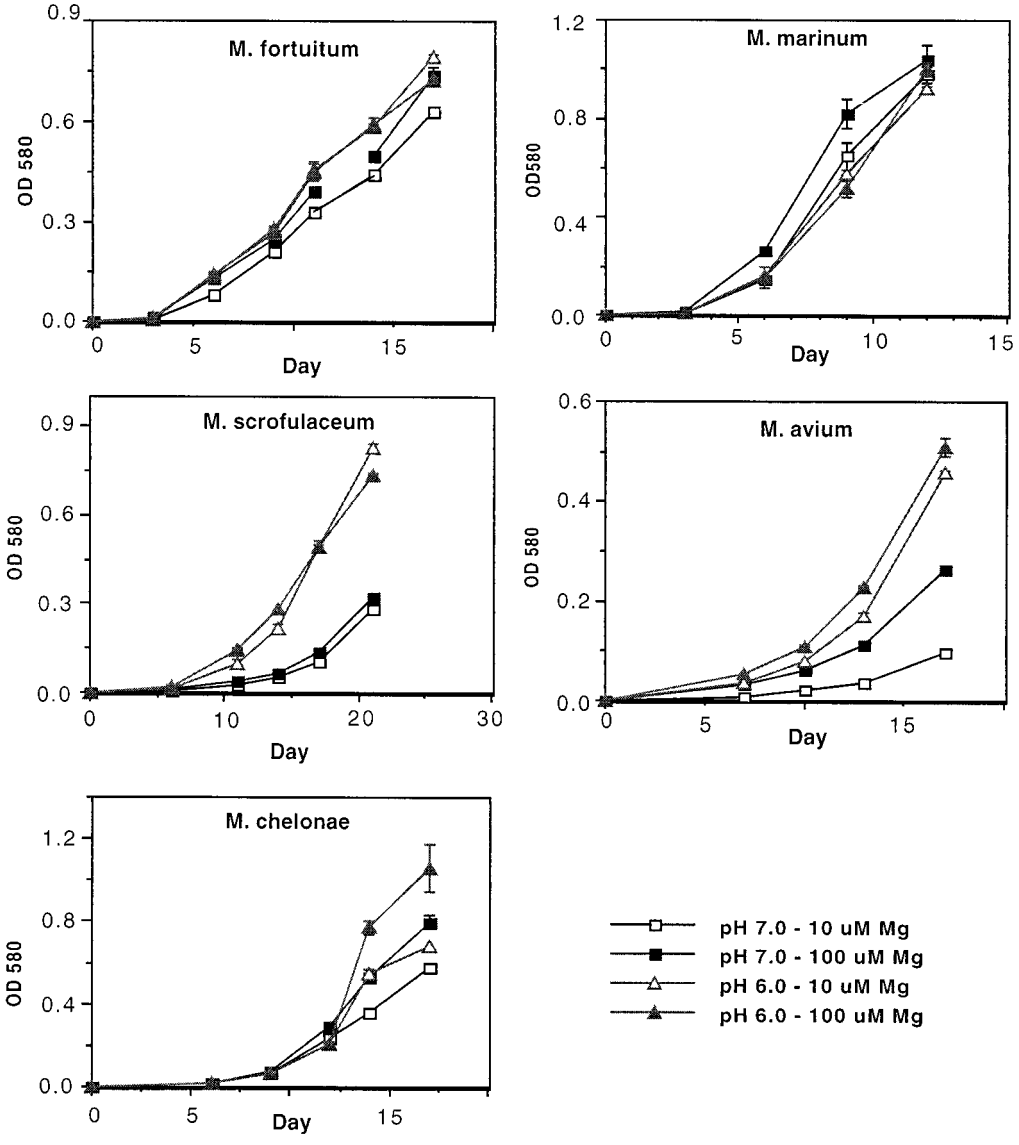

FIG. 4. Growth curves of mycobacteria which do not require higher concentrations of Mg^{2+} for growth in acidic medium. Growth of mycobacterial species in modified Sauton media buffered to the indicated pH and containing 10 or 100 μM Mg^{2+} is shown. Data are expressed as the mean and standard error of the mean from three cultures.

of the modified Sauton medium. K^+ and Na^+ transport systems are associated with an electroneutral exchange for protons which contributes to pH homeostasis in bacteria (5). We have identified one of the *M. tuberculosis* genes required for growth under low-Mg^{2+}, low-pH conditions. An *M. tuberculosis* mutants in the *mgtC* gene failed to grow in mildly acidic medium (pH 6.25) with limiting levels of Mg^{2+} (20 μM) (6). Furthermore, the *mgtC* mutant was attenuated for growth in human macrophages as well as for virulence in mice. These data suggest that the virulence of *M. tuberculosis* is dependent upon the growth of the bacteria in a mildly acidic low-Mg^{2+} environment. The concentration of Mg^{2+} in phagosomes has not been measured directly; however, it has been estimated to be in the low micromolar range (12). Thus, a limitation in Mg^{2+} within the phagosome would intensify the sensitivity of *M. tuberculosis* to moderately acidic pH.

TABLE 1. Growth of mycobacterial species in modified Sauton media

Species	Growth at pH 6.0	Elevated levels of Mg^{2+} required for growth at pH 6.0
M. tuberculosis	Poor	Yes
M. marinum	Good	No
M. kansasii	Good	Yes
M. avium	Good	No
M. smegmatis	Good	Yes
M. chelonae	Good	Partial
M. fortuitum	Good	No
M. scrofulaceum	Good	No

Among diverse mycobacterial species, there was a spectrum of tolerance to acid pH and low levels of Mg^{2+}. *M. tuberculosis* was the most restricted for growth at pH 6.0. Nontuberculosis mycobacterial species, which may grow in soil or aquatic environments, were much more acid tolerant and in fact *M. kansasii*, *M. scrofulaceum*, *M. avium*, and *M. chelonae* grew better

at pH 6.0 than at pH 7.0. Previous studies investigating the sensitivity of mycobacteria to acid pH have reported growth at a broad range of pH values (7, 18). These studies reported that the optimum growth of *M. tuberculosis* in Dubos medium was between pH 5.8 and pH 6.5, with growth observed at as low as pH 5.4 (18). The disparity in results between these studies and those presented here may be explained by the influence of the different media used since the sensitivity to extremes in pH can be masked in complex media (5). Dubos medium contains caseinate extract, while Sauton medium contains no protein extract. Our results suggest that in a environment containing simple nutrients, mycobacteria are more sensitive to acid.

The role of Mg^{2+} in the adaptation of *M. tuberculosis* to growth under moderately acidic conditions is unknown. Mg^{2+} may be participating directly in the maintenance of a neutral internal pH. The Mg^{2+}-dependent proton-translocating ATP synthase is reported to play a role in acid tolerance in bacteria (3). Homologues of the ATP synthase genes are present in the *M. tuberculosis* genome (9). Conversely, Mg^{2+} may not be directly involved in maintenance of a neutral internal pH. Instead, the Mg^{2+} transport systems of *M. tuberculosis* may function less efficiently under acidic conditions, and the bacteria may therefore require more Mg^{2+} in the surrounding environment for adequate uptake. Alternatively, under acidic conditions, greater amounts of Mg^{2+} may be required to perform the normal cellular functions of Mg^{2+} such as stabilization of membranes and acting as cofactors for enzymes. Ongoing studies to identify the function of the MgtC protein in *M. tuberculosis* should provide additional information on mechanisms involving Mg^{2+} which facilitate growth of *M. tuberculosis* under acidic conditions.

ACKNOWLEDGMENTS

This work was supported by NIH grants AI40075 and AI37005 and by undergraduate research funds from U.S. grants to A.K.

We thank E. Bearer, J. Fierer, S. Reed, and S. Sabat for mycobacterial strains used in this study.

REFERENCES

1. **Allen, B. W.** 1998. Mycobacteria: general culture methodology and safety considerations, p. 15–30. *In* T. Parish and N. Stoker (ed.), Mycobacteria protocols. Humana Press, Totowa, N.J.
2. **Armstrong, J. A., and P. D. Hart.** 1971. Response of cultured macrophages to *Mycobacterium tuberculosis*, with observations on fusion of lysosomes with phagosomes. J. Exp. Med. **134:**713–740.
3. **Bearson, S., B. Bearson, and J. W. Foster.** 1997. Acid stress responses in enterobacteria. FEMS Microbiol. Lett. **147:**173–180.
4. **Booth, I. R.** 1985. Regulation of cytoplasmic pH in bacteria. Microbiol. Rev. **49:**359–378.
5. **Booth, I. R.** 1999. The regulation of intracellular pH in bacteria. Novartis Found. Symp. **122:**19–28.
6. **Buchmeier, N., A. Blanc-Potard, S. Ehrt, D. Piddington, L. Riley, and E. Groisman.** 2000. A parallel intraphagosomal survival strategy shared by *Mycobacterium tuberculosis* and *Salmonella enterica*. Mol. Microbiol. **35:**1375–1382.
7. **Chapman, J. S., and J. S. Bernard.** 1962. The tolerances of unclassified mycobacteria. I. Limits of pH tolerance. Am. Rev. Respir. Dis. **86:**582–583.
8. **Clemens, D. L., and M. A. Horwitz.** 1995. Characterization of the *Mycobacterium tuberculosis* phagosome and evidence that phagosomal maturation is inhibited. J. Exp. Med. **181:**257–270.
9. **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, B. G. Barrell, et al.** 1998. Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. Nature **393:**537–544.
10. **Dilworth, M., and A. Glenn.** 1999. Problems of adverse pH and bacterial strategies to combat it. Novartis Found. Symp. **122:**4–14.
11. **Fields, P. I., R. V. Swanson, C. G. Haidaris, and F. Heffron.** 1986. Mutants of *Salmonella typhimurium* that cannot survive within the macrophage are avirulent. Proc. Natl. Acad. Sci. USA **83:**5189–5193.
12. **Garcia-del Portillo, F., J. W. Foster, M. E. Maguire, and B. B. Finlay.** 1992. Characterization of the micro-environment of *Salmonella typhimurium*-containing vacuoles within MDCK epithelial cells. Mol. Microbiol. **6:**3289–3297.
13. **Gomes, M. S., S. Paul, A. Moreira, R. Appleberg, M. Rabinovitch, and G. Kaplan.** 1999. Survival of *Mycobacterium avium* and *Mycobacterium tuberculosis* in acidified vacuoles of murine macrophages. Infect. Immun. **67:**3199–3206.
14. **Hackam, D. J., O. D. Rotstein, W. Zhang, S. Gruenheid, P. Gros, and S. Grinstein.** 1998. Host resistance to intracellular infection: mutation of natural resistance-associated macrophage protein 1 (Nramp1) impairs phagosomal acidification. J. Exp. Med. **188:**351–364.
15. **Jacobs, W. R., Jr., G. V. Kalpana, J. D. Cirillo, L. Pascopella, S. B. Snapper, R. A. Udani, W. Jones, R. G. Barletta, and B. R. Bloom.** 1991. Genetic systems for mycobacteria. Methods Enzymol. **204:**537–555.
16. **Lin, J., J. Frey, J. Slonczewski, and J. Foster.** 1995. Comparative analysis of extreme acid survival in *Salmonella typhimurium*, *Shigella flexneri*, and *Escherichia coli*. J. Bacteriol. **177:**4097–4104.
17. **Oh, Y. K., and R. M. Straubinger.** 1996. Intracellular fate of *Mycobacterium avium*: use of dual-label spectrofluorometry to investigate the influence of bacterial viability and opsonization on phagosomal pH and phagosome-lysosome interaction. Infect. Immun. **64:**319–325.
18. **Portaels, F., and S. Pattyn.** 1982. Growth of Mycobacteria in relation to the pH of the medium. Ann. Microbiol. **133B:**213–221.
19. **Sturgill-Koszycki, P. Schlesinger, P. Chakraborty, P. Haddix, H. Collins, A. Fok, R. Allen, S. Gluck, J. Heuser, and D. Russell.** 1994. Lack of acidification in *Mycobacterium* phagosomes produced by exclusion of the vesicular proton-ATPase. Science **163:**678–681.
20. **Xu, S., A. Cooper, S. Sturgill-Koszycki, T. v. Heyningen, D. Chatterjee, I. Orme, P. Allen, and D. Russell.** 1994. Intracellular trafficking in *Mycobacterium tuberculosis* and *Mycobacterium avium*-infected macrophages. J. Immunol. **153:**2568–2578.

Editor: E. I. Tuomanen