INFECTION AND IMMUNITY, June 1989, p. 1714–1718
0019-9567/89/061714-05$02.00/0

Vol. 57, No. 6

# Plasmid-Influenced Changes in *Mycobacterium avium* Catalase Activity

MICHELE L. PETHEL AND JOSEPH O. FALKINHAM III*

*Department of Biology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061*

Received 21 November 1988/Accepted 21 February 1989

**A virulent *Mycobacterium avium* strain, LR25, which carries three plasmids (18, 28, and 165 kilobases) and grows at 43°C was compared with its plasmid-free, avirulent segregant, strain LR163, to identify the basis for the latter's inability to grow at 43°C. The failure of mid-log-phase cultures of strain LR163 to grow at 43°C was dependent on the presence of high levels of culture aeration. In addition, highly aerated cultures of strain LR163 failed to grow at 37°C. Mid-log-phase cultures of strain LR163 had 30% of the catalase activity of strain LR25 and were more hydrogen peroxide (0.08%, wt/vol) susceptible. Catalase activity of strain LR25 was higher in cultures grown with high aeration than in those grown with almost no aeration. These data support the contention that plasmid-encoded genes influence *M. avium* catalase activity.**

Members of the *Mycobacterium avium*, *M. intracellulare*, and *M. scrofulaceum* group (*M. avium* complex) are slow-growing opportunistic human pathogens (20) found in high numbers in southeastern United States waters (10), soils (6), and aerosols (19). Recently, it has been noted that persons suffering from acquired immunodeficiency syndrome are at risk for *M. avium* complex infection (5). *M. avium* complex strains isolated from patients and natural aerosols have been shown to grow at 43°C and carry plasmids, unlike the majority of water and soil isolates (11, 17). In addition, the ability of *M. avium* complex strains to grow at 43°C was correlated with the presence of plasmids (11).

The implication that plasmids carry genetic determinants for virulence was supported by evidence that a plasmid-carrying *M. avium* complex strain, LR25, was of high virulence in beige mice, whereas its plasmid-free derivative, strain LR163, was of low virulence (12). The possible influence of oxygen metabolites on *M. avium* complex infections was demonstrated by the observation that significantly higher levels of both superoxide anion and hydrogen peroxide were released from mouse peritoneal macrophages infected with the plasmid-free derivative compared with the plasmid-carrying parent (12).

Because of the possible influence of plasmid-encoded genes on virulence and the correlation between the presence of plasmids and the ability to grow at 43°C, we sought to identify the basis for the ability of a plasmid-carrying strain of *M. avium* to grow at 43°C. The rationale of this approach was that growth at 43°C could serve as a marker for virulence.

## MATERIALS AND METHODS

**Mycobacterial strains.** *M. avium* LR25, which carries three plasmids of 18, 28, and 165 kilobases, was isolated from a patient with mycobacteriosis at the Veterans Administration Medical Center in Little Rock, Ark. (9). Its plasmid-free derivative, strain LR163, was isolated following neutral acriflavin exposure (9). Both strains used in this study were obtained from the Veterans Administration Medical Center, Little Rock, Ark.

**Media and preparation of inoculum.** Mycobacterial cultures and inocula were grown in Middlebrook 7H9 medium (BBL Microbiology Systems, Cockeysville, Md.) containing 1% (vol/vol) glycerol and 10% (vol/vol) oleic acid-albumin enrichment (MGE). The enrichment was prepared by mixing 8.5 g of NaCl and 50 g of bovine serum albumin, fraction V (Sigma Chemical Co., St. Louis, Mo.), in 1 liter of distilled water. After the NaCl and bovine serum albumin had dissolved, 0.6 ml of oleic acid (Sigma) was added, and the solution was mixed for 30 min. The solution was then filtered successively through 0.5-, 0.45-, and 0.2-μm Metricel membrane filters (Gelman Sciences, Inc., Ann Arbor, Mich.) and then filter sterilized by passage through a 0.2-μm Metricel membrane filter into a sterile flask.

The inocula for the experiments were grown in MGE at 37°C with daily vortexing in screw-cap tubes (18 by 150 mm) to a turbidity of 25 Klett units (late log phase), measured with a Klett-Summerson colorimeter (Klett Manufacturing Co., New York, N.Y.) with a blue filter, and stored at 4°C for a maximum of 2 weeks.

**Temperature shift growth experiments.** Cells were inoculated (1%, vol/vol) into 500-ml Nephelo culture flasks (Bellco Glass, Inc., Vineland, N.J.) containing 50 ml of MGE and incubated in a model G76 Gyrotory water bath shaker (New Brunswick Scientific Co., Inc., New Brunswick, N.J.) adjusted to 90 oscillations per min at 37°C. When the cultures attained mid- or late-log-phase growth, 25 ml of each culture was transferred aseptically to an identical sterile flask and incubated at 43°C in a second identical water bath shaker adjusted to 90 oscillations per min. The original flasks, each containing 25 ml of culture, were returned to the 37°C water bath.

Growth of cells was measured by increases in turbidity, total cell count, and viable cell count. Turbidity was measured with a Klett-Summerson colorimeter, using a blue filter. Total cell number was determined by using a Petroff-Hausser counting chamber (Hausser Scientific, Blue Bell, Pa.), and viable cell counts, expressed as CFU per milliliter of culture, were determined on Middlebrook 7H10 agar medium (BBL) containing 1% (vol/vol) glycerol and 10% (vol/vol) oleic acid-albumin enrichment, following dilution of the cultures.

**Measurement of catalase activity.** Cells were inoculated (1%, vol/vol) into 1-liter screw-capped flasks containing 600 ml of MGE. The cultures were incubated at 37°C with daily shaking until mid-log, late log, early stationary, or stationary

* Corresponding author.

phase. Cells were harvested by centrifugation at 10,000 × *g* for 30 min at 4°C, washed three times, and suspended in 20 ml of 0.05 M sodium phosphate buffer (pH 7.0). Cells were broken by two passages through a cold French pressure cell (Aminco J4-3398A; American Instrument Co., Silver Spring, Md.) at 18,000 to 20,000 lb/in$^2$. The lysates were cleared by centrifugation at 23,000 × *g* for 30 min at 4°C. The supernatant (crude extract) was saved at 4°C for the catalase assay, which was completed within 24 h after breaking the cells.

In addition to measurement of catalase activity for cells grown as described above, the influence of culture aeration on catalase activity of strain LR25 was measured. Cultures were grown in a less aerobic environment by inoculating cells (1%, vol/vol) into 250-ml bottles containing 300 ml of MGE. An aerobic growth environment was obtained by inoculating cells (1%, vol/vol) into 1-liter screw-capped flasks containing 600 ml of MGE. Cultures were grown to late log phase at 37°C and mixed daily to suspend the cells. Cultures were grown in a highly aerobic environment by inoculating cells (1%, vol/vol) into 1-liter screw-capped flasks containing 100 ml of MGE. The cultures were grown to late log phase at 37°C in a New Brunswick model G76 water bath shaker adjusted to 90 oscillations per min. Following growth to late log phase, all cultures were harvested and catalase activity of crude extracts was measured.

The method of Beers and Sizer (4), which monitors the degradation of hydrogen peroxide at 240 nm, was used to assay the catalase activity of the crude extracts. Catalase activity was reported as units per milligram of protein (1 U equals 1 μmol of $H_2O_2$ decomposed per min). The catalase assay was measured at 37 and 43°C, using a model 102 digital spectrophotometer (Hitachi, Ltd., Tokyo, Japan). A temperature-controlled cell housing (Hitachi, Ltd.) and a model FJ constant-temperature circulator (Haake, Inc., Saddle Brook, N.J.) were used to maintain the temperature. Cell-free extracts were prewarmed to the test temperature in a water bath before the assay was run. Protein concentration of each extract was determined by the method of Lowry et al. (15), using bovine serum albumin fraction V (Sigma) as the standard.

The data from the experiments studying the effect of culture age on catalase activity of strains LR25 and LR163 were analyzed with the Statistical Analysis System (SAS; SAS Institute, Inc., Raleigh, N.C.), using the following general linear model: $y_{ijkl} = \mu + \alpha_i + \beta_j + \sigma_k + \alpha\sigma_{ik} + e_{ijkl}$, where $\mu$ is the overall mean of catalase activity; $\alpha_i$ is the effect of strain differences; $\beta_j$ is the effect of catalase assay temperature; $\sigma_k$ is the effect of culture growth phase; $\alpha\sigma_{ik}$ is the effect of interaction between strain and culture growth phase; and $e_{ijkl}$ is random error associated with each *ijkl*th observation.

The data from the experiments studying the effect of aeration on catalase activity of strain LR25 were analyzed with SAS, using the following general linear model: $y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + e_{ijk}$, where $\mu$ is the overall mean of catalase activity; $\alpha_i$ is the effect of level of aeration; $\beta_j$ is the effect of catalase assay temperature; $\alpha\beta_{ij}$ is the effect of interaction between aeration and catalase assay temperature; and $e_{ijk}$ is random error associated with each *ijk*th observation.

**Isolation of soluble and particulate fractions.** Following recovery of the crude extract (above), it was centrifuged at 100,000 × *g* for 60 min at 4°C to separate the cytoplasmic membrane (pellet) and soluble fractions. Catalase activities of each were measured as described above.

**Polyacrylamide gel electrophoresis.** Samples of soluble fractions were examined for bands of catalase activity in polyacrylamide tube gels as described by Mayer and Falkinham (16).

**Hydrogen peroxide susceptibility.** A modification of the method of Subbaiah et al. (18) was used to measure the effect of hydrogen peroxide ($H_2O_2$) on colony-forming ability of cells of *M. avium* LR25 and LR163. Cells were grown to mid-log phase at 37°C, and 0.4 ml of culture was removed and added to 7.6 ml of 0.05 M sodium phosphate buffer (pH 7.0) in a 125-ml Erlenmeyer flask containing 0.08% (wt/vol) $H_2O_2$. A control lacking $H_2O_2$ was also prepared. A molar extinction coefficient ($\varepsilon_{240}$) of 43.6 mol$^{-1}$ cm$^{-1}$ was used to prepare a standardized $H_2O_2$ stock solution from which the 0.08% (wt/vol) $H_2O_2$ solution was prepared. Immediately and after 60, 120, and 180 min of incubation at either 37 or 43°C in water bath shakers adjusted to 90 oscillations per min, CFU per milliliter of serial dilutions in 0.05 M sodium phosphate buffer (pH 7.0) were determined on M7H10 agar medium containing 1% (vol/vol) glycerol and 10% (vol/vol) oleic acid-albumin enrichment. Colonies were counted after 15 days of incubation at 37°C.

The hydrogen peroxide susceptibility data were analyzed with SAS, using the following general linear model: $y_{ijklm} = \mu + b_i + \alpha_j + \beta_k + \sigma_l + \alpha\beta_{jk} + \alpha\sigma_{jl} + b\sigma_{il} + e_{ijklm}$, where $\mu$ is the overall mean of percent survival; $b_i$ is the effect of performing the assay on different days; $\alpha_j$ is the effect of different strains; $\beta_k$ is the effect of $H_2O_2$ exposure; $\sigma_1$ is the effect of $H_2O_2$ exposure temperature; $\alpha\beta_{jk}$ is the effect of interaction between strain and $H_2O_2$ exposure; $\alpha\sigma_{jl}$ is the effect of interaction between strain and $H_2O_2$ exposure temperature; $b\sigma_{il}$ is the effect of interaction between day of test and $H_2O_2$ exposure temperature; and $e_{ijklm}$ is random error associated with each *ijklm*th observation. The data were blocked by day due to slight variations in percent survivals that may have been the result of clumping of cells during plate counts.

## RESULTS

**Effect of temperature shift on growth of strains LR25 and LR163.** Preliminary experiments demonstrated that strain LR163, the plasmid-free segregant of strain LR25, failed to grow at 43°C on Middlebrook 7H10 medium containing 1% glycerol and 10% oleic acid-albumin, whereas strain LR25 grew. To identify the physiologic basis for failure to grow at 43°C, a series of temperature shift growth experiments was performed. Following a shift from 37 to 43°C, accomplished by dividing the culture equally between two flasks (one remaining at 37°C), mid-log-phase cultures of strain LR25 grew at 37 and 43°C, whereas mid-log-phase cultures of strain LR163 failed to grow at 43°C (Fig. 1). Surprisingly, cultures of strain LR163 also failed to grow at 37°C following removal of half (25 ml) of the culture volume (Fig. 1). In all experiments, changes in total cell number and CFU per milliliter paralleled changes in turbidity and are not shown. Because the extent of aeration was increased following removal of half of the culture volume, the failure of strain LR163 to grow at 37°C suggested that it might be oxygen sensitive. A number of growth shift experiments were performed to rule out trivial explanations (e.g., pipetting sensitivity). First, the entire LR163 culture volume grown at 37°C to mid-log phase was removed and transferred to another flask and incubated at 37°C. Such cultures continued to grow at the preshift rate, demonstrating that strain LR163 was not sensitive to pipetting (data not shown) and that cultures of the strain could grow when the culture volume was main-

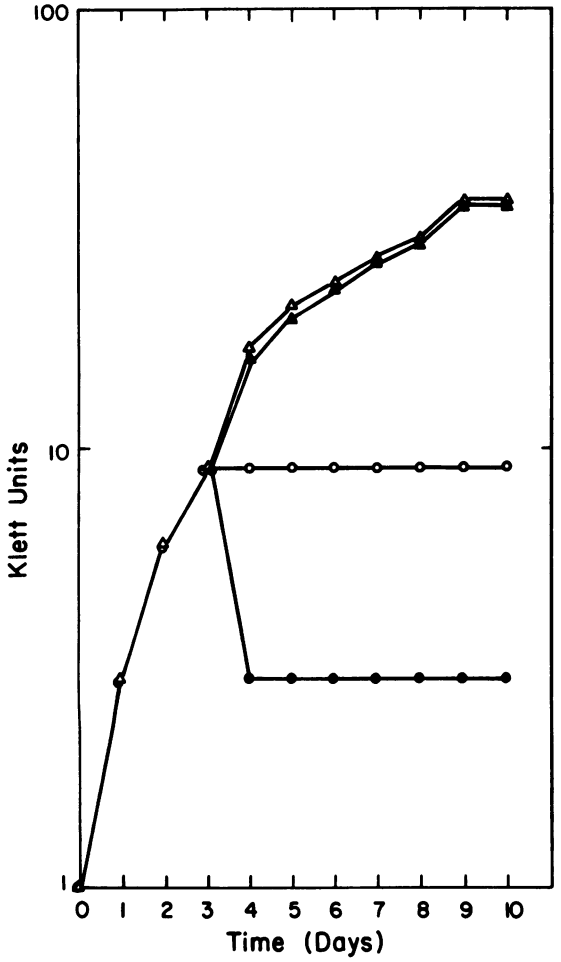


FIG. 1. Growth of *M. avium* LR25 (△, ▲) and LR163 (○, ●) incubated at 37°C and shifted to 43°C (▲, ●) or kept at 37°C (△, ○).

tained. Second, two identical 50-ml cultures of strain LR163 were incubated to mid-log phase and one was placed at 43°C (one remained at 37°C). Cultures continued to grow at 37 and 43°C (data not shown). In contrast to the results of shift experiments, cultures of strain LR163 failed to grow when inoculated and immediately incubated at 43°C (data not shown). The results of those reconstruction experiments led to the hypothesis that the inability of strain LR163 to grow at 43°C was possibly due to its sensitivity to oxygen or its toxic metabolites (e.g., $H_2O_2$).

**Catalase activity of strains LR25 and LR163.** Because of the role of catalase in protection of microorganisms against toxic oxygen metabolites (3), the catalase activities of strains LR25 and LR163 were measured at 37 and 43°C. Measurement at 43°C was included because the differences between the strains were more pronounced at 43°C (Fig. 1). Results show that crude extracts of mid-log-phase cultures of strain LR25 had significantly more catalase activity than those of strain LR163 measured at both 37 and 43°C ($P = 0.0001$) (Table 1). Extracts of mid-log-phase cells of strain LR25 had 3.3 (37°C) and 4.2 (43°C) times more catalase activity than extracts of strain LR163 (Table 1). The catalase activities of both strains were inhibited by 1 mM KCN and found only in the soluble cell fraction (data not shown).

Because growth stage had been shown to influence catalase activity of *Nocardia asteroides* (2) and the thermal stability of *M. avium*, *M. intracellulare*, and *M. scrofulaceum* catalase activity (16), the effect of growth stage on catalase activity of cells of strains LR25 and LR163 was investigated as well. As culture age increased, the catalase activity of strain LR25 fell and that of strain LR163 rose, and the difference in catalase activity between extracts of strains LR25 and LR163 became smaller (Table 1). However, cells from mid-log-, late-log-, and early-stationary-phase cultures of strain LR25 had significantly more catalase activity at both test temperatures than cultures of strain LR163 ($P = 0.0001$, 0.02, and 0.03, respectively). There was no significant difference in the catalase activities of stationary-phase cultures of the two strains (Table 1). This was observed at both 37 and 43°C.

TABLE 1. Catalase activity and effect of culture age on catalase activity of *M. avium* LR25 and LR163

| Culture age[a] | Temp of assay (°C) | Catalase activity (U/mg of protein)[b] | |
|---|---|---|---|
| | | Strain LR25 | Strain LR163 |
| Mid-log | 37 | 73.3 ± 17.3 | 21.9 ± 10.8 |
| | 43 | 50.1 ± 11.5 | 11.9 ± 6.6 |
| Late log | 37 | 48.3 ± 18.1 | 34.9 ± 13.4 |
| | 43 | 36.3 ± 12.5 | 22.4 ± 8.6 |
| Early stationary | 37 | 63.0 ± 11.8 | 54.5 ± 12.9 |
| | 43 | 41.8 ± 16.0 | 23.8 ± 15.3 |
| Stationary | 37 | 53.7 ± 12.8 | 55.1 ± 14.2 |
| | 43 | 32.0 ± 2.8 | 45.5 ± 10.0 |

[a] Klett readings: mid-log, 10; late log, 25; early stationary, 30; stationary, 36 Klett units.
[b] Means ± standard deviations of three assays are given.

**Effect of growth conditions on catalase activity of strain LR25.** In addition to culture age, the extent of culture aeration influenced catalase activity of strain LR25 (Table 2). The mean catalase activity of late-log-phase cultures grown under high aeration measured at 37°C was significantly higher than that of the moderately aerated ($P = 0.05$) and less aerated ($P = 0.02$) cultures also grown to late log phase. The mean catalase activities measured at 43°C of cells grown under the three conditions were not significantly different (Table 2).

**Electrophoresis of catalases of strains LR25 and LR163.** The increased catalase activity of strain LR25 was not due to the appearance of a new catalase activity band (data not shown) beyond those two catalase activity bands characteristic of *M. avium* strains (16). Further, distribution of staining intensity between the two bands in each strain did not differ (data not shown).

**Hydrogen peroxide susceptibility of strains LR25 and LR163.** If *M. avium* catalase activity was of biological

TABLE 2. Effect of culture aeration on catalase activity of *M. avium* LR25

| Culture aeration[a] | Temp of assay (°C) | Catalase activity (U/mg of protein)[b] |
|---|---|---|
| Less aerobic | 37 | 40.7 ± 7.6 |
| | 43 | 36.9 ± 2.3 |
| Aerbic | 37 | 48.3 ± 18.1 |
| | 43 | 36.3 ± 12.5 |
| Highly aerobic | 37 | 64.2 ± 12.5 |
| | 43 | 35.2 ± 9.6 |

[a] Culture aeration conditions are described in Materials and Methods.
[b] Means ± standard deviations of late-log-phase cultures (two assays) are given.

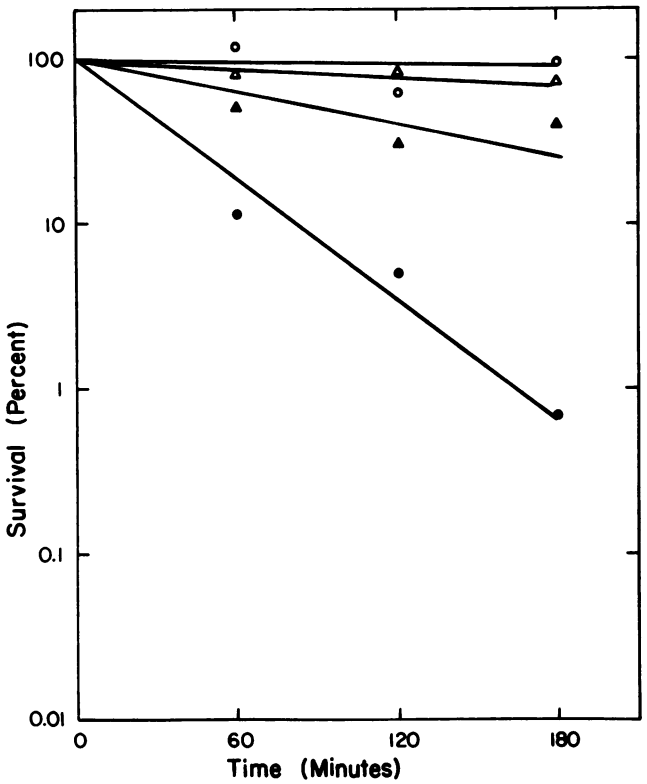


FIG. 2. Survival of cells of *M. avium* LR25 (△, ▲) and LR163 (○, ●) in 0.05 M sodium phosphate buffer, pH 7 (△, ○), and buffer containing 0.08% (wt/vol) $H_2O_2$ (▲, ●) at 37°C.

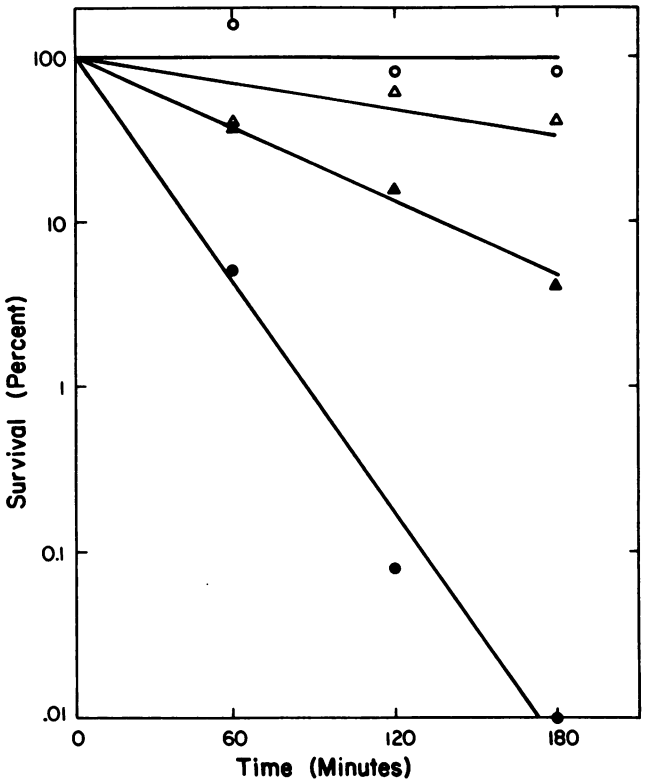


FIG. 3. Survival of cells of *M. avium* LR25 (△, ▲) and LR163 (○, ●) in 0.05 M sodium phosphate buffer, pH 7 (△, ○), and buffer containing 0.08% (wt/vol) $H_2O_2$ (▲, ●) at 43°C.

significance, cells of strain LR25 would be more resistant to hydrogen peroxide than those of strain LR163. Mid-log-phase cultures of strain LR25 were significantly more resistant to 0.08% hydrogen peroxide at 37°C (Fig. 2; $P = 0.05$) and 43°C (Fig. 3; $P = 0.004$) than those of strain LR163.

## DISCUSSION

The data demonstrate that a plasmid-carrying isolate of *M. avium*, strain LR25, had significantly more catalase activity than its plasmid-free segregant, strain LR163 (Table 1). Further, catalase activity of strain LR25 was influenced by culture age (Table 1) as observed for *N. asteroides* (2). In *N. asteroides*, there was more catalase activity in early-stationary-phase than in log-phase cells (2) unlike the virulent *M. avium* strain LR25, which had highest catalase activity in mid-log phase (Table 1). Catalase activity of strain LR25 measured at 37°C was increased by growth under high levels of aeration (Table 2). Surprisingly, aeration did not alter the catalase activity measured at 43°C (Table 2), perhaps because as culture age increases, the proportion of heat-resistant catalase activity of *M. avium* increases (16). The biologic significance of the increased catalase activity of strain LR25 was demonstrated by the fact that the strain was more resistant to hydrogen peroxide than its plasmid-free segregant, strain LR163, at both 37 and 43°C (Fig. 2 and 3). In contrast to our results, Gangadharam and Pratt (13) did not find a correlation between catalase activity and $H_2O_2$ susceptibility. However, they did not compare isogenic strains; thus, the responses of their strains could have been influenced by other factors (e.g., $H_2O_2$ permeation and pigmentation), as they suggested (13).

Because *M. avium* LR163 failed to grow at 43°C, it was originally thought that any difference between strains LR25 and LR163 would be more pronounced at 43 than at 37°C. Though catalase activity measured at 43°C was less than that measured at 37°C in all cases (Table 1), the greatest difference between strains LR25 and LR163 was found in mid-log-phase cultures (Table 1). Though mid-log-phase cultures of strain LR25 grew at both 37 and 43°C, mid-log-phase cultures of strain LR163 failed to grow at both temperatures following the temperature shift regimen (Fig. 1). The possibility that culture aeration influenced the ability of strain LR163 to grow at 37 and 43°C was supported by the observation that it could grow when the surface/volume ratio of cultures was maintained.

The low catalase activity of cells of strain LR163 was most likely responsible for its inability to grow at 37 and 43°C under highly aerobic conditions. Autoclaving and exposure to air have been shown to result in the formation of hydrogen peroxide in bacteriologic media (1, 7). Catalase specific activities of mid-log-phase cells of strain LR163 were significantly less than those of strain LR25 when measured at both 37°C (30%; Table 1) and 43°C (24%; Table 1). The possibility that the low specific activity of catalase limited the growth of strain LR163 was supported by the observation that late-log-phase cultures grew at 37 and 43°C when shifted following removal of half of the culture volume. The catalase specific activities of late-log-phase cultures of strain LR163 were 1.6 (37°C)- and 1.9 (43°C)-fold higher than those of mid-log-phase cultures (Table 1). However, the data do not prove that catalase activity is the sole determinant permitting growth of *M. avium* LR25 at 37 and 43°C under highly aerobic conditions. It is possible that other plasmid-encoded

gene functions (e.g., fatty acid composition of the cell membrane), in addition to those influencing catalase activity, contribute to the ability of strain LR25 to grow at 43°C.

The data provide a possible explanation for the greater virulence of strain LR25 compared with strain LR163 in the beige mouse (12). Hydrogen peroxide is a known agent of intracellular killing of bacteria by mammalian phagocytic cells, and microbial catalases have been shown to enhance survival in phagocytic cells (2). The higher catalase activity of strain LR25 could result in elevated survival in phagocytes and a lower release of hydrogen peroxide by phagocytes compared with strain LR163, as observed by Gangadharam et al. (12).

Based on the fact that no new bands of catalase activity were observed in extracts of strain LR25, it is unlikely that a plasmid encodes for a novel catalase. Since the highest mean catalase activity of strain LR25 is found during mid-log phase and that of strain LR163 is found during stationary phase, it is possible that a plasmid-encoded gene product modifies the regulation of catalase production. Alternatively, one of the plasmids of strain LR25 could contain an additional copy of one or both of the chromosomal catalase genes which now demonstrate a novel pattern of regulation.

## ACKNOWLEDGMENTS

We acknowledge the kind gift of *M. avium* LR25 and LR163 by Jack T. Crawford.

This work was supported by Public Health Service grant AI-23710 from the National Institute of Allergy and Infectious Diseases.

## LITERATURE CITED

1. **Barry, V. C., M. L. Conalty, J. M. Denneny, and F. Wilder.** 1956. Peroxide formation in bacteriological media. Nature (London) **178**:596–597.
2. **Beaman, B. L., C. M. Black, F. Doughty, and L. Beaman.** 1985. Role of superoxide dismutase and catalase as determinants of pathogenicity of *Nocardia asteroides*: importance in resistance to microbicidal activities of human polymorphonuclear neutrophils. Infect. Immun. **47**:135–141.
3. **Beaman, L., and B. L. Beaman.** 1984. The role of oxygen and its derivatives in microbial pathogenesis and host defense. Annu. Rev. Microbiol. **38**:27–48.
4. **Beers, R. F., Jr., and I. W. Sizer.** 1952. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J. Biol. Chem. **195**:133–140.
5. **Blaser, M. J., and D. L. Cohn.** 1986. Opportunistic infections in patients with AIDS: clues to the epidemiology of AIDS and the relative virulence of pathogens. Rev. Infect. Dis. **8**:21–30.
6. **Brooks, R. W., B. C. Parker, H. Gruft, and J. O. Falkinham III.** 1984. Epidemiology of infection by nontuberculous mycobacteria. V. numbers in eastern United States soils and correlation with soil characteristics. Am. Rev. Respir. Dis. **130**:630–633.
7. **Carlsson, J., G. Nyberg, and J. Wrethén.** 1978. Hydrogen peroxide and superoxide radical formation in anaerobic broth media exposed to atmospheric oxygen. Appl. Environ. Microbiol. **36**:223–229.
8. **Clare, D. A., M. N. Duong, D. Darr, F. Archibald, and I. Fridovich.** 1984. Effects of molecular oxygen on detection of superoxide radical with nitroblue tetrazolium and on activity stains for catalase. Anal. Biochem. **140**:532–537.
9. **Crawford, J. T., M. D. Cave, and J. H. Bates.** 1981. Evidence for plasmid-mediated restriction-modification in *Mycobacterium avium-intracellulare*. J. Gen. Microbiol. **127**:333–338.
10. **Falkinham, J. O., III, B. C. Parker, and H. Gruft.** 1980. Epidemiology of infection by nontuberculous mycobacteria. I. Geographic distribution in the eastern United States. Am. Rev. Respir. Dis. **121**:931–937.
11. **Fry, K. L., P. S. Meissner, and J. O. Falkinham III.** 1986. Epidemiology of infection by nontuberculous mycobacteria. VI. Identification and use of epidemiologic markers for studies of *Mycobacterium avium*, *M. intracellulare*, and *M. scrofulaceum*. Am. Rev. Respir. Dis. **134**:39–43.
12. **Gangadharam, P. R. J., V. K. Perumal, J. T. Crawford, and J. H. Bates.** 1988. Association of plasmids and virulence of *Mycobacterium avium* complex. Am. Rev. Respir. Dis. **137**: 212–214.
13. **Gangadharam, P. R. J., and P. F. Pratt.** 1984. Susceptibility of *Mycobacterium avium* to hydrogen peroxide. Am. Rev. Respir. Dis. **130**:309–311.
14. **Gregory, E. M., and I. Fridovich.** 1974. Visualization of catalase on acrylamide gels. Anal. Biochem. **58**:57–62.
15. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
16. **Mayer, B. K., and J. O. Falkinham III.** 1986. Catalase activity and its heat inactivation for differentiation of *Mycobacterium avium*, *Mycobacterium intracellulare*, and *Mycobacterium scrofulaceum*. Int. J. Syst. Bacteriol. **36**:207–212.
17. **Meissner, P. S., and J. O. Falkinham III.** 1986. Plasmid DNA profiles as epidemiological markers for clinical and environmental isolates of *Mycobacterium avium*, *M. intracellulare*, and *M. scrofulaceum*. J. Infect. Dis. **153**:325–331.
18. **Subbaiah, T. V., D. A. Mitchison, and J. B. Selkon.** 1960. The susceptibility to hydrogen peroxide of Indian and British isoniazid-sensitive and isoniazid-resistant tubercle bacilli. Tubercle **41**:323–333.
19. **Wendt, S. L., K. L. George, B. C. Parker, H. Gruft, and J. O. Falkinham III.** 1980. Epidemiology of infection by nontuberculous mycobacteria. III. Isolation of potentially pathogenic mycobacteria from aerosols. Am. Rev. Respir. Dis. **122**:259–263.
20. **Wolinsky, E.** 1979. Nontuberculous mycobacteria and associated diseases. Am. Rev. Respir. Dis. **119**:107–159.