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# Superoxide Dismutase Activity of *Mycobacterium avium*, *M. intracellulare*, and *M. scrofulaceum*

BRIAN K. MAYER AND JOSEPH O. FALKINHAM III*

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

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**Superoxide dismutase (EC 1.15.1.1) (SOD) activity has been detected in crude cell extracts of representative strains of the *Mycobacterium avium*, *M. intracellulare*, and *M. scrofulaceum* (MAIS) group. Polyacrylamide gel electrophoresis demonstrated a single SOD activity band for each of the MAIS strains, though there were differences in mobility. All *M. avium* and *M. intracellulare* and two of five *M. scrofulaceum* strains demonstrated a single activity band of identical mobility ($R_f$ = 0.83), while the SOD activity band for the three remaining *M. scrofulaceum* strains migrated farther ($R_f$ = 0.85). The differences in mobility correlated with differences in sensitivity to $NaN_3$ and $H_2O_2$. The SOD activities of the majority of the MAIS strains which displayed the slower-migrating activity band were inhibited 22 to 81% after 15 min of exposure to 5 mM $H_2O_2$, suggesting that both iron and manganese may be present in a single enzyme. The SOD activities of the three *M. scrofulaceum* strains which had the faster-migrating activity band were inhibited 100% after only 5 min of exposure to 5 mM $H_2O_2$ and exhibited greater sensitivity to 5 and 10 mM $NaN_3$, characteristics of an iron-containing SOD. A concentration of 1 mM KCN did not cause inhibition of enzyme activity in any of the MAIS strains tested. Extracellular SOD activity was detected in four of six MAIS strains and was shown to be identical in mobility to the SOD activity of the crude extracts.**

Superoxide dismutase (EC 1.15.1.1) (SOD), which catalyzes the dismutation of the superoxide radical to hydrogen peroxide and molecular oxygen, has been found in a number of bacterial species (8, 24, 31, 36) since it was first purified from bovine erythrocytes by McCord and Fridovich (29). Because the superoxide radical is a normal product of the univalent reduction of molecular oxygen, SODs are thought to be the primary defense against its potential cytotoxicity (14, 15). A study by Britton et al. (8) revealed that 17% of oxygen consumed by extracts of *Streptococcus faecalis*, whose SOD activity was suppressed by the addition of specific antibody, was associated with the production of superoxide radicals. The observation that induced levels of SOD correlated with greater resistance of bacteria to the toxic effects of hyperbaric oxygen (32) and superoxide-generating compounds, such as plumbagin (12), further supports its role in protection.

There are three distinct types of SODs which can be distinguished by their metal content. Most procaryotes produce a SOD with either iron or manganese as the prosthetic group, while eucaryotes have a copper-zinc cytoplasmic enzyme and a manganese-containing mitchondrial enzyme (5). Exceptions within the procaryotes include the copper-zinc SOD-producing bacteria *Photobacterium leiognathi* (34), *Pseudomonas diminuta*, and *Pseudomonas maltophilia* (35).

Within the genus *Mycobacterium*, SOD has been characterized for a few species. These include *Mycobacterium tuberculosis* (25), *M. phlei* (9), *M. smegmatis* (26), *M. lepraemurium* (21), and *M. leprae* (37). Currently there is no information on SOD activity from the *M. avium*, *M. intracellulare*, and *M. scrofulaceum* (MAIS) group. This group of organisms, whose origin is environmental (38), has been reported with increasing frequency as a cause of human disease (18, 33). Most recently it has been shown that a significant proportion of individuals suffering from acquired immunodeficiency syndrome have disseminated *M. avium* and *M. intracellulare* infections (39, 41).

Since MAIS organisms are capable of surviving and multiplying within the host lung while exposed to a high partial pressure of oxygen (38) and are capable of surviving the oxidative burst of activated macrophages (16), SOD activity may be important to their pathogenicity. As environmental pathogens, MAIS organisms may be exposed to photochemically generated superoxide radicals in surface waters exposed to sunlight (10), and SOD activity may aid in their survival.

## MATERIALS AND METHODS

**Bacterial strains.** Strains of mycobacteria used in this study (Table 1) were obtained from the National Jewish Hospital, Denver, Colo., and from the Statens Seruminstitut, Copenhagen, Denmark. Species assignments are based on DNA-DNA hybridization results (4, 22). *Streptococcus lactis* ATCC 11454 and *Alcaligenes faecalis* (unclassified) were obtained from the Virginia Polytechnic Institute microbiology stock culture collection.

**Growth of cells and preparation of crude extracts.** Cell cultures and inocula of MAIS strains were grown in Middlebrook 7H9 medium (BBL Microbiology Systems, Cockeysville, Md.) containing 0.44% (vol/vol) glycerol, 0.2% (vol/vol) dextrose, and 10% (vol/vol) oleic acid-albumin enrichment.

The MAIS inocula were grown in screw-cap tubes (16 by 150 mm) containing 10 ml of the above medium at 37°C for approximately 9 days and stored for use at 4°C for a maximum of 2 months. MAIS cells were inoculated (1% [vol/vol]) into 1-liter screw-cap flasks containing 300 ml of the enriched Middlebrook 7H9 medium and incubated with caps loose for 12 days at 37°C. The flasks were shaken by hand daily to resuspend the cells and to aerate the medium. Cultures of *S. lactis* and *A. faecalis* were grown in 1-liter screw-cap flasks containing 300 ml of brain heart infusion

* Corresponding author.

TABLE 1. SOD activity of MAIS strains

| Strain | Total no. of assays | Superoxide dismutase activity (U/mg of protein ± SD) |
|---|---|---|
| *M. avium*[a] | | |
| TMC 724[b] | 2 | 35.2 ± 4.6 |
| TMC 706[c] | 4 | 29.9 ± 3.1 |
| 13528-1079 | 4 | 27.1 ± 6.2 |
| TMC 701 | 2 | 27.7 ± 4.1 |
| TMC 721 | 2 | 14.6 ± 4.4 |
| Wild strain E46941/76 | 4 | 34.3 ± 2.3 |
| Susook | 3 | 21.5 ± 1.8 |
| J1868 | 2 | 25.3 ± 2.1 |
| Wild strain V3435/77 | 2 | 32.4 ± 7.4 |
| 4443-1237 | 2 | 22.4 ± 2.9 |
| *M. intracellulare*[d] | | |
| TMC 1403 | 2 | 20.0 ± 1.3 |
| TMC 1406[e] | 4 | 25.8 ± 4.2 |
| Manten 157 | 2 | 26.3 ± 4.0 |
| TMC 1405 | 4 | 25.5 ± 6.7 |
| ATCC 25169 | 4 | 20.6 ± 4.0 |
| Darden | 2 | 21.5 ± 2.2 |
| Mark Robert | 2 | 20.6 ± 1.8 |
| 2219, Altman | 2 | 19.8 ± 1.4 |
| *M. scrofulaceum*[f] | | |
| TMC 1312 | 4 | 22.1 ± 3.8 |
| TMC 1306 | 3 | 18.3 ± 1.5 |
| TMC 1302 | 3 | 14.6 ± 3.5 |
| TMC 1323[g] | 4 | 11.6 ± 2.9 |
| ATCC 19073 | 4 | 14.3 ± 3.6 |

[a] Total assays, 27; average ± standard deviation, 27.6 ± 6.7.
[b] Type strain of *M. avium*.
[c] Suggested IWGMT (International Working Group of Mycobacterial Taxonomy) working type.
[d] Total assays, 22; average ± standard deviation, 22.9 ± 4.5.
[e] Type strain of *M. intracellulare*.
[f] Total assays, 18; average ± standard deviation, 16.2 ± 4.8.
[g] Type strain of *M. scrofulaceum*.

broth (Difco Laboratories, Detroit, Mich.) on a rotary platform shaker for 18 h (8). After a period of growth, the cultures were streaked to confirm purity, and 300 ml of the log-phase cells was harvested by centrifugation at 10,000 × *g* for 10 min, washed once in 100 ml of 0.05 M potassium phosphate buffer (pH 7.8) containing 1 mM EDTA, and were suspended in a final volume of 10 ml of that buffer. Cells were broken by one passage 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 crude extracts were clarified by centrifugation at 23,000 × *g* for 30 min, and the supernatants were retained for enzyme assays.

Extracts used for SOD inhibition studies to indirectly determine metalloenzyme type were dialyzed (12,000- to 14,000-$M_r$ cutoff; Spectrapor; American Hospital Supply Corp., McGaw Park, Ill.) for 24 h at 4°C against 0.05 M potassium phosphate buffer (pH 7.8) containing 1 mM EDTA. The extracts then were centrifuged at 23,000 × *g* for 10 min to remove any precipitates.

The cultures used for determination of extracellular SOD activity were grown in 1-liter screw-cap flasks as previously described, with the exception that oleic acid-albumin enrichment was omitted and the length of incubation was increased to 21 days. After centrifugation at 10,000 × *g* for 10 min to remove cells, 200 ml of medium was concentrated to 10 ml by ultrafiltration through a PM-10 membrane (Amicon Corp., Danvers, Mass.). The concentrate was then dialyzed for 24 h at 4°C against 0.05 M sodium phosphate buffer (pH 7.0) and centrifuged at 23,000 × *g* for 10 min.

**Assay of SOD activity.** The enzyme was measured by the method of McCord and Fridovich (29). The amount of SOD required to inhibit the rate of reduction of cytochrome *c* by 50% was defined as 1 U of activity. A final concentration of 0.05 mM KCN was added to the assay system to inhibit any cytochrome oxidase which might be present in the extracts (11). Protein was determined by the method of Lowry et al. (27) with bovine serum albumin (fraction V) as the standard.

**PAGE.** Polyacrylamide gel electrophoresis (PAGE) was done as follows. Samples of crude cell extracts were examined for bands of SOD activity by using a 7.5% acrylamide resolving gel, a 4% spacer gel, and 0.022 M Tris–0.018 M glycine electrode buffer at pH 8.2. Samples contained 60 to 80 μl of crude extract (50 to 55 μg of protein) and 20 μl of a 10% (vol/vol) glycerol–1 mM EDTA–0.5 M sodium phosphate solution (pH 7.8), with a few crystals of bromophenol blue added as the running dye marker.

Electrophoresis was carried out using a vertical tank apparatus (SE600 series; Hoefer Scientific Instruments, San Francisco, Calif.) cooled with running water. Voltage was maintained at 150 V, using a constant-voltage power supply (model EC103; EC Apparatus Corp., St. Petersburg, Fla.) until the bromophenol blue tracking dye reached the bottom of the gel, approximately 5 h. The gels were then removed and stained for SOD activity by the Nitro Blue Tetrazolium method as described by Beauchamp and Fridovich (7).

**Inhibition of SOD activity.** The effect of KCN (1 mM final concentration) and $NaN_3$ (1, 5, and 10 mM final concentration) on SOD activity was determined by the addition of either directly to the assay mixture. At these concentrations neither azide nor cyanide inhibited the reduction of cytochrome *c*. The effect of $H_2O_2$ was determined by incubation of the cell extracts containing 5 mM $H_2O_2$ and 5 mM KCN at 22°C. The KCN was added to prevent the degradation of the added $H_2O_2$ by the catalase activity of the extracts (28). After different periods of incubation, a 0.1-ml sample was removed and assayed for SOD activity. Correction for the slight inhibition by $H_2O_2$ of cytochrome *c* reduction was made by addition of a slightly larger amount (12 to 13 μl instead of 10 μl) of xanthine oxidase to the assay mixture.

## RESULTS

**MAIS SOD activity.** The specific activity of cell extracts of MAIS reference strains is shown in Table 1. *M. avium* strains had an average enzyme activity of 27.6 ± 6.7 U/mg of protein (average ± standard deviation), *M. intracellulare* strains had an average of 22.9 ± 4.5 U/mg of protein, and the average SOD activity of the *M. scrofulaceum* strains was 16.2 ± 4.8 U/mg of protein. The SOD activity was stable under refrigeration for at least 7 days, but was lost upon freezing extracts at 0°C. Extracts which were dialyzed prior to freezing retained their activity.

**Electrophoresis of MAIS SOD.** Electrophoresis of crude extracts demonstrated a single band of SOD activity for each of the MAIS strains tested (Fig. 1). However, there were differences in mobility. *M. avium* E46941/76 and Susook, *M. intracellulare* TMC 1406 and ATCC 25169, and *M. scrofulaceum* TMC 1323 all had an activity band of identical mobility ($R_f$ = 0.83 [Fig. 1, lanes 1 to 5]), while the SOD of *M. scrofulaceum* ATCC 19073 migrated further ($R_f$ = 0.85; Fig. 1, lane 6). Of six *M. avium* strains and four *M. intracellulare* strains examined, all had identical mobility. Of the five *M. scrofulaceum* strains, two (TMC 1312 and TMC

1323) had SOD activity similar to that of *M. avium* and *M. intracellulare*, and three strains (TMC 1306, TMC 1302, and ATCC 19073) had the faster-migrating enzyme activity band.

**Characteristics of MAIS SOD activity.** The effect of various inhibitors on MAIS SOD activity is shown on Table 2. Extracts from bacterial species of known metallotypes were also tested for comparison. All of the *M. avium* and *M. intracellulare* strains studied had SOD activities which were moderately susceptible to 5 mM $H_2O_2$ and 1 to 10 mM $NaN_3$. The SODs from *M. scrofulaceum* ATCC 19073, TMC 1302, and TMC 1306 were completely inactivated after 5 min of incubation with 5 mM $H_2O_2$ and exhibited greater sensitivity to 10 mM $NaN_3$, while *M. scrofulaceum* TMC 1312 and TMC 1323 were more resistant to both treatments. KCN (1 mM) did not inhibit any of the MAIS SOD activities.

**Extracellular SOD activity.** Enzyme activity was found in the concentrated medium after growth and removal of the cells. The activity levels of the concentrates for *M. avium* 13528-1079 and V3435/77 were 0.8 and 0.7 U/mg of protein, respectively. *M. intracellulare* Manten 157 and Darden had slightly higher extracellular SOD activities of 1.6 and 1.5 U/mg of protein, respectively. *M. scrofulaceum* TMC 19073 and TMC 1323 also had measurable activities, with values of 1.0 and 1.5 U/mg of protein, respectively. Examination of concentrated medium by PAGE demonstrated a single band of SOD activity which correlated in mobility with the activity band of crude extracts. Assays of concentrated medium from *M. scrofulaceum* TMC 1306 and TMC 1312 failed to demonstrate any detectable SOD activity (<0.3 U/mg of protein).

## DISCUSSION

The average SOD activities of MAIS cell extracts (Table 1) were slightly higher than values reported for most other species of mycobacteria. These include the following: *M. tuberculosis*, 11.0 U/mg (25); *M. smegmatis* TAKEO, 12.0 U/mg (26); *M. leprae*, 0.17 U/mg (37); and *M. phlei*, 5.9 U/mg (37). The SOD activity of *M. lepraemurium* seems to vary, depending on whether the organism is grown in vivo or in vitro. Ichihara et al. (21) reported that the cell extract of *M. lepraemurium* grown in vitro (1% Ogawa egg yolk medium) had a specific activity of 180 U/mg, while Wheeler and Gregory (37) reported that *M. lepraemurium* grown in vivo (mice) had an activity of only 14.3 U/mg. This disparity (possibly due to differences in culture conditions) is important because of the high DNA homology of *M. lepraemurium* and *M. avium* (22).

Electrophoresis of crude cell extracts showed a single band of SOD activity with all MAIS strains examined. However, there were differences in mobility (Fig. 1). All *M. avium* and *M. intracellulare* strains and two of five *M. scrofulaceum* strains tested had a SOD activity band of identical mobility ($R_f$ = 0.83; Fig. 1, lanes 1 to 5). The three remaining *M. scrofulaceum* strains had a single, faster-migrating SOD activity band ($R_f$ = 0.85; Fig. 1, lane 6).

SODs have been differentiated into three classes based on the type of metal ion that predominates as the prosthetic group. These classes are copper-zinc-, manganese-, and iron-containing SODs. Differentiation is possible indirectly by studies of inactivation and inhibition of enzyme activity in crude cell extracts. The copper-zinc SODs are inhibited by 1 mM KCN, while the manganese and iron forms are not (20). Irreversible inhibition of copper-zinc- (19) and iron-containing (1) SODs occurs in the presence of 5 mM $H_2O_2$, which does not affect the manganese-containing enzyme.

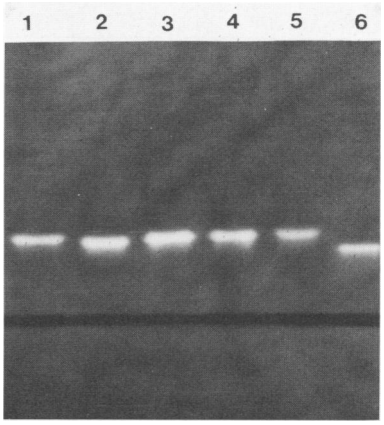


FIG. 1. PAGE of MAIS cell extracts stained for superoxide dismutase activity. Lanes: 1, *M. avium* E46941/76; 2, *M. avium* Susook; 3, *M. intracellulare* TMC 1406; 4, *M. intracellulare* ATCC 25169; 5, *M. scrofulaceum* TMC 1323; 6, *M. scrofulaceum* ATCC 19073.

Addition of 10 mM $NaN_3$ will inhibit all three enzymes, but to different degrees. The iron-containing SOD is most sensitive, followed by the manganese and then the copper-zinc form (30).

The data presented in Table 2 suggest that MAIS SODs possess characteristics of both manganese- and iron-containing enzymes. An iron-containing SOD should have been inactivated completely by incubation with 5 mM $H_2O_2$, as with the control strains (*A. faecalis*, 96% inhibited; *Bacillus fragilis*, 91% inhibited), while a manganese-containing SOD should have been resistant (*S. lactis*, 12% inhibited). The SOD activities of extracts from *M. avium* and *M. intracellulare* strains (Table 2) were intermediate in their resistance (22 to 51% inhibition after exposure to 5 mM $H_2O_2$ for 15 min). SOD activity levels of the *M. scrofulaceum* strains, with the exception of TMC 1323, were even more sensitive to $H_2O_2$. *M. scrofulaceum* ATCC 19073, TMC 1302, and TMC 1306 were 100% inhibited after only 5 min of incubation with 5 mM $H_2O_2$, and the enzyme from *M. scrofulaceum* TMC 1312 was 81% inhibited after 15 min. The SODs of the *M. scrofulaceum* strains, which exhibited greater sensitivity to $H_2O_2$, were also more sensitive to 10 mM $NaN_3$. These characteristics are more indicative of iron-containing SODs.

A similar pattern of inhibition of MAIS SOD activities by $H_2O_2$ was also apparent in polyacrylamide gels (data not shown). When 5 mM $H_2O_2$ and 1 mM KCN were added to the riboflavin and Nitro Blue Tetrazolium solutions (for assaying SOD activity in polyacrylamide gels [6]), the activity bands of *M. scrofulaceum* TMC 1302 and TMC 1312 were completely inhibited, while *M. scrofulaceum* TMC 1323 still displayed a faint band. The SOD activity bands of *M. avium* Susook and *M. intracellulare* TMC 1406 were inhibited approximately 50% but were still clearly visible. By comparison, *A. faecalis*, which displayed a single SOD activity band, was completely inhibited, and the SOD activity band of *S. lactis* was not significantly inhibited at all.

Interestingly, all of the strains which displayed the slower migrating band were also intermediate in their sensitivity to $H_2O_2$ and $NaN_3$, while the SOD activity from the *M. scrofulaceum* strains with the faster-migrating SOD band were much more sensitive (Table 2 and Fig. 1).

Inhibition studies on SOD in crude extracts from *Nocardia asteroides*, also an intracellular pathogen, gave

TABLE 2. MAIS SOD inhibition

| Strain | Sp act (U/mg) | % Inhibition by 5 mM $H_2O_2$ | | | % Inhibition by $NaN_3$ (mM): | | | % Inhibition by 1 mM KCN |
|---|---|---|---|---|---|---|---|---|
| | | 1′ | 5′ | 15′ | 1 | 5 | 10 | |
| *M. avium* | | | | | | | | |
| E46941/76 | 33.5 | 9 | 9 | 22 | 9 | 13 | 34 | 0 |
| Susook | 23.5 | 35 | 35 | 35 | 15 | 26 | 32 | −8 |
| *M. intracellulare* | | | | | | | | |
| TMC 1406 | 29.8 | 32 | 32 | 44 | 35 | 23 | 40 | 9 |
| ATCC 25169 | 22.6 | 14 | 38 | 51 | 26 | 16 | 35 | −31 |
| *M. scrofulaceum* | | | | | | | | |
| TMC 1323 | 11.6 | 33 | 44 | 44 | 6 | 17 | 22 | −45 |
| TMC 1312 | 17.5 | 57 | 57 | 81 | 14 | 14 | 39 | −24 |
| ATCC 19073 | 17.3 | 67 | 100 | 100 | 34 | 47 | 62 | −25 |
| TMC 1302 | 16.8 | 81 | 100 | 100 | 16 | 30 | 46 | −22 |
| TMC 1306 | 16.9 | 54 | 100 | 100 | 14 | 38 | 48 | 0 |
| *A. faecalis* Fe SOD[a] | 14.3 | ND[b] | 96 | ND | 0 | 31 | 48 | 5 |
| *B. fragilis* VPI 2393 Fe SOD[c] | 810.0[d] | 28 | 68 | 91 | 74 | 95 | 95 | −23 |
| *Streptococcus lactis* ATCC 11454 Mn SOD[e] | 20.3 | ND | 12 | 12 | ND | ND | 19 | 9 |

[a] Fe SOD, Iron-containing SOD (8).
[b] ND, Not done.
[c] Purified by E. M. Gregory (32).
[d] Units per milliliter.
[e] Mn SOD, Manganese-containing SOD (8).

results similar to those reported here for the slow migrating form. Treatment with 20 mM $NaN_3$ and 5 mM $H_2O_2$ resulted in 41 and 40% inhibition, respectively (6). Analysis by atomic absorption spectroscopy subsequently detected almost equimolar concentrations of Mn, Fe, and Zn (6). *M. phlei* SOD also contains relatively large proportions of all three metals (9). Atomic absorption spectroscopy measured 1.2, 1.7, and 0.8 g-atoms/mol of enzyme of Fe, Mn, and Zn, respectively (9). Studies of SOD produced by other species of mycobacteria have reported that *M. leprae* (37) and *M. smegmatis* TAKEO (26) both produce a manganese-containing SOD, while *M. tuberculosis* produces an iron-containing SOD (25). Atomic absorption analysis of SOD from *M. lepraemurium* (21) has shown that the enzyme contains manganese (1.3 g-atoms/mol) and a small but significant amount of iron (0.3 g-atoms/mol). Since *M. lepraemurium* and *M. avium* have a high degree of DNA-relatedness (22), our suggestion that the SODs of a majority of MAIS strains contain both iron and manganese is not unrealistic.

Examination of concentrated medium from some MAIS cultures demonstrated the presence of SOD activity. PAGE of concentrated medium in which these strains were grown demonstrated a single band of SOD activity which correlated in mobility with the activity of crude extracts. Concentrated medium in which *M. scrofulaceum* TMC 1306 or TMC 1312 was grown failed to exhibit any measurable activity by either the cytochrome *c* reduction assay or by PAGE. It is possible that the extracellular SOD activity could be due to autolysis of cells. However, the fact that repeated cultures of some *M. scrofulaceum* strains did not exhibit extracellular SOD activity, even though cultured under identical conditions, suggests that the extracellular activity may indeed be due to secretion of the enzyme. More extensive work needs to be done to confirm the observation.

SOD activity of concentrated culture medium has been reported for *M. tuberculosis* (25) and *N. asteroides* (6), both intracellular pathogens. Immunofluorescent staining of live cells of *N. asteroides* indicated the association of SOD with the outer cell envelope (6). Conversely, examination of concentrated culture media of rarely pathogenic *M. smegmatis* (25) and nonpathogenic strains of *N. asteroides* (6) failed to detect any extracellular SOD activity.

It has been suggested that the secretion of SOD by *N. asteroides*, as well as its association with the outer cell envelope, could provide protection against killing by superoxide radicals (6) which are produced during active phagocytosis (2, 3). Further, Filice et al. (13) have shown that *N. asteroides* is resistant to the phagocytic attack of human neutrophils and monocytes. The fact that exogenously added SOD has protected bacteria against phagocytic attack (23, 40) also illustrates the importance of SOD.

A study by Gangadharam and Pratt (16) has demonstrated that alveolar and peritoneal mouse macrophages readily phagocytosed *M. intracellulare* in vitro, but could not indefinitely prevent growth of the ingested bacilli, regardless of their state of activation. The production and secretion of SOD by certain MAIS strains may therefore serve a protective function against phagocytic killing. It would be interesting to determine whether SOD activity is associated with the outer cell surface of MAIS organisms, as demonstrated with *N. asteroides*. This association could provide protection to MAIS present in aquatic habitats (17) where photochemically generated superoxide radicals may be present (10).

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## LITERATURE CITED

1. **Asada, K., K. Yoshikawa, M.-A. Takahashi, Y. Maeda, and K. Enmanji.** 1975. Superoxide dismutases from a blue-green algae, *Plectonema boryanum*. J. Biol. Chem. **250**:2801–2807.
2. **Babior, B. M.** 1984. Superoxide and oxidative killing by phagocytes. Dev. Biochem. **26**:190–207.
3. **Babior, B. M., R. S. Kipnes, and J. T. Curnutte.** 1973. Biological defense mechanisms. The production by leukocytes of superoxide, a potential bactericidal agent. J. Clin. Invest. **52**:741–744.
4. **Baess, I.** 1983. Deoxyribonucleic acid relationships between different serovars of *Mycobacterium avium*, *Mycobacterium intracellulare* and *Mycobacterium scrofulaceum*. Acta Pathol. Microbiol. Immunol. Scand. Sect. B. Microbiol. **91**:201–203.
5. **Bannister, J. V., and G. Rotilio.** 1984. A decade of superoxide dismutase activity. Dev. Biochem. **26**:146–189.
6. **Beaman, B. L., S. M. Scates, S. E. Moring, R. Deem, and H. P. Misra.** 1983. Purification and properties of a unique superoxide dismutase from *Nocardia asteroides*. J. Biol. Chem. **258**:91–96.
7. **Beauchamp, C., and I. Fridovich.** 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal. Biochem. **44**:276–287.
8. **Britton, L., D. P. Malinowski, and I. Fridovich.** 1978. Superoxide dismutase and oxygen metabolism in *Streptococcus faecalis* and comparisons with other organisms. J. Bacteriol. **134**: 229–236.
9. **Chikata, Y., E. Kusunose, K. Ichihara, and M. Kusunose.** 1975. Purification of superoxide dismutase from *M. phlei*. Osaka City Med. J. **21**:127–136.
10. **Cooper, W. J., and R. G. Zika.** 1983. Photochemical formation of hydrogen peroxide in surface and ground waters exposed to sunlight. Science **220**:711–712.
11. **Crapo, J. D., J. M. McCord, and I. Fridovich.** 1978. Preparation and assay of superoxide dismutases. Methods Enzymol. **53**:382–393.
12. **DiGuiseppi, J., and I. Fridovich.** 1982. Oxygen toxicity in *Streptococcus sanguis*. The relative importance of superoxide and hydroxyl radicals. J. Biol. Chem. **257**:4046–4051.
13. **Filice, G. A., B. L. Beaman, J. A. Krick, and J. S. Remington.** 1980. Effects of human neutrophils and monocytes on *Nocardia asteroides*: failure of killing despite occurrence of the oxidative metabolic burst. J. Infect. Dis. **142**:432–438.
14. **Fridovich, I.** 1974. Superoxide dismutases. Adv. Enzymol. **41**:35–97.
15. **Fridovich, I.** 1978. The biology of oxygen radicals. The superoxide radical is an agent of oxygen toxicity; superoxide dismutases provide an important defense. Science **201**:875–880.
16. **Gangadharam, P. R. J., and P. F. Pratt.** 1983. In vitro response of murine alveolar and peritoneal macrophages to *Mycobacterium intracellulare*. Am. Rev. Respir. Dis. **128**:1044–1047.
17. **George, K. L., B. C. Parker, H. Gruft, and J. O. Falkinham III.** 1980. Epidemiology of infection by nontuberculous mycobacteria. II. Growth and survival in natural waters. Am. Rev. Respir. Dis. **122**:89–94.
18. **Gorse, G. J., R. D. Fairshter, G. Friendly, L. D. Maza, G. R. Greene, and T. C. Cesario.** 1983. Nontuberculous mycobacterial disease. Experience in a Southern California hospital. Arch. Intern. Med. **143**:225–228.
19. **Hodgson, E. K., and I. Fridovich.** 1975. The interaction of bovine erythrocyte superoxide dismutase with hydrogen peroxide: inactivation of the enzyme. Biochemistry **14**:5294–5299.
20. **Hoffner, E. K., and J. E. Coleman.** 1973. Cu(II)-carbon binding in cyanide complexes of copper enzymes. J. Biol. Chem. **248**:6626–6629.
21. **Ichihara, K., E. Kusunose, M. Kusunose, and T. Mori.** 1977. Superoxide dismutase from *Mycobacterium lepraemurium*. J. Biochem. **81**:1427–1433.
22. **Imaeda, T., L. Barksdale, and W. F. Kirchheimer.** 1982. Deoxyribonucleic acid of *Mycobacterium lepraemurium*: its genome size, base ratio, and homology with those of other mycobacteria. Int. J. Syst. Bacteriol. **32**:456–458.
23. **Johnston, R. B., Jr., B. B. Keele, Jr., H. P. Misra, J. E. Lehmeyer, L. S. Webb, R. L. Baehner, and K. V. Rajagopalan.** 1975. The role of superoxide anion generation in phagocytic bactericidal activity. Studies with normal and chronic granulomatous disease leukocytes. J. Clin. Invest. **55**:1357–1372.
24. **Keele, B. B., Jr., J. M. McCord, and I. Fridovich.** 1970. Superoxide dismutase from *Escherichia coli* B. A new manganese-containing enzyme. J. Biol. Chem. **245**:6176–6181.
25. **Kusunose, E., K. Ichihara, Y. Noda, and M. Kusunose.** 1976. Superoxide dismutase from *Mycobacterium tuberculosis*. J. Biochem. **80**:1343–1352.
26. **Kusunose, M., Y. Noda, K. Ichihara, and E. Kusunose.** 1976. Superoxide dismutase from *Mycobacterium* species, strain Takeo. Arch. Microbiol. **108**:65–73.
27. **Lowry, O. H., N. J. Rosebrough, O. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
28. **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.
29. **McCord, J. M., and I. Fridovich.** 1969. Superoxide dismutase. An enzymatic function for erythrocuprein (hemocuprein). J. Biol. Chem. **244**:6049–6055.
30. **Misra, H. P., and I. Fridovich.** 1978. Inhibition of superoxide dismutase by azide. Arch. Biochem. Biophys. **189**:317–322.
31. **Moore, E. R. B., E. P. Norrod, and P. Jurtshuk, Jr.** 1984. Superoxide dismutases of *Azotobacter vinelandii* and other aerobic, free-living nitrogen-fixing bacteria. FEMS Microbiol. Lett. **24**:261–265.
32. **Privalle, C. T., and E. M. Gregory.** 1979. Superoxide dismutase and $O_2$ lethality in *Bacteroides fragilis*. J. Bacteriol. **138**: 139–145.
33. **Schaefer, W. B., K. J. Birn, P. A. Jenkins, and J. Marks.** 1969. Infection with the Avian-Battey group of mycobacteria in England and Wales. Br. Med. J. **2**:412–415.
34. **Steffens, G.-J., J. V. Bannister, W. H. Bannister, L. Flohe, W. A. Gunzler, S.-M. A. Kim, and F. Otting.** 1983. The primary structure of Cu-Zn superoxide dismutase from *Photobacterium leiognathi*: evidence for a separate evolution of Cu-Zn superoxide dismutase in bacteria. Hoppe-Seyler's Z. Physiol. Chem. **364**:675–690.
35. **Steinman, H. M.** 1985. Bacteriocuprein superoxide dismutases in pseudomonads. J. Bacteriol. **162**:1255–1260.
36. **Vance, P. G., B. B. Keele, Jr., and K. V. Rajagopalan.** 1972. Superoxide dismutase from *Streptococcus mutans*: isolation and characterization of two forms of the enzyme. J. Biol. Chem. **247**:4782–4786.
37. **Wheeler, P. R., and D. Gregory.** 1980. Superoxide dismutase, peroxidatic activity, and catalase in *Mycobacterium leprae* purified from armadillo liver. J. Gen. Microbiol. **121**:457–464.
38. **Wolinsky, E.** 1979. Nontuberculous mycobacteria and associated diseases. Am. Rev. Respir. Dis. **119**:107–159.
39. **Wong, B., F. F. Edwards, T. E. Kiehn, E. Whimbey, H. Donnelly, E. M. Bernard, J. W. M. Gold, and D. Armstrong.** 1985. Continuous high-grade *Mycobacterium avium-intracellulare* bacteremia in patients with acquired immune deficiency syndrome. Am. J. Med. **78**:35–40.
40. **Yost, F. J., Jr., and I. Fridovich.** 1974. Superoxide radicals and phagocytosis. Arch. Biochem. Biophys. **161**:395–401.
41. **Zakowski, P., S. Fligiel, O. G. W. Berlin, and B. Lamar Johnson, Jr.** 1982. Disseminated *Mycobacterium avium-intracellulare* infection in homosexual men dying of acquired immunodeficiency syndrome. J. Am. Med. Assoc. **248**:2980–2982.