Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 7924–7928, September 1992
Microbiology

Multicellular oxidant defense in unicellular organisms

MUCHOU MA AND JOHN W. EATON*

Division of Experimental Pathology, Department of Pathology and Laboratory Medicine, Albany Medical College, A-81, 47 New Scotland Avenue, Albany, NY 12208

Communicated by David W. Talmage, May 8, 1992

ABSTRACT **Although catalase is thought to be a major defense against hydrogen peroxide (H_2O_2), the catalase activity within individual *Escherichia coli* fails to protect against exogenous H_2O_2. Contrary to earlier reports, we find that dilute suspensions of wild-type and catalase-deficient *E. coli* are identical in their sensitivity to H_2O_2, perhaps because even wild-type, catalase-positive *E. coli* cannot maintain an internal/external concentration gradient of this highly diffusible oxidant. However, concentrated suspensions or colonies of catalase-positive *E. coli* do preferentially survive H_2O_2 challenge and can even cross-protect adjacent catalase-deficient organisms. Furthermore, high-density catalase-positive—but not catalase-negative—*E. coli* can survive and multiply in the presence of competitive, peroxide-generating streptococci. These observations support the concept that bacterial catalase may defend colonial, but not individual, *E. coli* against environmental H_2O_2. Group protection by the activity of enzymes that mitigate oxidative stress may have been a driving force in the evolution of multicellular organisms.**

Biologically hazardous reactive oxygen species, such as hydrogen peroxide (H_2O_2), arise from a variety of chemical (1–3) and metabolic (4–7) reactions. Accordingly, all aerobic organisms possess enzymatic systems that protect against H_2O_2 cytotoxicity. These systems include reduced glutathione and associated enzymes and catalases. The relative importance of glutathione-associated enzymes versus catalases may depend on the source and extent of H_2O_2 exposure (8–11). For many kinds of bacteria, catalase may be most important in protection against high H_2O_2 concentrations because catalases generally have very high turnover numbers (12). Furthermore, many bacteria such as *Escherichia coli* lack glutathione peroxidase (13) and cannot use glutathione for enzymatic catabolism of H_2O_2. In fact, glutathione appears dispensable in the defense of *E. coli* against H_2O_2 (14). Although other metabolic pathways for H_2O_2 detoxification may exist in *E. coli* (15, 16), the quantitative importance of these alternative mechanisms is unclear.

Therefore, that microbial catalase is reported to be an effective defense against relatively high concentrations of exogenous H_2O_2 is not surprising (17–24). These earlier findings are, however, puzzling when one compares the ready diffusibility of H_2O_2 with the small cellular dimensions of most microbes. That is, H_2O_2 should so rapidly permeate small organisms that even very high intracellular catalase activity might not produce an outside/inside concentration gradient. To investigate this apparent ambiguity, we have carefully tested the susceptibility of catalase-deficient *E. coli* to killing by H_2O_2. The results indicate that bacterial catalase does not protect isolated organisms against H_2O_2 challenge but does favor the survival of high-density and colonial *E. coli*. Such group enzymatic defense in unicellular organisms may have been a driving force in the evolution of multicellularity.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Reagents. Brain heart infusion broth, Todd-Hewitt broth, Lennox L agar (LB agar), and Bactoagar were obtained from GIBCO/BRL. The bicinchoninic acid protein microassay was from Pierce. All other enzymes and chemicals were purchased from Sigma.

Bacterial Strains and Culture Conditions. A catalase-deficient mutant strain of *E. coli* K-12 [UM1, hereafter, cat(−)] and its parent wild-type [CSH7, hereafter cat(+)] (17) were provided by P. C. Loewen (University of Manitoba). *E. coli* were grown statically in brain heart infusion broth or M9 minimal salts medium supplemented with 10 mM glucose (M9/glucose) (25) at 37°C in room air overnight (18–20 hr) to early stationary-phase (OD_{600} = 2.2–2.4). Thiamin was not added to the growth medium because these strains of *E. coli* were phenotypically Thi^+, probably from a spontaneous Thi^- → Thi^+ reversion. Bacteria in midexponential growth were obtained by diluting these stationary-phase cultures 1:100 in prewarmed fresh brain heart infusion broth and incubated further until the OD_{600} of the culture reached 0.5–1.0. Cells were harvested from the culture by centrifugation (10,000 × *g* for 10 min at 2°C), washed thrice in cold phosphate-buffered saline (140 mM NaCl/10 mM Na_2HPO_4/10 mM KH_2PO_4, pH 7.2) or in M9/glucose before being resuspended in the indicated buffers, and the absorbance at 600 nm was measured. The correlation of OD_{600} with bacterial concentration was affirmed by direct microscopic counts and by colony-forming units obtained by growth on LB agar. Bacterial stocks were maintained on LB agar plates, stored at 2–4°C. The catalase status of colonies was constantly monitored by direct catalase assay and by H_2O_2 drop tests.

Streptococcal strains were obtained from the American Type Culture Collection (*Streptococcus sobrinus*; 33478), G. Germaine (University of Minnesota) (*Streptococcus mitis*, 9811), and E. L. Thomas (University of Tennessee) (*Streptococcus mutans* OMZ176 and *S. mutans* GS5). Organisms were cultured in Todd-Hewitt broth statically at 37°C either in room air (*S. mutans*) or in a candle extinction jar (*S. sobrinus* and *S. mitis*) and washed as described for *E. coli*.

H_2O_2 Killing of *E. coli*. Suspensions of *E. coli* were diluted to the concentration of 5×10^7 cells per ml (concentrated) or 500 cells per ml (dilute) in M9/glucose and prewarmed to 37°C before being exposed to H_2O_2 at the indicated concentrations. Bacterial viability, or, more accurately, ability of the bacteria to replicate, was assayed by pour-plating diluted aliquots (in triplicate) of the H_2O_2/bacteria mixture in bovine catalase-supplemented (100 units per ml) LB agar at different times during incubation. Killing experiments were performed not only in pure cat(+) and pure cat(−) *E. coli* suspensions but also were carried out in mixtures of cat(+) and cat(−) *E. coli* as indicated. To differentiate cat(+) from cat(−) *E. coli* after H_2O_2 exposure of such mixtures, bacteria of both strains were made resistant to ampicillin by pUC118 (a derivation of

Abbreviations: LB agar, Lennox L agar; M9, minimal salts medium; M9/glucose, M9/10 mM glucose; cat(−), catalase-deficient mutant; cat(+), wild-type strain CSH7.

*To whom reprint requests should be addressed.

pUC18-carrying ampicillin resistance) transformation. Transformed cat(+) *E. coli* were admixed with nontransformed cat(−) organisms (or vice versa) before H_2O_2 exposure, and the surviving bacteria were plated in LB agar with or without ampicillin supplementation (30 μg/ml).

For the preparation of *E. coli* colonies, ≈500 individual organisms were pour-plated in 10 ml of molten LB agar and allowed to solidify on a precisely horizontal platform to ensure equal thickness of the agar layer. After preincubation at 37°C for various periods of time, the embedded microcolonies were exposed to 1.0 mM H_2O_2 in an agar overlay (20 ml of 1.5% Bactoagar in M9/glucose). After solidification, the Petri dishes were further incubated for 48 hr at 37°C before viable colony-forming units were enumerated. Control plates containing bovine catalase (100 units per ml) were also exposed to H_2O_2 under identical conditions.

Coculture of *E. coli* and Streptococci. Stationary-phase *S. mutans* OMZ176 were resuspended to an OD_{650} of 1.0 in M9 medium/50 mM glucose. These suspensions of streptococci were incubated aerobically (10–12 ml in 50-ml Erlenmeyer flasks shaken on a rotary platform at 135 rpm) at 37°C for 60 min, and then *E. coli* were added at 5×10^7 organisms per ml. The fate of the cocultured *E. coli* was determined by pour-plating in LB agar supplemented with erythromycin (0.4 μg/ml), conditions that suppress streptococcal but not *E. coli* growth. The H_2O_2 concentration of the coculture supernatant was assayed in parallel. Interactions between colonies of streptococci and *E. coli* were also tested. *S. mitis* were sparsely inoculated on plates of presolidified Todd-Hewitt agar (1.5%) and incubated in a candle extinction jar at 37°C for 4–5 days, by which time each plate bore several streptococcal colonies of 3- to 4-mm diameter. *E. coli* were suspended at 10^6 cells per ml in molten M9 agar (1.5%) supplemented with 50 mM glucose and overlain onto the plate bearing mature streptococcal colonies. After overnight incubation in air at 37°C, the diameter of growth-inhibitory zones surrounding each streptococcal colony was determined.

Catalase Assay. Catalase activity was assayed polarographically according to the method of Rorth and Jensen (26) with bacterial suspensions or lysates as samples. Lysis of *E. coli* was achieved by sonication (55–60 W for 3 min at 4°C). In the absence of added H_2O_2, the oxygen tension of bacterial suspensions, either cat(+) or cat(−), remained the same in room-air-equilibrated buffer for at least 10 min. Therefore, oxygen consumption by intact cells in the catalase assay system did not compromise the results. Bacterial suspensions or lysates were mixed with H_2O_2 (starting concentration = 10 mM) in nitrogen-purged buffer supplemented with 10 mM glucose, and the initial (1–3 min) rates of oxygen evolution were measured and recorded with a modified Clark O_2 electrode at 24°C. Appropriate dilutions of bovine liver catalase were used as a standard.

H_2O_2 Assay. H_2O_2 was determined by horseradish peroxidase-catalyzed oxidation of phenol red (27). Briefly, samples were mixed with phosphate-buffered saline containing horseradish peroxidase (8.5 purpurogallin units per ml) and phenol red (0.28 mM) and incubated at 24°C for 10 min before being alkalinized by adding 1.0 M NaOH. Absorbance of the reaction mixture at 610 nm was measured and correlated with values obtained by using appropriate dilutions of reagent H_2O_2. The concentration of reagent H_2O_2 was determined by UV absorbance at 230 nm (molar absorptivity = 81 $M^{-1} \cdot cm^{-1}$). The accumulation of H_2O_2 by streptococci in aerobic culture was estimated by inoculating stationary-phase organisms into M9 medium/50 mM glucose at a concentration of 5×10^9 colony-forming units per ml (OD_{650} = 1.0). The culture was aerated (12 ml in 50-ml Erlenmeyer flasks shaken on a rotatory platform at 135 rpm) and incubated at 37°C. At the indicated time points, H_2O_2 in the culture supernatant was determined.

Protein Assay. Protein concentrations were determined by using the bicinchoninic acid protein microassay (28), using bovine serum albumin as the standard.

RESULTS

Catalase Activity of Intact and Lysed *E. coli*. In agreement with previous reports (29, 30), our own direct measurements on intact and lysed *E. coli* indicate no significant difference in internal versus external H_2O_2 concentrations at millimolar peroxide concentrations. The apparent catalase activities of cat(+) *E. coli* suspensions before (intact) and after sonication (lysate; >99.9% of the cells lysed as judged microscopically) were bacterial protein at 8.71 ± 0.97 and 9.67 ± 1.34 units per mg (n = 4), respectively. Thus, it appears that, at higher H_2O_2 concentrations, *E. coli* catalases cannot maintain an appreciable H_2O_2 gradient across the cell envelope. It should be noted, however, that a H_2O_2 permeability barrier at the level of the cell envelope has been proposed for other microbial species (31–33).

H_2O_2 Sensitivity of Suspensions of cat(+) and cat(−) *E. coli*. If wild-type *E. coli* are, indeed, incapable of sustaining an internal/external gradient of peroxide, then, contrary to earlier reports (17–24), cat(+) and cat(−) *E. coli* should be equally susceptible to H_2O_2-mediated cytotoxicity. To test this hypothesis, we exposed dilute (500 organisms per ml) suspensions of stationary-phase cat(+) and cat(−) *E. coli* to 1.0 mM H_2O_2 and followed the kinetics of replication inactivation (hereafter referred to as killing). Under these conditions, there is no detectable difference in the killing of cat(+) versus cat(−) organisms by exogenous H_2O_2 (Fig. 1*A*). As shown in Fig. 1*A*, *Inset*, at such low bacterial numbers neither cat(+) nor cat(−) *E. coli* suspensions clear appreciable amounts of the exogenous H_2O_2. Similarly, brief (15 min) exposure of dilute *E. coli* suspensions to various concentrations (10^{-7} M to 4×10^{-2} M) of H_2O_2 reveals identical sensitivity of cat(+) and cat(−) organisms to H_2O_2, the LD_{50} for both strains being ≈2.5 mM (data not shown). Because bacterial catalase activity is known to vary with different growth and metabolic states (34, 35), we also examined H_2O_2 sensitivity of exponential-phase and minimal-medium-growth (M9/glucose) organisms. Again, H_2O_2 killing in both strains is identical (data not shown). Therefore, intracellular catalase does not protect dilute suspensions of *E. coli* against external H_2O_2.

However, entirely different results are obtained when more concentrated (5×10^7 organisms per ml) suspensions of cat(+) and cat(−) *E. coli* are similarly exposed to 1.0 mM H_2O_2. In this case, organismal catalase clearly confers survival advantage (Fig. 1*B*). Unlike dilute suspensions of *E. coli* that cannot clear H_2O_2 (Fig. 1*A Inset*), concentrated cat(+) *E. coli* efficiently catabolize added H_2O_2 (Fig. 1*B Inset*) and, therefore, survive. This concentration-dependent resistance to H_2O_2 cytoxicity is not observed in cat(−) *E. coli* (Fig. 1*B*). Furthermore, when equal numbers of cat(+) and cat(−) *E. coli* are admixed in dilute suspensions and then exposed to H_2O_2, once again, there is no difference in the rate or extent of H_2O_2-dependent killing of the two strains (Fig. 2*A*). However, concentrated cat(+) *E. coli* will actually cross-protect cat(−) organisms in the same incubation mixture (Fig. 2*B*).

H_2O_2 Sensitivity of Colonial cat(+) and cat(−) *E. coli*. The preceding results suggested that colonies of cat(+) *E. coli* might survive challenge by exogenous H_2O_2. We, therefore, grew individual bacterial colonies for increased periods of time (during which the colonies progressively enlarge as a result of bacterial multiplication) before challenge with H_2O_2. As microcolonies of cat(+) *E. coli* increase in size, substantial resistance to H_2O_2 develops, whereas cat(−) microcolonies are killed by H_2O_2, regardless of their size (Fig. 3). If the generation time of *E. coli* embedded in such an artificial

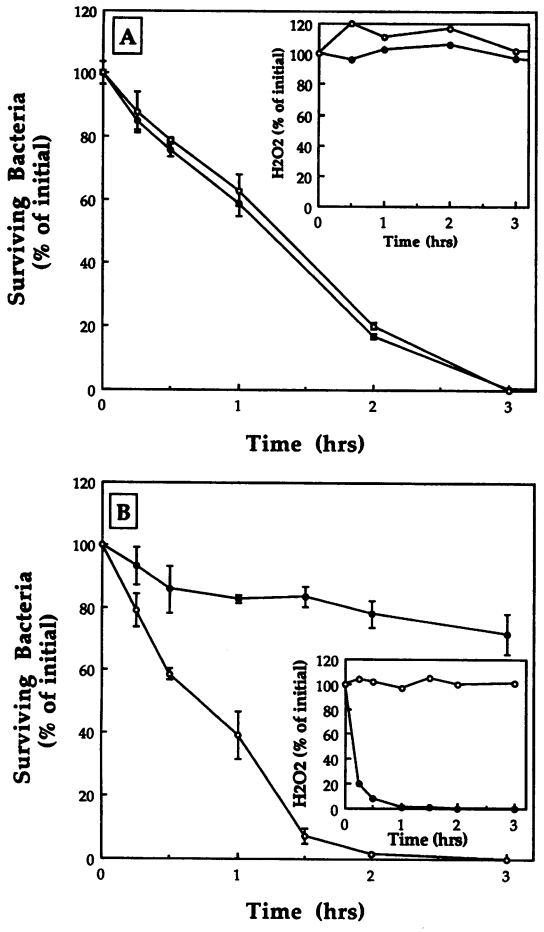

FIG. 1. Kinetics of replication inactivation ("killing") of dilute (*A*) and concentrated (*B*) suspensions of cat(+) (●) and cat(−) (○) *E. coli*. Early stationary-phase bacteria were suspended to ≈5 × 10^2 cells per ml (*A*) or ≈5 × 10^7 cells per ml (*B*) in M9/glucose. At zero time, H_2O_2 was added to a final concentration of 1.0 mM, and the bacterial suspensions were then incubated at 37°C. Surviving organisms were quantitated by pour-plating appropriate dilutions in LB agar supplemented with bovine liver catalase at 100 units per ml. After 24–48 hr of growth at 37°C, colonies were counted. Each point represents the mean of triplicate determinations, and vertical lines show ± 1 SD. (*Insets*) Suspension-phase H_2O_2 concentration during incubations of cat(+) (●) and cat(−) (○) *E. coli* as measured by the peroxidase/phenol red reaction (27).

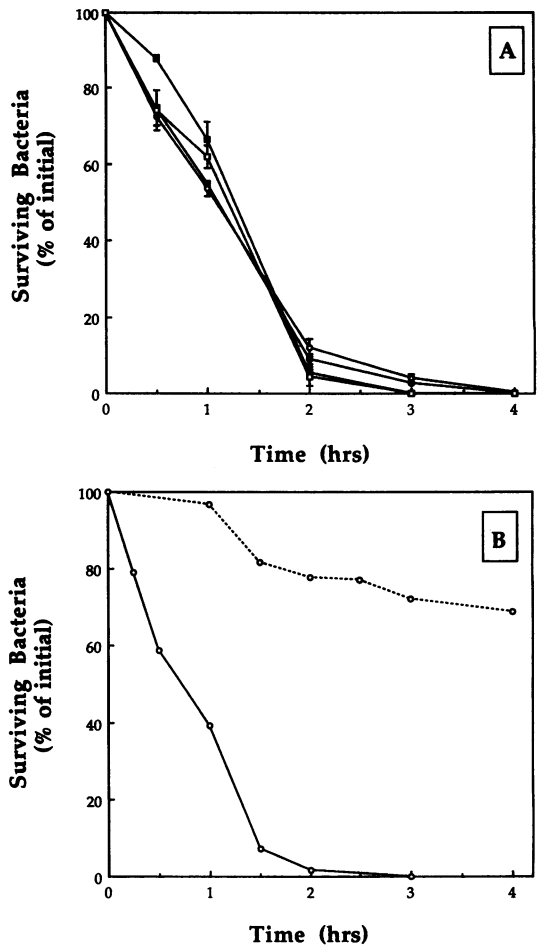

FIG. 2. H_2O_2 killing of heterogeneous dilute (*A*) or concentrated (*B*) suspensions of cat(+) and cat(−) *E. coli*. The two strains of *E. coli* were made resistant to ampicillin by pUC118 (Amp^r) transformation. (*A*) Dilute suspensions (≈5 × 10^2 cells per ml) of untransformed cat(+) and transformed cat(−) bacteria, or vice versa, were prepared in M9/glucose, and equal volumes of these suspensions were admixed immediately before adding 1.0 mM H_2O_2. After incubation at 37°C for the indicated times, cat(+) and cat(−) survivors were differentiated by pour-plating in LB agar containing 100 units of catalase per ml with or without ampicillin (30 μg/ml). Identity of the colonies was further confirmed by the H_2O_2 drop test (36). ●, Untransformed cat(+); ■, transformed cat(+); ○, untransformed cat(−); □, transformed cat(−). (*B*) Survival of concentrated (≈5 × 10^7 cells per ml) Amp^r-transformed cat(−) *E. coli* alone (—) or admixed with equal numbers of cat(+) *E. coli* (---). Each point represents the mean of triplicate determinations, and vertical lines show ± 1 SD.

environment is 30 min (37), the cat(+) microcolonies acquire significant resistance to H_2O_2 (survival increasing to >90% at 6 hr) after ≈12 generations. The population of each microcolony during this growth period should reach ≈4000 bacteria. In contrast to the small dimensions of an individual organism, a spherical colony of 4000 organisms should have a diameter of ≈40 μm.

Competitive Interactions of cat(+) and cat(−) *E. coli* with Peroxidogenic Streptococci. The above results suggested that, in an environment contaminated by H_2O_2 generated by other microorganisms, catalase might be critical for the survival of *E. coli*. Indeed, when an agar overlay containing *E. coli* is poured onto established colonies of H_2O_2-releasing *S. mitis*, large zones of *E. coli* growth inhibition are observed for cat(−) *E. coli*, whereas the cat(+) bacteria grow in close proximity to the streptococcal colonies (Fig. 4). Even in liquid culture, there is a profound difference in the ability of cat(−) and cat(+) organisms to compete with these streptococci. Under these conditions, cat(+) *E. coli* proliferate after a short lag period, despite the presence of numerous H_2O_2-producing streptococci. By contrast, cat(−) *E. coli* fail to grow and eventually die (Fig. 5). This competitive advantage of cat(+) *E. coli* is clearly related to catalase-dependent protection from environmental H_2O_2. When bovine catalase is added to the liquid coculture system, even the cat(−) organisms survive and multiply (Fig. 5). Furthermore, the ability of different species and strains of streptococci to suppress and kill cocultured cat(−) *E. coli* is directly correlated with their various H_2O_2-accumulating potentials (data not shown).

DISCUSSION

It is difficult to imagine that the catalase activity within small unicellular organisms might be an effective protection against exogenous H_2O_2. In view of the highly diffusible nature of this oxidant, it is unlikely that intraorganismal catalase could sustain an internal/external concentration gradient. Indeed, direct measurements of the "apparent" catalase activity within intact and lysed *E. coli* yield very similar values.

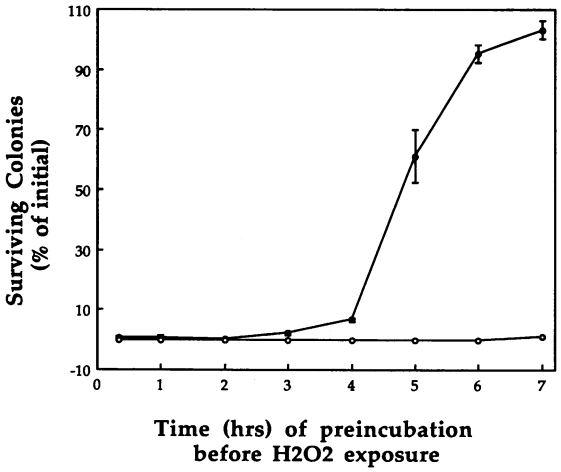

FIG. 3. Enhanced resistance of colonial cat(+) *E. coli* to H_2O_2 killing. Known numbers (≈500 colony-forming units per plate) of cat(+) (●) or cat(−) (○) *E. coli* were pour-plated in 10 ml of LB agar and incubated at 37°C. After various periods of preincubation, 20 ml of molten 1.5% Bactoagar containing freshly added H_2O_2 (final concentration, 1.0 mM) was overlaid on the LB agar-embedded bacterial microcolonies. Surviving colonies were counted after 48 hr of incubation and expressed as percentage survival of control plates either not exposed to an H_2O_2-containing overlay or supplemented with catalase (100 units per ml). Data from one representative experiment are shown with error bars of ± 1 SD from counts of triplicate plates. The growth rates of cat(+) and cat(−) *E. coli*, as measured by either turbidometry in brain heart infusion broth or visually as colonies in LB agar, were identical.

Because the rate of reaction of catalase with H_2O_2 (at least between 1 and 40 mM) is an almost linear function of substrate concentration (12), this result indicates that the catalase activity within intact *E. coli* cannot lower the effective intracellular concentrations of H_2O_2 and, therefore, cannot prevent H_2O_2-mediated damage to the organism. In direct support of this conclusion [but contrary to a number of earlier reports (17–24)], we find that dilute suspensions of cat(+) *E. coli* are as readily killed by bulk-phase H_2O_2 as are

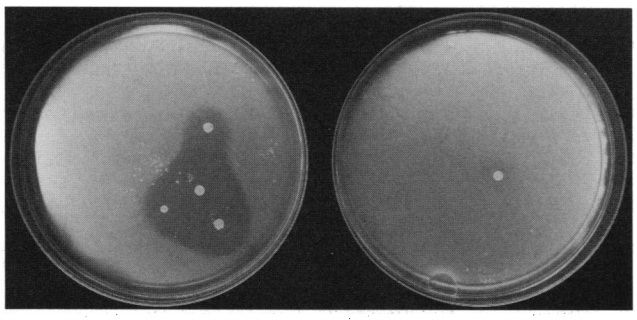

FIG. 4. Preferential inhibition of the growth of cat(−) *E. coli* by H_2O_2-generating colonies of *S. mitis*. *S. mitis* was first cultured overnight in Todd-Hewitt broth at 37°C in a candle extinction jar, then sparsely inoculated on presolidified Todd-Hewitt agar (1.5%) plates, and allowed to grow for 4 to ≈5 days, by which time the streptococcal colonies were ≈3–4 mm in diameter. *E. coli* were prepared as described in the legend for Fig. 1 and resuspended at ≈10^6 organisms per ml in molten M9 agar (1.5%) supplemented with 50 mM glucose. Ten milliliters of *E. coli*-containing top agar was overlaid onto the plate bearing the streptococcal colonies. After overnight incubation at 37°C in room air, clear growth-inhibitory zones appear surrounding each streptococcal colony on the cat(−) plate (*Left*). In contrast, the plate overlaid with cat(+) *E. coli* (*Right*) shows no such zone of inhibition of *E. coli*. The important role of H_2O_2 in this experimental system was confirmed by addition of catalase (100 units per ml) in the top agar, which prevented development of any detectable zone of inhibition (data not shown).

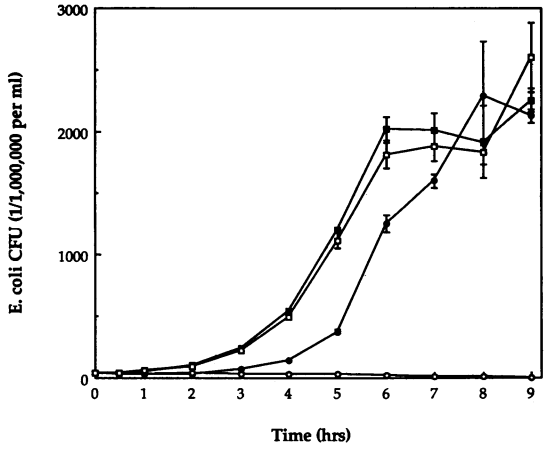

FIG. 5. Competitive elimination of cat(−) *E. coli* by *S. mutans* OMZ176. Early stationary-phase cultures of *E. coli* and *S. mutans* were grown separately in M9/glucose and Todd-Hewitt broth, respectively, and used as inocula. Streptococci were inoculated into prewarmed M9/50 mM glucose, at ≈5 × 10^9 colony-forming units (CFU) per ml (OD_{650} = 1.0), aerated (12 ml in 50-ml Erlenmeyer flasks shaken on rotatory platform at 135 rpm), and preincubated at 37°C. After 60 min of preincubation, cat(+) or cat(−) *E. coli* were inoculated at 5 × 10^7 organisms per ml, and their viability was followed by pour-plating in LB agar supplemented with erythromycin (0.4 μg/ml), which suppresses streptococcal but not *E. coli* growth. ●, cat(+) without catalase; ○, cat(−) without catalase; ■, cat(+) with catalase; □, cat(−) with catalase. Each point represents the results of triplicate determinations, and vertical lines show ± 1 SD. *E. coli* were inoculated at zero time when [H_2O_2] was 0.258 mM, which increased to 0.801 mM in cat(−) coculture in 9 hr. In the control experiment, catalase (100 units per ml) was added immediately before streptococcal inoculation, which completely prevented both H_2O_2 accumulation and cytotoxicity to cat(−) *E. coli*.

their cat(−)—but otherwise isogenic—counterparts. This result is very simply explained by the inability of organismal catalase to (*i*) maintain an internal/external concentration gradient and (*ii*) catabolically decrease the concentration of the bulk-phase H_2O_2.

On the other hand, the catalase activity of cat(+) *E. coli* does protect high-density or colonial bacteria from external H_2O_2, whereas cat(−) *E. coli* show persistent sensitivity to H_2O_2, regardless of density. In these conditions, cat(+) organisms have high aggregate catalase activity, are able rapidly to catabolize environmental peroxide, and, therefore, survive. Interestingly, this group protection can extend to neighboring cat(−) *E. coli*, lending further support to the idea that catalase may function to protect groups of bacteria, rather than discrete, isolated organisms.

In the laboratory setting, bacteria are generally treated as unicellular organisms, whereas, in the natural world, many microbial species, including bacteria, live a more colonial existence (38). Colonic *E. coli*, for example, are interspersed with many other species of enteric flora in the form of a dense biofilm adjacent the intestinal epithelial surface (39, 40). In natural environments like this, one important source of exogenous H_2O_2 might be that generated by competitive microorganisms of other genera (41–43). Streptococci and lactobacilli, found in significant numbers in human feces, generate substantial amounts of H_2O_2 (44). In fact, peroxidogenic streptococci, known to be frequent inhabitants of the oral cavity, suppress growth of other microbial species (45–52).

We have, therefore, studied interactions between cat(+)/cat(−) *E. coli* and peroxidogenic streptococci. When an agar overlay containing *E. coli* is poured onto established colonies of H_2O_2-releasing *S. mitis*, large zones of *E. coli* growth inhibition are observed for cat(−) *E. coli*, whereas the cat(+)

bacteria grow in close proximity to the streptococcal colonies. Even in liquid culture, there is a profound difference in the ability of cat(−) and cat(+) organisms to compete with these streptococci. Under these conditions, cat(+) *E. coli* proliferate after a short lag period, despite the presence of numerous H_2O_2-producing streptococci. By contrast, cat(−) *E. coli* fail to grow and eventually die. This competitive advantage of cat(+) *E. coli* is clearly related to catalase-dependent protection from environmental H_2O_2. When catalase is added to the liquid coculture system, even the cat(−) organisms survive and multiply (Fig. 5). Furthermore, the ability of different species and strains of streptococci to suppress and kill cocultured cat(−) *E. coli* is directly correlated with their various H_2O_2-accumulating potentials (data not shown).

Unfortunately, the present work does not elucidate the mechanism(s) whereby H_2O_2 is cytotoxic to *E. coli*. Earlier investigations suggest that low (1–3 mM) concentrations of H_2O_2, such as those used in our work, may cause lethality via transition metal-mediated DNA damage (whereas this may not be true of higher H_2O_2 concentrations) (53–55). If so, catalase-dependent group protection evidently works through prevention of irreparable H_2O_2-mediated damage to organismal DNA.

In summary, we find that one particular oxidant-defense enzyme—catalase—does not protect individual *E. coli* against bulk-phase H_2O_2. However, highly concentrated or colonial cat(+) *E. coli* are resistant to external H_2O_2, whereas cat(−) *E. coli* show persistent sensitivity to H_2O_2, regardless of density. From an evolutionary perspective, it is probably important that the catalase status of *E. coli* determines the ability of aggregates of these organisms to compete with H_2O_2-generating microbial competitors, such as streptococci. Furthermore, rival bacteria are not the only source of concentrated exogenous H_2O_2 that may be encountered in nature. When unicellular prokaryotes first populated the pristine surface water on precambrian Earth, the thin atmosphere likely allowed massive x-ray and UV radiation to infiltrate these aquatic systems (56). Such intense irradiation, especially in the presence of green photoautotrophs and organic molecules, may rapidly generate millimolar H_2O_2 concentrations (1, 57–59). The catalase elaborated by small unicellular organisms may protect the group—rather than the individual—against attack by such environmental H_2O_2. We speculate that this enzymatic "safety in numbers" may have been an important driving force in the evolution of multicellular organisms.

We thank Drs. O. R. Brown, G. Germaine, P. C. Loewen, W. S. Sauerbier, J. Haldane, and E. L. Thomas for providing materials used in these studies and for offering helpful advice and also thank Ms. Diane Konzen for manuscript preparation. This work was supported by National Institutes of Health Grant AI-25625.

1. Frey, H. E. & Pollard, E. C. (1966) *Radiat. Res.* **28**, 668–676.
2. Peyton, G. R. & Glaze, W. H. (1987) in *Photochemistry of Environmental Aquatic Systems*, eds. Zika, R. G. & Cooper, W. J. (Am. Chem. Soc., Washington), pp. 76–88.
3. Cooper, W. J. & Zika, R. G. (1983) *Science* **220**, 711–712.
4. Boveris, A. & Chance, B. (1973) *Biochem. J.* **134**, 707–716.
5. Foerder, C. A., Klebanoff, S. J. & Shapiro, B. M. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 3183–3187.
6. Weiss, S. J. & LoBuglio, A. F. (1982) *Lab. Invest.* **47**, 5–18.
7. Dupuy, C., Virion, A., Ohayon, R., Kaniewski, J., Deme, D. & Pommier, J. (1991) *J. Biol. Chem.* **266**, 3739–3743.
8. Jones, D. P., Eklow, L., Thor, H. & Orrenius, S. (1981) *Arch. Biochem. Biophys.* **210**, 505–516.
9. Starke, P. E. & Farber, J. L. (1985) *J. Biol. Chem.* **260**, 10099–10104.
10. Gaetani, G. F., Galiano, S., Canepa, L., Ferraris, A. M. & Kirkman, H. N. (1989) *Blood* **73**, 334–339.
11. Eaton, J. W. (1991) *J. Lab. Clin. Med.* **118**, 3–4.
12. Chance, B. (1949) *J. Biol. Chem.* **179**, 1311–1330.
13. Penninckx, M. J. & Jaspers, C. J. (1982) *Bull. Inst. Pasteur* **80**, 291–301.
14. Greenberg, J. T. & Demple, B. (1986) *J. Bacteriol.* **168**, 1026–1029.
15. Jacobson, F. S., Morgan, R. W., Christman, M. F. & Ames, B. N. (1989) *J. Biol. Chem.* **264**, 1488–1496.
16. Sample, A. K. & Czprynski, C. J. (1991) *Infect. Immun.* **59**, 2239–2244.
17. Yoshpe-Purer, Y. & Henis, Y. (1976) *Appl. Environ. Microbiol.* **32**, 465–469.
18. Filice, G. A. (1983) *J. Infect. Dis.* **148**, 861–867.
19. Barbado, C., Ramirez, M., Blanco, M. A., Lopez-Barea, J. & Pueyo, C. (1983) *Curr. Microbiol.* **8**, 251–253.
20. Gregory, E. M. & Fanning, D. D. (1983) *J. Bacteriol.* **156**, 1012–1018.
21. Loewen, P. C. (1984) *J. Bacteriol.* **157**, 622–626.
22. Meir, E. & Yagil, E. (1984) *Curr. Microbiol.* **11**, 13–18.
23. Mauel, J. (1990) *J. Leukocyte Biol.* **47**, 187–193.
24. Abril, N. & Pueyo, C. (1990) *Environ. Mol. Mutagen.* **15**, 184–189.
25. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY), 2nd Ed., Vol. 3, p. A3.
26. Rorth, M. & Jensen, P. K. (1967) *Biochim. Biophys. Acta* **139**, 171–173.
27. Pick, E. & Keisari, Y. J. (1980) *Immunol. Methods* **38**, 161–170.
28. Smith, P. K., Krohn, R. I., Hermanson, G. T., Mallia, A. K., Gartner, F. H., Provenzana, M. D., Fujimoto, E. K., Goeke, N. M., Olson, B. J. & Klenck, D. C. (1985) *Anal. Biochem.* **150**, 76–85.
29. Engel, M. S. & Adler, H. I. (1961) *Radiat. Res.* **15**, 269–275.
30. White, D. C. (1962) *J. Bacteriol.* **83**, 851.
31. Herbert, D. & Pinsent, J. (1948) *Biochem. J.* **43**, 193–202.
32. Clayton, R. K. (1959) *Biochim. Biophys. Acta* **36**, 35–39.
33. Howard, D. H. (1983) *Infect. Immun.* **39**, 1161–1166.
34. Yoshpe-Purer, Y., Henis, Y. & Yashphe, J. (1977) *Can. J. Microbiol.* **23**, 84–91.
35. Hassan, H. M. & Fridovich, I. (1978) *J. Biol. Chem.* **253**, 6445–6450.
36. McLeod, J. W. & Gordon, J. (1922) *Biochem. J.* **16**, 499–506.
37. Woldringh, C. L. & Nanninga, N. (1985) in *Molecular Cytology of Escherichia coli*, ed. Nanninga, N. (Academic, New York), pp. 161–197.
38. Shapiro, J. A. (1988) *Sci. Am.* **259**, 82–89.
39. Savage, D. S. (1980) in *Bacterial Adherence*, ed. Beachey, E. H. (Chapman & Hall, London), pp. 31–59.
40. Hamilton, W. A. (1987) in *Ecology of Microbial Communities*, eds. Fletcher, M., Gray, T. R. G. & Jones, J. G. (Cambridge Univ. Press, New York), pp. 361–385.
41. McLeod, J. W., Gordon, J. & Pyrah, L. N. (1923) *J. Pathol. Bacteriol.* **26**, 127–128.
42. Cook, F. D. & Quadling, C. (1962) *Can. J. Microbiol.* **8**, 933–935.
43. Thomas, E. L. (1985) in *The Lactoperoxidase System: Chemistry and Biological Significance*, eds. Pruitt, K. M. & Tenovuo, J. O. (Dekker, New York), pp. 179–202.
44. Drasar, B. S. (1988) in *Role of the Gut Flora in Toxicity and Cancer*, ed. Rowland, I. R. (Academic, New York), pp. 23–38.
45. Kraus, F. W., Nickerson, J. F., Perry, W. I. & Walker, A. P. (1957) *J. Bacteriol.* **73**, 727–735.
46. Holmberg, K. & Hollander, H. O. (1973) *Arch. Oral Biol.* **18**, 423–434.
47. Germaine, G. & Tellefson, L. M. (1982) *Infect. Immun.* **38**, 1060–1067.
48. Thomas, E. L. & Pera, K. A. (1983) *J. Bacteriol.* **154**, 1236–1244.
49. Donoghue, H. D., Perrons, C. J. & Hudson, D. E. (1985) *Arch. Oral Biol.* **30**, 519–523.
50. Allen, B. W. (1985) *J. Med. Microbiol.* **19**, 227–235.
51. Carlsson, J., Edlund, M.-B. K. & Lundmark, S. K. E. (1987) *Oral Microbiol.* **2**, 15–20.
52. Hillman, J. D. & Shivers, M. (1988) *Arch. Oral Biol.* **33**, 395–401.
53. Imlay, J. A. & Linn, S. (1987) *J. Bacteriol.* **169**, 2967–2976.
54. Greenberg, J. T. & Demple, B. (1988) *EMBO J.* **7**, 2611–2617.
55. Imlay, J. A. & Linn, S. (1988) *Science* **240**, 1302–1309.
56. Levine, J. S. (1985) in *The Photochemistry of Atmospheres, Earth, the Other Planets, and Comets*, ed. Levine, J. S. (Academic, New York), pp. 3–38.
57. McCormick, J. P., Fischer, J. R., Pachlatko, J. P. & Eisenstark, A. (1976) *Science* **191**, 468–469.
58. Wang, R. J. & Nixon, B. T. (1978) *In Vitro* **14**, 715–722.
59. Zepp, R. G., Skurlatov, Y. I. & Pierce, J. T. (1987) in *Photochemistry of Environmental Aquatic Systems*, eds. Zika, R. G. & Cooper, W. J. (Am. Chem. Soc., Washington), pp. 215–224.