Characterization of a catalase gene from *Aeromonas veronii*, the digestive-tract symbiont of the medicinal leech

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The catalase gene *katA* of the medicinal leech symbiont *Aeromonas veronii* bv. sobria was cloned, sequenced, and functionally characterized. Southern hybridization, using an *A. veronii* *katA*-specific hybridization probe, suggested the presence of a single gene copy in many *Aeromonas* species. *A. veronii* *katA* consisted of 1446 nt encoding a protein with a high degree of similarity to the small-subunit group III bacterial catalases. A catalase-null mutant (JG186) was constructed through gene-replacement mutagenesis. In the parent strain (HM21R), catalase activity was only detected in extracts of cells grown to early exponential phase following H2O2 induction, in which the ability to induce activity was inversely related to optical density. In contrast, induced JG186 cells were very sensitive to oxidative stress, with survival being affected even at low H2O2 concentrations. In contrast to the findings of previous reports of other symbiotic systems, the catalase mutant was not defective in its ability to competitively colonize or persist within its host, in both co-inoculation and sole-colonization assays. This body of evidence suggests either that oxidative stress, in the form of H2O2 exposure, is not encountered by the microbial partner under the examined symbiotic conditions or that compensatory mechanisms exist. The data suggest that although many colonization factors reoccur, each symbiotic system has also evolved specific mechanisms that affect symbiont–host dynamics.

INTRODUCTION

Physiological adaptations typically reflect the selection pressures encountered within ecological niches. For example, catalases are responsible for the decomposition of hydrogen peroxide to water and oxygen (2H2O2 → 2H2O + O2), and have presumably evolved to protect against cellular damage arising from exposure to H2O2. The two main factors that influence the expression of catalase gene(s) in bacteria are the exposure to sublethal H2O2 levels during exponential growth (Barnes et al., 1999) and entry into stationary phase (reviewed by Loewen, 1997). Reactive oxygen species (ROS) may also be powerful microbicidal weapons implemented by eukaryotic cells during infection. Highly diffusible oxidants, such as H2O2, enter membranes and can damage a variety of cell targets (Franzon et al., 1990; Hassett & Cohen, 1989), including DNA, RNA, proteins and lipids, leading to the destruction of microbes. In addition, ROS are generated continuously during aerobic growth by the electron transport chain, leading to the production of H2O2 (Cabisco et al., 2000; Gonzalez-Flecha & Demple, 1995). The toxicity of H2O2 necessitates that aerobic microbes or those whose life cycle relies on host infection encode a functional catalase within their genomes.

*Aeromonas veronii* is a facultative anaerobe which has a propensity to colonize the digestive tracts of a variety of hosts, including humans, leeches and mosquitoes, with manifestations of infection ranging from pathogenesis to mutualism (Graf et al., 2006; Janda & Abbott, 1998). Recently culture-independent analysis of the digestive-tract microbiota of the medicinal leech (*Hirudo verbana*, one of three species within the *Hirudo medicinalis* complex; Hirudinea: Arhynchobdellida: Hirudinidae) (Apakupakul et al., 1999; Siddall & Burreson, 1998; Siddall et al., 2001; Trontelj & Utevsky, 2005) has revealed the presence of a currently uncultured *Rikenella*-like species (Worthen et al., 2006; Kikuchi & Graf, 2007). These two symbionts are the dominant members of the leech digestive-tract microbiota.
Putative functions for the *Aeromonas* symbiont within the medicinal leech include: (i) aiding in the digestion of the blood meal; (ii) providing essential nutrients lacking in the exclusive blood diet, such as B-complex vitamins; (iii) providing ‘colonization resistance’, in which *A. veronii* prevents colonization by other potentially harmful microorganisms, thus preventing the putrefaction of blood and permitting long-term storage; and (iv) priming the microenvironment for the obligate anaerobic *Rikenella*-like symbiont (Worthen et al., 2006; reviewed by Graf, 2002).

Analogous to vertebrate innate immune responses, the introduction of Gram-negative bacteria and their by-products into leech wounds activates an inflammatory response involving the infiltration of macrophage-like cells (de Equi-leor et al., 1999, 2000a, b). During phagocytosis or following stimulation with a wide variety of agents, macrophages undergo respiratory bursts that are characterized by the production and release of ROS into the extracellular milieu (Forman & Torres, 2002; Park, 2003). ROS production is essential for the increased bactericidal capability of stimulated macrophages (Johnston & Kitagawa, 1985). Bacteria can protect themselves against host-produced ROS by upregulating the genes encoding protective enzymes, such as superoxide dismutase, peroxidase, and catalase. While the importance of these protective enzymes is well established for pathogens (Franzon et al., 1990; Mandell, 1975; Zheng et al., 1992), they have also been shown to be crucial for the successful colonization of the light organ of the Hawaiian bobtail squid *Euprymna scolopes* by its extracellular, mutualistic symbiont *Vibrio fischeri* (Visick & Ruby, 1998), and for the regulation of the infection of root nodules by the nitrogen-fixing *Sinorhizobium meliloti* (Santos et al., 2001).

We report the cloning and functional characterization of the *A. veronii* gene encoding the antioxidant enzyme catalase *katA*. We further examine the presence of *katA* homologues in other *Aeromonas* species. Implementing a targeted mutant analysis approach, the importance of *katA* for *A. veronii* survival following exposure to damaging oxidative-stress conditions, and for proliferation and persistence within the medicinal leech, was evaluated.

**METHODS**

**Bacterial strains, plasmids and growth conditions.** The sources and properties of the bacterial strains and plasmids used in this study are listed in Table 1. The bacteria were grown at 200 r.p.m. in Luria–Bertani (LB) medium at 37 and 30 °C for *Escherichia coli* and *Aeromonas* species, respectively (Sambrook & Russell, 2001). The growth rates were determined in LB containing rifampicin (Rf) at 30 °C.

The *A. veronii* strain HM21R is a spontaneous Rf* mutant derived from HM21, an isolate from the leech digestive tract (Graf, 1999). HM21RS was derived from HM21R by plating 10⁵ cells from an overnight culture on LB [Rf, streptomycin (Sm)] plates. Where appropriate, antibiotics were added at the following concentrations: ampicillin (Ap), 100 μg ml⁻¹; chloramphenicol (Cm), 1 μg ml⁻¹ for *A. veronii* and 10 μg ml⁻¹ for *E. coli*; kanamycin (Km), 100 μg ml⁻¹; Sm, 100 μg ml⁻¹; and Rf, 100 μg ml⁻¹ for *Aeromonas* selection and 10 μg ml⁻¹ for maintenance.

**Animals.** The medicinal leeches used in this study were obtained from LeechesUSA and Zaugg GmbH (Biebertal, Germany). The animals were starved for at least 3 months prior to delivery and maintained without feeding in leech tanks at 25 ± 1 °C (Graf, 1999).

**Isolation of genomic DNA.** Genomic DNA was isolated using a modification of the cetyltrimethylammonium bromide (CTAB) method (Nelson & Selander, 1994) by preheating the samples to 65 °C for 5 min prior to lysis with SDS. For large-scale DNA isolation, the volume was increased 30-fold, samples were preheated to 65 °C for 10 min, and the precipitated DNA was isolated using a glass Pasteur pipette.

**Construction of *A. veronii* genomic library.** Genomic DNA was partially digested with Sau3A and separated by electrophoresis in low-melting-point agarose. DNA fragments (6–5–7.6 kb) were purified by phenol extraction and cloned into the dephosphorylated BamHI site of pBSIIKS+. The ligations were transformed into *E. coli* XL-1 Blue MR cell super competent cells (Stratagene) through heat shock at 42 °C.

**Complementation of the *E. coli* rpoS mutant.** Purified plasmid DNA from the *A. veronii* genomic library was transformed into calcium-competent *E. coli* rpoS mutant ZK918 (Sambrook & Russell, 2001). Transformsants were plated on MacConkey agar (Ap, Km) and screened for red colonies. Strains exhibiting this phenotype were picked and patched onto MacConkey agar plates, where the colour phenotype was reassessed. The colonies were additionally examined for the production of catalase by observing the release of O₂ bubbles following the addition of 3% H₂O₂ (Supplementary Fig. S1).

**Characterization of the complementing plasmids.** The complementing plasmids were isolated using Plasmid Mini or Plasmid Midi kits (Qiagen) and sequenced using a combination of subcloning and primer walking (Central DNA Sequencing Facility, Department of Clinical Research, University of Berne, and the Biotechnology Center, University of Connecticut). The DNA sequences were aligned using Vector NTI (Invitrogen) and compared to the databases using the BLAST 2.2.13 algorithm (Altschul et al., 1997).

**Southern analysis.** DNA (*Aeromonas hydrophila* ATCC 14715, *Aeromonas salmonicida* CDC 0434-84, *Aeromonas caviae* ATCC 15468, *Aeromonas media* CDC 0862-83, *Aeromonas eucrenophila* ATCC 23309, *Aeromonas sobria* CIP 7433, *A. veronii* bv. sobria CDC 0437-84, *Aeromonas veronii* bv. sobria HM21, *A. veronii* bv. veronii ATCC 35624, *Aeromonas schubertii* ATCC 43700 and *Aeromonas allosaccharophila* LMG 140549) was digested with *PstI* for 6 h and separated by agarose gel electrophoresis. The loading of an equivalent amount of DNA in the various lanes was confirmed by ethidium bromide staining. The DNA was transferred onto a nylon membrane through overnight capillary transfer and fixed to the membrane with 0.4 M NaOH (Sambrook & Russell, 2001).

A 743 bp hybridization probe was PCR-amplified from HM21 DNA using PkatF, 5′-TCG ACA ACA ACA ACA GCC TCA C-3′, and PkatR1, 5′-CAC CTC CAC ACA GAG TTT CCA GC-3′, as described below, and subsequently labelled using the Amersham ECL Direct System (Amersham Life Science). The membrane was prehybridized at 42 °C in the Amersham ECL direct hybridization buffer. After 1 h, the probe was added and allowed to hybridize overnight. The membrane was washed twice for 20 min at 42 °C in the primary wash buffer and twice for 5 min in the secondary wash buffer. The
membrane was incubated with detection reagents 1 and 2 for 1 min and exposed to X-ray film.

**Construction of the catalase mutants.** For the construction of the catalase mutants, two internal fragments of katA were amplified by PCR using PkatF and either PkatR1 (amplifying a 743 bp product and resulting in DkatAS) or PkatR2, 5'-AGG AGA AGA GTC GCC CTT G-3' (amplifying a 966 bp product and resulting in DkatAL). Single amplicons were obtained for each of the two primer sets. The PCR products were blunt-ended using T4 DNA polymerase and ligated into SmaI-digested pBSII, yielding pJG54 (containing the shorter PCR product, DkatAS) and pJG55 (containing the longer PCR product, DkatAL). The cloned fragments were introduced into the pBS-dependent R6K origin of replication suicide vector pKAS32, using XbaI–EcoRI ends, resulting in pJG57 (DkatAS) and pJG58 (DkatAL). The suicide plasmids were transformed into S17-1 pir and introduced into HM21R by conjugation with shaking (Simon et al., 1983). Donor (5 × 10^7 c.f.u. between 0.4 and 0.8 OD_{600}) and recipient (2 × 10^8 c.f.u. between 0.25 and 0.4 OD_{600}) cells were harvested and spotted on an LB agar plate. Following an overnight incubation at 30 °C, transconjugants were selected on LB agar (Rf, Km).

**Zymography.** Whole-cell lysates were prepared from the cells as described in Methods, Sensitivity to H_{2}O_{2}, below. The cells were lysed by sonication (three bursts, 15 s) and centrifuged at 4 °C for 30 min (12 000 g). Supernatants were loaded onto a 10%, w/v, acrylamide gel and separated at 150 V for 45 min. The gel was washed extensively in double-distilled (dd) H_{2}O and for 10 min in 0.0155 M H_{2}O_{2}.

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**Table 1. Strains and plasmids used in this study**

<table>
<thead>
<tr>
<th>Designation</th>
<th>Relevant characteristics</th>
<th>Source or reference</th>
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<tr>
<td><strong>A. veronii bv. sobria strains</strong></td>
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<tr>
<td>HM21</td>
<td>Wild-type, isolated from <em>H. medicinalis</em></td>
<td>Graf (1999)</td>
</tr>
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<td>HM21R</td>
<td>Spontaneous Rf’ derivative of HM21</td>
<td>Graf (1999)</td>
</tr>
<tr>
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<td>Spontaneous Sm’ derivative of HM21R</td>
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<tr>
<td>JG186</td>
<td>HM21R, katA1 (katA* : pJG58), Rf’, Km’</td>
<td>This study</td>
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<td><strong>Other Aeromonas species</strong></td>
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<td>Ap’, ColE1, high-copy-number cloning vector</td>
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<td>pBSIIKS+ containing ΔKatS in the SmaI site, Ap’</td>
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<tr>
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<td>pBSIIKS+ containing ΔKatL in the SmaI site, Ap’</td>
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<td>pKAS46 containing ΔKatS, Ap’, Km’</td>
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</tr>
<tr>
<td>pRRSR1</td>
<td>pMMB207-katA</td>
<td>This study</td>
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*Type strain.
Subsequently, the gel was stained in a solution containing 1%, w/v, ferric chloride and 1%, w/v, potassium ferricyanide for the visualization of catalase activity (Barnes et al., 1999).

**Analysis of catalase activity.** Paired 5 ml aliquots of cultures were removed at various optical densities and either induced with 0.05 mM H2O2 or not induced. The aliquots were incubated for an additional 30 min before being placed on ice. A crude cell extract was prepared by sonication. Specific catalase activity was determined by measuring the removal of H2O2 from the crude cell extract for 60 s and dividing by the amount of protein, as determined by a Lowry protein assay (Lowry et al., 1951; Beers & Sizer, 1952; Visick & Ruby, 1998). Briefly, the cell pellet was resuspended in phosphate buffer, pH 7.0, containing 5 mM EDTA, 10%, v/v, glycerol and 25 μM PMSF. The change in A240 was monitored four consecutive times (15 s per period) following the addition of 0.5 volumes of 59 mM H2O2 to 1 volume of crude extract. The mean rates were converted to U mg⁻¹ min⁻¹.

**Reverse transcription of katA.** Total RNA was isolated from 2.5 x 10⁸ A. veronii (JG186 and HM21R) cells grown to stationary or early mid-exponential phase, employing the Qiagen RNeasy protocol for total RNA isolation from bacteria. An aliquot of the mid-exponential-phase cells was exposed to a sublethal dose of H2O2 (0.05 mM) for 30 min at 30 °C. The optional on-column RNase-free DNase I (Qiagen) was used to remove contaminating DNA. After RNA isolation, traces of contaminating DNA were further eliminated with an RNase-free DNase I treatment. Random hexamer primers, iScript reverse transcriptase (Bio-Rad) and 0.5 μg total RNA were utilized for first-strand cDNA synthesis. Subsequent PCR was performed with katAP, 5'-GAC AAC ACC CTG CAC AGC-3', and katAR, 5'-CGC TCA TTG GCC TTG-3'. The absence of DNA contamination was verified by PCR using RNA template lacking a reverse-transcription step.

**Sensitivity to H2O2.** The sensitivity of the katA mutant JG186 and its parent strain HM21R to H2O2 was determined for cells prepared in three different ways. The cells were grown either to stationary or to early exponential phase. A portion of the early exponential-phase cells was exposed to a sublethal dose of H2O2 (0.05 mM) for 30 min at 30 °C. The absolute and relative levels of each strain were monitored over time by sacrificing animals at predetermined time points and plating serial dilutions on antibiotic-containing plates. CI was calculated as follows:

\[
CI = \frac{\text{Mutant output}}{\text{Wild-type output}} - \frac{\text{Mutant input}}{\text{Wild-type input}}
\]

A CI of 1 indicates that the mutant colonizes to the same level as the competitor strain, while CI < 1 indicates that the mutant has a colonization defect. A two-tailed, one-sided t test was used to test whether the CI differed from 1. Student’s t test was performed to determine whether colonization levels significantly differed for mutants introduced in sole-colonization assays versus competition assays. Significant differences (P ≤ 0.05) are reported.

**RESULTS**

**Isolation of plasmids that complement the rpoS mutant ZK918**

The initial goal of this study was to isolate a functional rpoS homologue from a genomic library of A. veronii bv. sobria by complementing the E. coli rpoS mutant ZK918 (Bohannon et al., 1991). This mutant has two phenotypes that can be easily screened for and that have been complemented in other studies by rpoS homologues from Vibrio cholerae and Pseudomonas putida (Ramos-Gonzalez & Molin, 1998; Yildiz & Schoolnik, 1998). One of these phenotypes is the red colouration of colonies on MacConkey agar due to lactose fermentation that is dependent on the transcription of the σ52-dependent promoter fusion bolA1–lacZ. The second phenotype is the release of O2 bubbles by colonies after the addition of H2O2 due to the expression of the σ52-dependent HPII catalase katE.

Competent ZK918 were transformed with a genomic library from A. veronii. Of the 9000 transformants screened, three exhibited the red colouration and release of O2 after the addition of H2O2. The complementing plasmids pMA2, pMA5 and pEP2 were isolated and reintroduced into the rpoS mutant ZK918. All three plasmids again complemented ZK918, indicating that
DNA sequence analysis

The complementing region was sequenced in both directions using a combination of primer walking and subcloning. Database searches with the sequence of the cloned Aeromonas DNA using BLASTN and BLASTX showed no similarity to any sigma factors, suggesting that the inserted DNA does not encode a sigma factor. Further sequence analysis revealed one large ORF (ORF1) and two smaller ORFs (ORF2 and ORF3) that are transcribed in the opposite direction (Fig. 1b).

ORF1 consisted of 1446 nt encoding 482 amino acids with a predicted molecular mass of 54 kDa and isoelectric point (pI) of 5.98. Putative −10 and −35 regions for the E. coli housekeeping sigma factor were found 27 and 52 bp, respectively, upstream of the presumptive start codon. A potential Shine–Dalgarno sequence (AGGAGA) was also detected 7 bp upstream of the presumptive start codon. Downstream of the stop codon a GC-rich interrupted dyad symmetry followed by a run of Ts was found, consistent with the stem–loop structure of a rho-independent terminator.

Comparison of the nucleotide sequence from ORF1 with the public databases using BLASTX revealed sequence similarity to the group III small-subunit bacterial catalasases (Klotz et al., 1997), including HktE from Pasteurella multocida (70 % identity and 81 % similarity), a putative catalase from Photobacterium profundum (71 % identity and 79 % similarity) and the catalasases of Bordetella pertussis, Bordetella parapertussis and Bordetella bronchiseptica (69 % identity and 83 % similarity). Furthermore, the deduced amino acid sequence was conserved along the entire protein length with a slightly lower degree of sequence identity at the carboxyl terminus. In addition, a PROSITE search revealed that the two regions conserved in functional catalasases are also present in A. veronii protein. Catalase region 1 is responsible for haem binding and contains a conserved tyrosine residue (RLFS_Y.DTQ), while catalase region 2, a section of the catalytic site, contains a conserved histidine residue (F.R....ER..H..GSG) (Fig. 1c).

In accordance with the high sequence identity, the retention of conserved residues and the release of bubbles following exposure to H₂O₂, the smallest plasmid that restored both phenotypes of ZK918 was pMA7. (b) Genetic map of the katA region of pMA7. Sequence analysis revealed one large ORF (ORF1) encoding katA, and two smaller ORFs (ORF2 and ORF3). Large arrows indicate the locations and directions of transcription of the identified genes. (c) Deduced amino acid sequence of A. veronii katA. The boxed region denotes catalase region 1, and the conserved tyrosine residue is in white on black type. The underlined region denotes catalase region 2, part of the catalytic site that contains a conserved histidine residue (asterisked).
following the addition of H₂O₂ in the complemented rpoS mutant ZK918, we designated the gene as katA (accession no. EF028076).

ORF2 had sequence similarity to a putative acetyltransferase of *Bacillus subtilis*, whereas ORF3 was homologous to a DNA gyrase inhibitor. A comparison of the *A. veronii* katA locus with the *A. hydrophila* genome revealed conservation in genetic organization (Seshadri et al., 2006).

### Presence of catalase genes in *Aeromonas* species

We were interested in determining whether other *Aeromonas* species possess a similar catalase gene. An internal fragment of katA was amplified and used as a probe for Southern analysis of nine *Aeromonas* species (Fig. 2). With the exception of *A. schubertii*, all of the *Aeromonas* species examined revealed one band that hybridized to the katA probe, suggesting that these eight species possess a similar catalase gene. Interestingly, the *Aeromonas* leech isolate HM21 katA band co-migrated with that of *A. veronii* bv. veronii rather than that of *A. veronii* bv. sobria.

### Construction of katA mutants

Deletion mutants were constructed by introducing internal fragments of katA, located on a suicide vector, through homologous recombination into the *A. veronii* chromosome. This results in two copies of katA with each having a deletion in either the 5’ or the 3’ region. The presumptive mutants were screened for inability to release air bubbles after the addition of H₂O₂, and the disruption of the katA gene in four of the Km⁺ and catalase-minus strains, JG185 and JG186 (ΔkatAL derived) and JG183 and JG184 (ΔkatAS derived), was confirmed by Southern blotting using a katA-specific probe. A shift in the molecular mass of the bands that hybridized with the katA probe was observed, indicating that the plasmid had incorporated into the katA locus (data not shown). We chose JG186 for further functional characterization. The growth rate of JG186 in LB did not differ from that of HM21R and HM21RS under similar conditions.

### Detection of catalase activity

Facultative or obligate aerobic bacteria can possess multiple catalases. Our Southern analysis suggested the presence of only one catalase, but we wanted to verify catalase activity independently of sequence similarity through zymography. Whole-cell lysates of the parent strain and JG186 were obtained from cells grown to early exponential phase and stationary phase, and from early exponential-phase cells that were exposed to a sublethal concentration of H₂O₂ for 30 min (induced). Catalase activity was detected only from lysates of induced early exponential-phase HM21R cells (Fig. 3). No other samples exhibited catalase activity, further supporting the presence of only one *A. veronii* catalase that is inducible by oxidative stress. Furthermore, these results also demonstrated that JG186, the katA mutant, does not produce detectable levels of catalase.

The specific catalase activity (U mg⁻¹ min⁻¹) of the parent strain and JG186, with and without exposure to sublethal levels of H₂O₂, was monitored over a range of cell densities (Fig. 4 and data not shown). No catalase activity above background was ever detected for JG186. Interestingly, the ability of the wild-type strain to induce the catalase activity

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**Fig. 2.** Southern blot analysis of *Aeromonas* species DNA hybridized with a 743 bp *A. veronii* katA fragment. Ah, *A. hydrophila* ATCC 14715; As, *A. salmonicida* CDC 0434-84; Ac, *A. caviae* ATCC 15468; Am, *A. media* CDC 08262-83; Ae, *A. eucrenophila* ATCC 23309; Aso, *A. sobria* CIP 7433; Avs, *A. veronii* bv. sobria CDC 04374-84; Avv, *A. veronii* bv. veronii ATCC 35624; Asc; *A. schubertii* ATCC 43700; Aa, *A. allosaccharophila* LMG 140549; Hm, *A. veronii* bv. sobria HM21.

**Fig. 3.** Zymogram analysis of catalase activity of HM21R and JG186 through cell growth. Catalase activity was specifically detected from HM21R early exponential-phase lysates that had been induced and subsequently challenged with H₂O₂. No other samples demonstrated catalase activity. Stat., stationary; EL, early exponential; Ind. EL, induced early exponential.
was inversely related to the optical density of the culture. It is possible that the specific catalase activity from cells at low optical density can be elevated by an underestimation of total protein level. Reverse-transcriptional analysis further confirmed katA expression by H2O2-induced HM21R (data not shown).

Sensitivity to H2O2

The sensitivity and protective response of the wild-type strain (HM21R) to oxidative stress was determined by exposing the cells to increasing concentrations of H2O2 (0.01–10 mM) and monitoring their survival. The cells were grown to early exponential phase or stationary phase, and subsequently a portion of the early exponential-phase cells was exposed to a sublethal concentration of H2O2 (induced cells). Non-induced HM21R early exponential-phase cells (Fig. 5a) were less resistant to increasing H2O2 concentrations than induced (Fig. 5b) and stationary-phase (Fig. 5c) cells. Both induced and stationary-phase HM21R were adversely affected commencing at 1 mM H2O2, with survival rates of 88 and 70 %, respectively (Fig. 5b, c).

We then determined the importance of katA in providing oxidative stress protection by exposing JG186 to similar concentrations of H2O2. Interestingly, no survival differences between JG186 and HM21R were detected in stationary-phase cells (Fig. 5c). In contrast to induced HM21R, induced JG186 cells were much more sensitive to H2O2, with survival negatively affected at concentrations as low as 0.1 mM. These results demonstrated that katA is critical in providing an inducible protection against exogenous H2O2 in vitro. However, in a similar manner to HM21R, non-induced early exponential-phase JG186 cells were less resistant than their induced counterparts, with survival dropping to 2 % (Fig. 5a) in comparison to 38 % (Fig. 5b) at 0.5 mM H2O2. These results suggest that A. veronii bv. sobria retains another inducible mechanism in addition to katA to safeguard against oxidative stress, and that during stationary phase, protection is provided through catalase-independent means.
Colonization of the medicinal leech

In symbiotic relationships, mechanisms that control the spatial (anatomical) localization and the proliferation of the symbiotic flora are critical for the maintenance of homeostasis (Rio et al., 2006). One common mechanism employed to control infections of pathogenic bacteria is to create an environment of oxidative stress that kills sensitive bacteria. Catalase has been shown to be required for the normal symbiotic competence of V. fischeri and Eu. scolopes, being induced by both oxidative stress and the approach to stationary phase (Visick & Ruby, 1998). Accordingly, we were interested to determine whether the approach to stationary phase (Visick & Ruby, 1998).

The DISCUSSION

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microbial partner during the colonization of the leech digestive tract or that other compensatory mechanisms exist, such as the previously mentioned peroxidases. Examining whether catalase is crucial for *A. veronii* infection in other associations, such as the cooperative zebrafish digestive tract, and whether virulence is attenuated in the mouse model may provide further insights. Similarly, *B. pertussis* does not require catalase for persistence within human polymorphonuclear leukocytes (PMNs), although phagocytosis is associated with a respiratory burst that involves the generation of $O_2$ and $H_2O_2$ (DeShazer et al., 1994). It is possible that $O_2$ introduced with the fresh blood meal is removed rapidly in the leech digestive tract by the aerobic metabolism of *Aeromonas*, generating an environment that does not permit the generation of ROS and is suitable for the anaerobic *Rikenella*-like symbiont that likely does not possess antioxidant enzymes. Synergistic interactions between the two microbes resulting in enhanced mixed-species, polysaccharide-embedded microcolony formation have been suggested to occur within the leech digestive tract (Kikuchi & Graf, 2007). The apparent lack of oxidative stress suggests that other mechanisms must be responsible for ensuring the specificity, establishment and maintenance of this unusually simple digestive-tract community.

**ACKNOWLEDGEMENTS**

We thank Eva Pitek and Cuong Phang for help with initial experiments and Adam Silver and Rolf Troller for excellent technical assistance. This research was supported by the National Science Foundation Career award MCB 0448052 to J. G.

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Edited by: J. G. Shaw