

Interspecific Quorum Sensing Mediates the Resuscitation of Viable but Nonculturable Vibrios

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Entry and exit from dormancy are essential survival mechanisms utilized by microorganisms to cope with harsh environments. Many bacteria, including the opportunistic human pathogen *Vibrio vulnificus*, enter a form of dormancy known as the viable but nonculturable (VBNC) state. VBNC cells can resuscitate when suitable conditions arise, yet the molecular mechanisms facilitating resuscitation in most bacteria are not well understood. We discovered that bacterial cell-free supernatants (CFS) can awaken preexisting dormant vibrio populations within oysters and seawater, while CFS from a quorum sensing mutant was unable to produce the same resuscitative effect. Furthermore, the quorum sensing autoinducer AI-2 could induce resuscitation of VBNC *V. vulnificus* *in vitro*, and VBNC cells of a mutant unable to produce AI-2 were unable to resuscitate unless the cultures were supplemented with exogenous AI-2. The quorum sensing inhibitor cinnamaldehyde delayed the resuscitation of wild-type VBNC cells, confirming the importance of quorum sensing in resuscitation. By monitoring AI-2 production by VBNC cultures over time, we found quorum sensing signaling to be critical for the natural resuscitation process. This study provides new insights into the molecular mechanisms stimulating VBNC cell exit from dormancy, which has significant implications for microbial ecology and public health.

At least 68 species of bacteria have been shown to enter a state of dormancy in which cells are viable but nonculturable (VBNC) (1), allowing them to evade harmful environmental stresses while maintaining the ability to return to an active state when the stress is alleviated (2). These cells are viable, as evidenced by their intact cell membranes and continued gene expression; however, they fail to replicate on routine laboratory media (2, 3). While in the VBNC state, these cells are able to resist a variety of normally fatal stresses, including antibiotic treatment (4), and they possess the ability to resuscitate within a host, potentially leading to disease initiation (5). Since these cells are not detectable by standard bacterial culture methods, the VBNC phenomenon is of significant public health and food safety concern. While there has been considerable growth in our knowledge of this dormancy state (1), very little is known regarding the molecular mechanisms for the resuscitation of VBNC cells.

Pathogenic vibrios exhibit seasonal fluctuations in abundance in water and in shellfish (6, 7), with their highest levels of culturability and incidences of infection observed during warm summer months (8, 9). The VBNC state is believed to be associated with this seasonal abundance in the environment, with culturability decreasing during winter months, when some cells enter the VBNC state, and increasing during warmer months, when these cells begin to resuscitate (10).

The opportunistic human pathogen *Vibrio vulnificus* is part of the normal microflora of the estuarine environment and is found concentrated within bivalves and other organisms that inhabit these waters (11, 12). *V. vulnificus* classically expresses its virulence after the ingestion of raw or undercooked shellfish (11), or by entry into open wounds (13), severely harming individuals with liver disease or immunodeficiency (14–16). With a case fatality rate of up to 50% (15), the majority (95%) of deaths associated with seafood in the United States are caused by this pathogen (7, 17). The VBNC state of *V. vulnificus* is easily studied *in vitro* by induction of dormancy using low-temperature incubation and of

resuscitation by a simple temperature upshift (18), making it an ideal model organism for the study of this physiological state.

We previously found that upon addition of exogenous *V. vulnificus* or *Escherichia coli* cells to oysters, the culturability of the normal flora of *Vibrio* spp. within these oysters dramatically increased and remained high even after the added bacterial populations had been purged by the oysters (19). This indicated that preexisting and naturally present VBNC populations within these oysters resuscitated in response to the added metabolically active bacteria. In a related study, environmental water samples were enriched with the quorum sensing molecules CAI-1 and AI-2 to enhance the culturability of *Vibrio cholerae* (20). These findings led us to test the hypothesis that the increase in the culturability of *Vibrio* spp. upon the addition of exogenous metabolically active cells to oysters is due to quorum sensing signaling.

The phenomenon known as quorum sensing is the production of and response to signals (autoinducers) generated by bacteria of the same or other species in an effort to gauge the population density in a certain environment or to evaluate the cells' environmental location (21). This leads to global changes in gene expression (22) and has been shown to orchestrate virulence (23) and fitness factor production (24). For a more in-depth explanation of quorum sensing, the reader is directed to a recent review (25). Interestingly, some environmental factors that have been shown to affect quorum sensing, such as temperature, salinity, and pH, have independently been shown to play a role in VBNC dynamics

Received 7 January 2014 Accepted 4 February 2014

Published ahead of print 7 February 2014

Editor: M. W. Griffiths

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doi:10.1128/AEM.00080-14

(2, 26). Given these findings, we asked whether quorum sensing autoinducers mediate the resuscitation of VBNC *V. vulnificus*.

Here we demonstrate that the interspecific quorum sensing molecule AI-2 can resuscitate VBNC cells both *in vivo* and *in vitro*. Most importantly, we report for the first time that AI-2 is required by VBNC cells to resuscitate and that this quorum sensing-mediated process occurs naturally within VBNC cultures when environmental conditions are permissive, leading to resuscitation. Furthermore, we propose a molecular mechanism for the resuscitation of VBNC cells and show that it can be targeted to manipulate resuscitation.

MATERIALS AND METHODS

Strains and growth conditions. Strains used in this study were *V. vulnificus* CMCP6, C7184, JDO1, and AH1, *V. parahaemolyticus* Sak-11, *V. harveyi* BB170, and *E. coli* K-12. All strains were stored at -80°C in Bacto Luria broth (LB) (BD, NJ) containing 20% glycerol. All strains were grown in Bacto heart infusion (HI) broth (BD, NJ) for 24 h at 30°C with aeration. Strains JDO1 and AH1 are *luxS* and *rpoS* mutants, respectively, and were derived from the parent strain C7184. JDO1 is a *luxS* mutant that is unable to produce 4,5-dihydroxy-2,3-pentanedione, an essential precursor to the autoinducer AI-2 (27), and AH1 is unable to produce the stress-related sigma factor RpoS (28). JDO1 and AH1 were grown with 2 and 15 $\mu\text{g}/\text{ml}$ chloramphenicol, respectively. All bioluminescence-based quorum sensing assays were performed using autoinducer bioassay (AB) medium (29).

Resuscitation of dormant cells in oysters. *Crassostrea virginica* oysters were collected from Hoop Pole Creek, Atlantic Beach, NC, during September 2012. Oysters were maintained in 30-liter aerated aquaria containing 20‰ artificial seawater (ASW) (Instant Ocean; Aquarium Systems, Mentor, OH) and were fed Phyto-Feast daily (Reed Mariculture Inc., Campbell, CA).

Cell-free supernatants (CFS) were obtained from overnight cultures of *V. vulnificus* C7184 and JDO1 by filter sterilization (0.22- μm syringe-driven filter; Millipore, Tullagreen, Ireland). To confirm the sterility of CFS, 100- μl aliquots of CFS were spread plated onto HI agar. Oysters were placed in 3-liter tanks (4 oysters per treatment condition) of 20‰ ASW with aeration. CFS was added to oyster tanks at a 1:100 (vol/vol) ratio. After 24 h, oysters were shucked using a sterile knife and homogenized in 20‰ ASW (1:1 [wt/vol]), using sterile blender cups (Waring, Torrington, CT). Individual oyster homogenates were serially diluted in phosphate-buffered saline (PBS) and spread plated onto ChromAgar Vibrio (ChromAgar, Paris, France) to selectively quantify *Vibrio* species. This medium was employed to quantify total vibrios, as it has been demonstrated previously to indiscriminately allow for growth of all *Vibrio* species tested (30). Oysters which were incubated with CFS were compared to control oysters that were supplemented only with ASW. As an additional control, some oysters were supplemented with HI broth to ensure that nutrient carryover had no effect on vibrio levels.

Resuscitation of dormant cells in seawater. Natural seawater (NSW) was collected from Core Creek, Beaufort, NC, and stored at 20°C in sterile 50-ml conical tubes. Resuscitation experiments were performed within 24 h of collection. To eliminate nutrient carryover, 1 ml of overnight bacterial culture was washed twice with 15‰ ASW and incubated at 30°C for 24 h prior to collection of CFS by filter sterilization as described above. This nutrient-free CFS was added to the NSW at a 1:100 (vol/vol) ratio. The mixture was incubated at 20°C for 24 h before serially diluting and plating of the mixture on ChromAgar Vibrio. The fold change in CFU after 24 h in NSW supplemented with CFS was compared to that for controls that received only sterile 15‰ ASW. Experiments were performed in triplicate.

Production and resuscitation of *in vitro* VBNC cells. VBNC cultures were produced as previously described (18). Briefly, *V. vulnificus* was grown overnight in HI broth, added to fresh HI broth at a 1:100 (vol/vol)

ratio, and grown to logarithmic phase (optical density at 610 nm [OD_{610}], 0.15 to 0.25). Cells were subsequently washed twice with 15‰ ASW to remove any nutrients, resuspended in 15‰ ASW at a 1:100 (vol/vol) ratio, and stored statically in a 4°C incubator. Cultures were quantified daily until *V. vulnificus* was no longer culturable on HI agar (<10 CFU/ml detectable).

To resuscitate VBNC cells, cultures were transferred from the 4°C incubator to room temperature (20°C). Cultures were immediately supplemented with nutrient-free CFS (collected as described above) from either *V. vulnificus*, *V. parahaemolyticus*, or *E. coli*, which are known to produce AI-2, at a 1:100 (vol/vol) ratio, or they were supplemented with 15‰ ASW at a 1:100 (vol/vol) ratio as a control. Additionally, we utilized nutrient-free CFS from a *V. vulnificus luxS* mutant strain (JDO1) unable to produce AI-2. These mixtures were serially diluted in PBS and plated at regular intervals onto HI agar to monitor resuscitation. Resuscitation curves were compared to those for control resuscitation cultures that received ASW. Three biological replicates were used for each treatment.

Use of synthetic autoinducer to induce resuscitation. VBNC cultures were prepared as described above. The synthetic autoinducer (*s*)-4,5-dihydroxy-2,3-pentanedione (DPD) (OMM Scientific, Dallas, TX) spontaneously forms the quorum sensing molecule AI-2 in liquid suspension (31). AI-2 was prepared by adding a 10-fold molar excess of boric acid to DPD, making a 50 μM DPD stock solution. To resuscitate VBNC cells, dormant cultures of C7184, JDO1, and AH1 were moved to 20°C , and AI-2 was immediately added to each culture, to a final concentration of 250 nM. Cultures were serially diluted and plated onto HI agar at regular intervals to monitor resuscitation. Resuscitation times were compared to those for control resuscitation cultures that received only ASW. Experiments were performed in triplicate.

Use of quorum sensing inhibitor to prevent resuscitation. Cinnamaldehyde is a quorum sensing inhibitor that has been shown to prevent binding of LuxR, the master regulator of quorum sensing, to DNA (32). We used a cinnamaldehyde concentration of 150 μM , which is noninhibitory to growth, as previously reported (32). Cinnamaldehyde-supplemented VBNC cultures (prepared as described above) were allowed to resuscitate by incubation at 20°C . Cultures were serially diluted and plated onto HI agar at regular intervals to monitor resuscitation. Resuscitation curves were compared to those for control resuscitation cultures that received an equal volume of ASW. Experiments were performed in triplicate.

Bioluminescence-based quorum sensing assay. The bioluminescence-based quorum sensing assay was performed as previously described (33), with slight modifications. Briefly, *Vibrio harveyi* strain BB170, which is bioluminescent only in response to AI-2 signals, was grown overnight on AB agar at 30°C . Cells were then removed using a sterile swab, diluted in AB broth to an OD_{610} of 0.7 to 0.8, and then incubated with shaking at 30°C until the OD_{610} reached 1.1. A 1:5,000 dilution of this culture in fresh AB broth was prepared to create the working solution. Nutrient-free CFS was added to the working solution at a 1:10 (vol/vol) ratio for a total of 1.5 ml and was incubated at 30°C with shaking for 4 h. After incubation, 200 μl of sample was transferred to a flat-bottom 96-well plate and read using a GloMax 96 microplate luminometer (Promega, Madison, WI). Background luminescence was assayed by quantifying control samples that contained 15‰ ASW and the working solution of BB170 at a 1:10 (vol/vol) ratio for a total of 1.5 ml. Data are presented as sample luminescence levels (arbitrary units) with the average background luminescence subtracted. Experiments were performed in triplicate.

Validation of resuscitation. To validate that during *in vitro* resuscitation experiments cells were truly resuscitating rather than growing from a small, undetectable population, theoretical generation times for resuscitating cells were calculated using equation 1:

$$\text{Theoretical generation time (min)} = \frac{60}{\left[\frac{\log(C_f) - \log(C_i)}{\log 2} \right]} \quad (1)$$

where C_i and C_f are the starting and ending CFU values, respectively, for

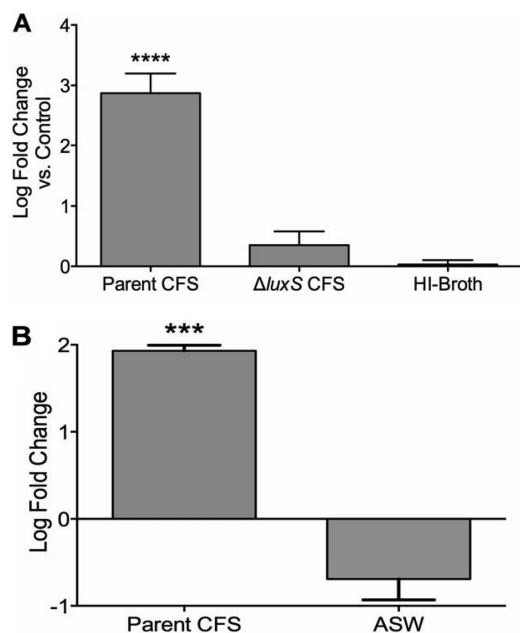


FIG 1 Increase in the culturability of *Vibrio* spp. in oysters and seawater upon addition of CFS. (A) Log fold change in culturability of *Vibrio* spp. in oyster tissues after 24 h of treatment with CFS from *V. vulnificus* C7184 (parent) or JDO1 (*luxS* mutant) relative to that of control oysters that received artificial seawater (ASW). Wild-type CFS significantly increased the culturability of *Vibrio* spp. in oysters (one-way ANOVA; $P < 0.0001$), whereas CFS from the *luxS* mutant did not. Since HI broth had little effect on culturability, nutrient addition did not affect the culturability of *Vibrio* spp. Error bars represent SD for 4 oysters. (B) Log fold change in levels of *Vibrio* spp. in NSW after 24 h (relative to that at 0 h) of treatment with either CFS from *V. vulnificus* C7184 or the ASW control (unpaired *t* test; $P = 0.0004$). Error bars represent SD for 3 NSW samples.

the 1-h time interval being analyzed. This method of validating resuscitation has been utilized by others (18, 20, 34) to demonstrate that the observed increase in culturability is indeed due to resuscitation rather than regrowth of a small culturable population.

Statistical analysis. Data were analyzed using unpaired Student's *t* test, or one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test for multiple comparisons. Statistical significance was determined using an alpha level of 0.05. Error bars in all figures represent standard deviations (SD). All data were analyzed using GraphPad Prism (version 6.0; GraphPad Software Inc.).

RESULTS AND DISCUSSION

Addition of CFS increases culturability of vibrios in oysters and seawater. We showed previously that the addition of exogenous bacteria to oysters dramatically increased the culturability of the resident *Vibrio* spp. in oyster tissues, likely through the resuscitation of a dormant population (19). To evaluate whether this increase was in response to extracellular factors, CFS collected from *V. vulnificus* (C7184) was added to oysters, and the culturability of *Vibrio* spp. present among the members of the oyster microflora was monitored. We found that upon addition of CFS from AI-2-producing *V. vulnificus* cells to live oysters, the culturability of *Vibrio* spp. within these oysters increased significantly ($P < 0.0001$), indicating that an extracellular factor promoted *in vivo* resuscitation of *Vibrio* spp. (Fig. 1A). In contrast, the addition of nutrients (HI broth) to oysters did not result in a considerable increase in culturability (Fig. 1A). We also observed that a quorum

sensing mutant lacking the ability to produce AI-2 was unable to produce the same effect (Fig. 1A), suggesting that the extracellular resuscitation-promoting factor was AI-2. A similar increase in culturability was observed when CFS was added to natural seawater (NSW) (Fig. 1B). These results provide evidence for the hypothesis that resuscitation of *V. vulnificus* from the VBNC state is a quorum sensing-related phenomenon.

Addition of CFS or AI-2 induces early resuscitation of *in vitro* VBNC cultures. If AI-2 mediates the resuscitation of VBNC cells present within oysters and seawater, then the addition of AI-2 to *in vitro* VBNC cultures would also be expected to induce early resuscitation. *V. vulnificus* can normally be resuscitated within 7 to 9 h after a temperature upshift from 4°C to 20°C (18). Therefore, we hypothesized that the addition of AI-2 to VBNC cultures immediately after a temperature upshift would cause resuscitation to occur much earlier. The addition of CFS from wild-type AI-2-producing *V. vulnificus*, *V. parahaemolyticus*, and *E. coli* bacteria induced resuscitation of VBNC cells in as little as 3 h, whereas CFS from a *V. vulnificus luxS* mutant deficient in AI-2 production was unable to induce a faster resuscitation than that of the control (~8 h) (Fig. 2A). Since CFS is composed of various components aside from AI-2, we tested the ability of synthetic AI-2 to induce resuscitation. Synthetic AI-2 was also able to induce early resuscitation of VBNC cells (Fig. 2A), indicating that AI-2 alone is able to induce the reactivation of dormant *V. vulnificus* cells during a temperature upshift.

Quorum sensing inhibitor delays resuscitation of *in vitro* VBNC cultures. To test whether the action of AI-2-mediated resuscitation was through the master quorum sensing regulator, SmcR (a LuxR homolog) (35), we employed an inhibitor of LuxR, hypothesizing that this would inhibit the resuscitation of VBNC cells. The LuxR inhibitor, cinnamaldehyde, was able to delay resuscitation by at least 40 h (Fig. 2B). This indicates that the resuscitation-promoting ability of autoinducers is effective, at least partially, through the quorum sensing regulatory pathways and that the inhibition of quorum sensing can hinder the resuscitation process.

Validation of resuscitation. To validate that the observed increase in culturability was in fact resuscitation rather than a regrowth of a small population of undetectable cells, we calculated the theoretical generation times required to yield such an increase if the cells were actually growing rather than resuscitating. In all cases, the generation times required to produce the observed increases in culturability were 13.9 min or less (Table 1). We determined *V. vulnificus* to have a generation time of 30.7 ± 0.17 min during logarithmic growth under nutrient-rich conditions (HI broth); thus, it is nearly impossible for this species to divide at a higher rate under the nutrient-depleted (ASW), nonaerated, room temperature conditions employed in our resuscitation studies. These resuscitation rates are much higher than normal rates of division would allow and therefore support our claim that these cells were truly resuscitating rather than growing from a small number of culturable cells.

Resuscitation occurs in response to increased AI-2. Since VBNC cultures artificially supplemented with autoinducers promoted resuscitation, it was speculated that this AI-2-mediated process might also occur naturally during a temperature upshift. To test this, the production of AI-2 and the culturability of cells during resuscitation from the VBNC state following a temperature upshift were monitored simultaneously. During resuscita-

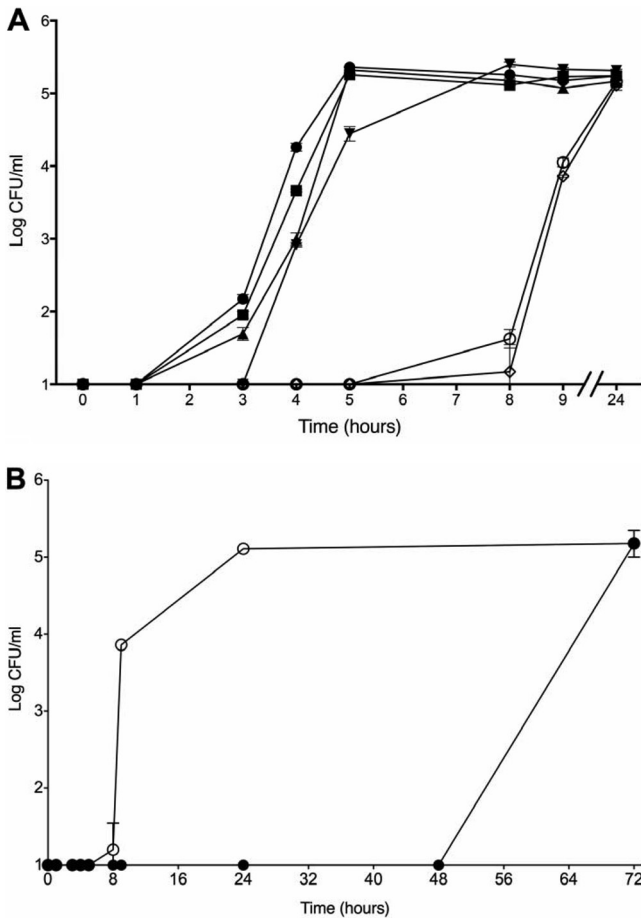


FIG 2 Resuscitation of VBNC *V. vulnificus* supplemented with CFS, synthetic AI-2, or cinnamaldehyde. (A) Culturability of *V. vulnificus* after temperature upshift and supplementation with CFS from *V. vulnificus* C7184 (parent; closed circles), JDO1 (*luxS* mutant; open circles), *V. parahaemolyticus* (closed squares), or *E. coli* (closed triangles) or with synthetic AI-2 (closed downward-facing triangles). Control cultures (open diamonds) received ASW. All wild-type CFS and synthetic AI-2 induced early resuscitation of VBNC cells, while CFS from the *luxS* mutant did not. Error bars represent SD for 3 replicates. (B) Culturability of *V. vulnificus* after a temperature upshift, with (closed circles) and without (open circles) 150 μ M cinnamaldehyde. Error bars represent SD for 3 replicates.

tion, there was a rapid increase in AI-2 levels, reaching a maximum value 5 h after temperature upshift, even though initiation of culturability was not detected until ca. 7 h (Fig. 3). Interestingly, in repeated studies, resuscitation was detected only after AI-2

TABLE 1 Theoretical generation times required for the observed increase in culturability during *Vibrio vulnificus* resuscitation

Treatment	Theoretical generation time (min)
<i>Vibrio vulnificus</i> CFS	8.66
<i>Vibrio parahaemolyticus</i> CFS	10.57
<i>Escherichia coli</i> CFS	13.88
<i>Vibrio vulnificus</i> JDO1 CFS	7.48
Synthetic AI-2	11.77
Control ^a (no CFS)	7.01

^a Untreated VBNC *Vibrio vulnificus* resuscitation.

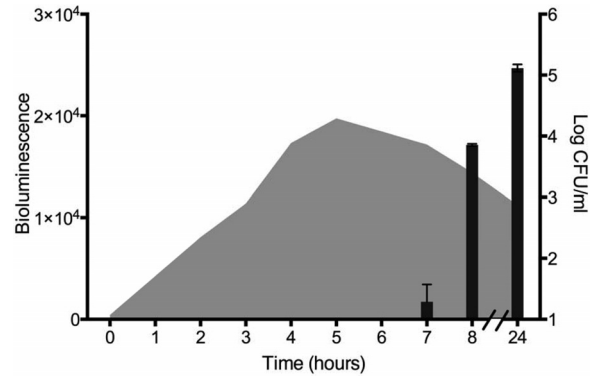


FIG 3 Production of AI-2 during temperature upshift-induced resuscitation from the VBNC state. The data depicted show the culturability of *V. vulnificus* cells (black bars) as they resuscitated from the VBNC state, with corresponding AI-2 levels (gray curve) in these cultures as measured by the AI-2 bioluminescence assay.

reached such a maximum, independent of the temporal occurrence of the maximum, suggesting that AI-2 levels must reach a threshold value before resuscitation can begin. It is interesting that AI-2 levels were able to increase before culturability could be detected following the temperature upshift. We postulate that either VBNC cells are able to produce AI-2 before they become culturable or a small, undetectable population signals to the dormant population to initiate resuscitation. A recent study by Buerger et al. (36) provided evidence for Epstein's (37) microbial scout hypothesis, which proposes that a small portion of a dormant population resuscitates stochastically (becomes "scouts"), independent of environmental signals. If environmental conditions are not permissive, these scouts are thought to die. However, if acceptable environmental conditions are sensed, then these scouts may signal to their dormant counterparts that growth-permissive conditions have been met (36). We propose that, at least for *V. vulnificus*, and likely other bacteria, quorum sensing autoinducers are the signals employed by the scouts to rouse the rest of the dormant population. This hypothesis is substantiated by our observed initial increase in AI-2 production without detectable culturability, indicating that scout cells, present at levels below our detection limits, had stochastically resuscitated and were signaling to the rest of the population, via quorum sensing, that acquiescent conditions had been reached. Since our studies have shown that resuscitation in response to autoinducers is not species specific, active cells may function as scouts for a dormant population of a different genus or species through AI-2 signaling. To confirm that resuscitation during a temperature upshift occurs in response to AI-2, the ability of VBNC cultures of the *V. vulnificus luxS* mutant to resuscitate was examined. The mutant was unable to resuscitate from the VBNC state during a temperature upshift unless the culture was supplemented with exogenous AI-2, again indicating that AI-2 is required for resuscitation to occur in *V. vulnificus* (Fig. 4A).

Resuscitation mechanism. It has previously been demonstrated that *V. vulnificus* cells in the VBNC state are nonculturable on laboratory media largely due to reduced catalase activity. This sensitizes the cells to the presence of reactive oxygen species and therefore prevents replication of these cells on media that routinely contain H₂O₂ (38). Furthermore, catalase production resumes upon resuscitation from the VBNC state, allowing renewed

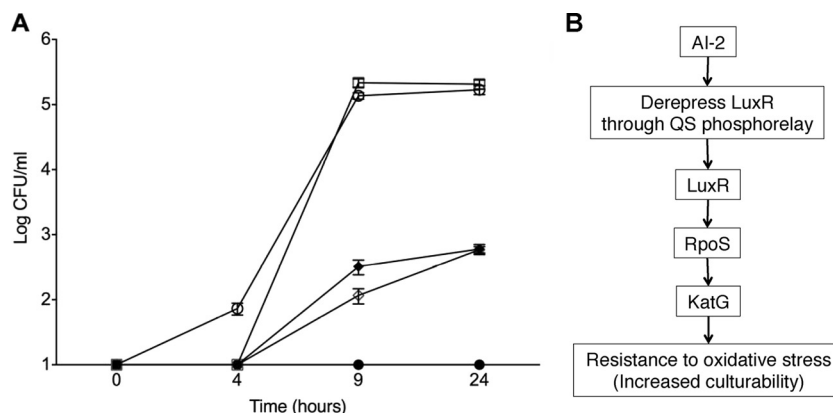


FIG 4 Resuscitation of *V. vulnificus* *luxS* and *rpoS* mutants during temperature upshift. (A) Culturability of *luxS* mutant (JDO1; closed circles), *rpoS* mutant (AH1; closed diamonds), and parent strain (C7184; open squares) after a resuscitation-inducing temperature upshift. JDO1 was unable to resuscitate within 24 h unless it was supplemented with exogenous AI-2 (open circles), indicating the requirement of AI-2 for the resuscitation of VBNC *V. vulnificus*. AH1 displayed a delayed ability to resuscitate, likely due to decreased survival at 4°C. Importantly, resuscitation of AH1 was not affected by the addition of AI-2 (open diamonds), indicating the importance of RpoS for AI-2-mediated resuscitation. (B) Proposed mechanism of resuscitation. An increase in AI-2 signaling causes the derepression of LuxR, a positive regulator of RpoS. Increased RpoS activity leads to an increase in production of catalase (KatG), which allows cells to be culturable on routine media containing H₂O₂.

culturability (39). The stress-related alternate sigma factor RpoS is known to be necessary for the production of catalase (40) and to play an important role in resuscitation from the VBNC state (41). Furthermore, Joëlsson et al. (42) reported that LuxR enhances the expression of *rpoS*. Another study found that expression of *rpoS* significantly increased during resuscitation from the VBNC state (43). Here we found that a *V. vulnificus* *rpoS* mutant had a restricted ability to resuscitate within 24 h. Importantly, the addition of AI-2 to a VBNC culture of the *rpoS* mutant did not induce early resuscitation (Fig. 4A) like that observed with VBNC cells of the wild-type parent. This finding indicates that RpoS is an important factor in AI-2-mediated resuscitation. Combining the existing literature with our current findings, we propose a model of resuscitation in which increased AI-2 signaling stimulates expression of *rpoS* through the action of LuxR. This subsequently induces the expression of catalase (*katG*), thereby allowing cells to overcome the toxic properties of H₂O₂ in bacterial media and to return to a culturable state (Fig. 4B). However, it cannot be excluded that quorum sensing has an effect on other processes related to dormancy, such as toxin-antitoxin regulation, which has been studied extensively in persister cell formation, another type of bacterial dormancy (44).

Conclusions. A previous study demonstrated that artificially supplementing VBNC cells with quorum sensing autoinducers can resuscitate dormant *Vibrio cholerae* cells to improve their detectability (20). Here we provide evidence that interspecific quorum sensing autoinducers have the capability of resuscitating dormant bacterial populations both *in vivo* and *in vitro*. For the first time, we have elucidated the active interplay between autoinducer levels and VBNC dynamics in *V. vulnificus*.

Specifically, we found that VBNC cells resuscitate in response to a quorum sensing signal that is produced by cells within a dormant population when permissive conditions are reached. As proposed by Manina and McKinney (45), with the advent of real-time single-cell analysis technology, the role of quorum sensing in the resuscitation of dormant microorganisms may be studied with much greater resolution. This study may provide important insights for clinical intervention and for the development of inno-

vative antimicrobial agents in the fight against drug-tolerant dormant cells. Overall, this study provides a greater understanding of the mechanisms employed by bacteria to exit from dormancy, which helps to explain the seasonality exhibited by bacterial populations in natural environments. These findings have important implications for microbial ecology and public health.

ACKNOWLEDGMENTS

We thank Bonnie L. Bassler for kindly providing the *V. harveyi* strains and Matthew W. Parrow for providing the synthetic AI-2 used in this study.

This material is based upon work supported by the U.S. Department of Agriculture, NIFA, under agreement 2010-65201-30959.

Any opinions, findings, conclusion, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.

We declare no conflicts of interest for this article.

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