Cholera is an acute diarrheal disease characterized by the passage of voluminous rice water stools. *Vibrio cholerae* strains belonging to serogroups O1 and O139 continue to cause seasonal cholera outbreaks that affect highly populated regions in Asia, Africa, and Latin America. The bacterium adheres to and colonizes the small intestine and secretes cholera toxin, which causes the major clinical symptoms of the disease. The quorum-sensing regulator HapR coordinates cell density-dependent expression of virulence factors (23). At a high cell density, expression of HapR switches the metabolism of *V. cholerae* from a “virulence mode” characterized by the expression of cholera toxin and toxin-coregulated pilus to an “exit mode” typified by expression of hemagglutinin/protease (13, 39). HA/protease in turn promotes detachment of *V. cholerae* to initiate new infection foci or exit from the host (5, 12).

The fate of *V. cholerae* outside the human host is still unresolved. *V. cholerae* strains belonging to non-O1 and non-O139 serogroups are known to be part of the microbial flora of estuarine and coastal waters, from which they are commonly isolated (9, 11). Interestingly, virulence factors typical of O1 and O139 pathogenic strains have also been found dispersed among non-O1 non-O139 strains isolated in areas where O1 and O139 pathogenic strains are not endemic (9). The existence of an aquatic reservoir of O1 and O139 toxigenic strains has not been established. However, the capacity of these strains to survive and persist in estuarine and brackish waters is widely accepted (9, 11). A better understanding of the mechanisms that *V. cholerae* employs to survive in the environment between epidemics could be important to the control of this disease.

The ability of *V. cholerae* to associate with phytoplankton and zooplankton and its ability to form biofilms on biotic and abiotic surfaces have been proposed to play important roles in environmental survival (16, 29). Survival in a dormant viable but not culturable stage has been proposed (37). However, it is not clear to what extent these mechanisms are found specifically in toxigenic O1 and O139 strains. Cyclical cholera outbreaks have been correlated with numerous environmental factors (15), but the genetic and biochemical determinants of the correlations remain unknown.

It has recently been reported that *V. cholerae* biosynthesizes very large amounts of inorganic polyphosphate (poly-P) (25). The enzyme polyphosphate kinase (PPK) catalyzes the reversible transfer of a terminal phosphate from ATP to poly-P (1, 2, 18). This enzyme can act as a nucleotide diphosphate kinase, or it can transfer a pyrophosphoryl group to GDP to generate the stringent response mediator guanosine tetraphosphate (ppGpp) (19). *Escherichia coli* polyphosphate kinase (ppk) mutant was defective in polyphosphate biosynthesis. Deletion of ppk had no significant effect on production of cholera toxin, hemagglutinin/protease, motility, biofilm formation, and colonization of the sucking mouse intestine. The wild type and mutant had similar growth rates in rich and minimal medium and exhibited similar phosphate uptake and alkaline phosphatase induction. In contrast to ppk mutants from other gram-negative bacteria, the *V. cholerae* mutant survived prolonged starvation in LB medium and artificial seawater basal salts. The ppk mutant was significantly more sensitive to low pH, high salinity, and oxidative stress when it was cultured in low-phosphate minimal medium. The ppk mutant failed to induce catalase when it was downshifted to phosphorus-limiting conditions. Furthermore, the increased sensitivity of the ppk mutant to environmental stressors in phosphate-limited medium correlated with a diminished capacity to synthesize ATP from intracellular reservoirs. We concluded that polyphosphate protects *V. cholerae* from environmental stresses under phosphate limitation conditions. It has been proposed that toxigenic *V. cholerae* can survive in estuaries and brackish waters in which phosphorus and/or nitrogen can be a limiting nutrient. Thus, synthesis of large polyphosphate stores could enhance the ability of *V. cholerae* to survive in the aquatic environment.
26, 38), inactivation of the \textit{V. cholerae} ppk gene did not reveal stress-related phenotypes (25). Finally, inorganic poly-P has been shown to affect motility in several bacterial pathogens, including \textit{E. coli}, \textit{Pseudomonas aeruginosa}, \textit{Klebsiella pneumoniae}, \textit{V. cholerae}, and \textit{Salmonella} spp (28).

Recently, \textit{V. cholerae} was reported to make 100 times more poly-P than \textit{E. coli} makes in response to extracellular phosphate (25). The presence of large poly-P stores in \textit{V. cholerae} was explained by reduced expression of poly-P exophosphatase (3, 25). Surprisingly, despite the accumulation of large amounts of poly-P, the \textit{V. cholerae} ppk mutant exhibited very few altered phenotypes compared to ppk mutants of \textit{E. coli}, \textit{Salmonella}, and \textit{Pseudomonas} (25). This raises an intriguing question. Why does \textit{V. cholerae} make such large amounts of poly-P?

In the present study we addressed this question by constructing a \textit{V. cholerae} mutant with a deletion in the ppk gene. The ppk mutant was significantly more sensitive to low pH, salinity, or \textit{H}_{2}\text{O}_{2} in minimal medium with a low phosphate content. In this study we investigated the mechanism of this phenomenon, and below we discuss the potential significance of this finding to the ecology of cholaera.

**Materials and Methods**

**Strains and media.** \textit{V. cholerae} C6709-1 (El Tor, Peru, 1991 isolate) was used as the wild-type strain to construct a ppk mutant. \textit{V. cholerae} AC-W66 (32, 34) and ABJ41 (33) are lacZ- and relA-defective derivatives of C6709-1, respectively. Strain ABJ2 contains a hapA lacZ fusion integrated into the chromosome of AC-W66 (32). E. coli strains TOP10 (Invitrogen), SEY327, and SM40plasmid (24) were grown in LB medium. \textit{V. cholerae} was grown in LB medium or in MOPS (mopholinepropanesulfonic acid) minimal medium (Tecnovka Laboratories) containing 0.4% D-glucose (MOPS-G medium) supplemented with 2 mM or 0.1 mM inorganic phosphate (K_{2}HPO_{4}).

**Construction and analysis of a \textit{V. cholerae} ppk deletion mutant.** Chromosomal DNA was purified from \textit{V. cholerae} C6709-1 using a QIAGEN DNeasy kit. A DNA fragment containing sequences 5'- of the ppk open reading frame (ORF) was amplified with primers 5'-AAAAGCATGCAAGCGAATACTACGGTC and 5'-TGTTGATCCGCTTCTGTAGATCG using an Advantage 2 PCR kit (BD Biosciences Clontech). A second fragment containing sequences immediately 3'- of the ppk ORF was amplified using primers 5'-GAAGAGTCCAGAAAGATCCGATTGCT and 5'-GGCGATGGCTCCACCGATGGAAT. The amplicons were sequentially cloned in pUC19 as Sacl-BamH1 and BamH1-Sphl fragments and were confirmed by DNA sequencing. A SacI-SphI fragment containing chromosomal sequences flanking the ppk gene was subcloned in pCVD442 (8) digested with the enzymes mentioned above. The resulting suicide vector was constructed in SEY327pir, transferred to strain SM10 constructed in SEY327pir, and mobilized to \textit{E. coli} \textit{pir}. The suicide vector was introduced into the \textit{V. cholerae} C6709-1 using a QIAGEN DNeasy kit. The amplicon was cloned in pUC19 and subsequently sequenced. A SacI-SphI fragment containing the lacZ locus was amplified from strain ABJ37 using primers 5'-ATGCGTGATGGTATGATGGCCTCCA and 5'-GGATTCGCCGCAAGATATTACTG. The amplicon was cloned in pCVD442 and sequenced using M13 forward and reverse primers.

**Virulence studies.** Cholera toxin contents were measured by a GM1 enzyme-linked immunosorbent assay as described previously (31). Production of HA/ protease was detected using an azoceain assay (5, 13) and was expressed in azoceain units per unit of optical density at 600 nm (OD_{600}) of the culture. For motility, strains were stabbed into LB medium containing 0.3% agar (swarm agar). Biofilm formation was measured by the crystal violet staining method (39). To compare the abilities of our wild type and the ppk mutant to colonize the suckling mouse intestine, mixtures (1:1) of C6709-1 and AC-W66 and of ABJ37 and AC-W66 were orally inoculated into 4- to 5-day-old CD-1 mice. After 16 h of incubation mice were sacrificed by cervical dislocation, and the small intestine was homogenized in phosphate-buffered saline (pH 7.4) and plated on LB agar containing X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) (20 mg/ ml). The competitive index was calculated from the ratio of the mutant to the wild type after intraintestinal growth divided by the input ratio.

**Measurement of inorganic phosphate uptake.** Phosphate uptake was measured as described by Ogawa et al. (25). Briefly, \textit{V. cholerae} was grown in LB medium for 24 h at 37°C, and the cells were collected by centrifugation, washed with phosphate-free MOPS-G minimal medium, and resuspended in 5 ml of the same medium at an OD_{600} of 0.1. The cells were then incubated for 2 h at 37°C in a rotary shaker (250 rpm). Carrier-free \textit{32P}-labeled K_{2}HPO_{4} (Amersham Biosciences) was added to a specific activity of 20 μCi/ml, and the preparation was incubated as described above. Samples (0.1 ml) were taken at different times, and the cells were collected on 0.45-μm Costar Spin-X centrifuge tube filters (Corning Inc.). The filters were washed three times with phosphate-free MOPS-G medium, and the radioactivity was measured by a Beckman Coulter LS 6500 scintillation counter.

**Measurement of poly-P biosynthesis.** Poly-P biosynthesis was assessed by measuring the incorporation of \textit{32P} into high-molecular-weight poly-P, binding to glassmilk (Qibiogene, California), and thin-layer chromatography (TLC) (4). \textit{V. cholerae} was grown in LB medium for 24 h at 37°C, and cells were collected by centrifugation, resuspended in MOPS-G medium containing 0.1 m{}^{3}{}^{-1}{} P phosphate and 20 μCi/ml of carrier-free K_{2}HPO_{4}, and incubated for 4 h at 37°C as described above. After incubation, aliquots (1 ml) of cell suspension were centrifuged and extracted with 0.5 ml of guanidine isothiocyanate in 50 mM Tris (pH 7.0) at 95°C for 5 min. The extract was then treated with 15 μl of 20% sodium dodecyl sulfate (SDS), 0.5 ml of 95% ethanol, and 5 μl of glassmilk. The tubes were incubated for 1 min with vortexing and centrifuged to pellet the glassmilk. Next, the glassmilk was washed twice with 5 ml M Tris (pH 7.4) to 50 mM NaCl—5 mM EDTA—50% ethanol (wash buffer), resuspended in 50 μl of 50 mM Tris (pH 7.4)—10 mM MgCl_{2}, containing 20 μg/ml of DNase and RNase, and incubated for 15 min at 37°C. The glassmilk was subsequently washed once with 150 μl of guanidine isothiocyanate, then with 95% ethanol, and finally twice with wash buffer. Poly-P was eluted from the glassmilk with 50 ml of 50 mM Tris (pH 8.0) at 95°C for 2 min. Five microliters of the poly-P solution was applied to polyethyleneimine-cellulose F TLC plates (EMD Chemicals, Inc.) and developed with 0.75 M KH_{2}PO_{4} (pH 3.5). The plates were air dried, and high-molecular-weight poly-P was visualized by autoradiography. In this assay, high-molecular-weight poly-P stayed at the origin.

**Alkaline phosphatase and catalase assays.** Alkaline phosphatase was detected as described by Manoil and Beckwith (21) using p-nitrophenol phosphate as the substrate. Briefly, cells were diluted in 50 mM Tris (pH 8.0) and permeabilized with SDS-chloroform. The reaction was started by addition of substrate and was stopped by addition of 0.5 ml of 0.1 M K_{2}HPO_{4}. Optical densities at 420 nm were recorded, and Miller units were calculated as described elsewhere (22). To measure catalase activity, \textit{V. cholerae} cells were grown in MOPS-G minimal medium containing 2 mM or 0.1 mM inorganic phosphate, and samples (5 ml) were collected at different times. Cells were collected and lysed by 0.2 ml of Celllytic B cell lysis reagent (Sigma Chemical Co.). Catalase activity was determined with a catalase assay kit (Sigma Chemical Co.) and was expressed in micromoles of H_{2}O_{2} decomposed per minute per milligram of protein.

**Western blot analysis.** Cells grown in MOPS-G medium containing 0.1 mM phosphate were collected by centrifugation and lysed with SDS-chloroform. The reaction was started by addition of substrate and was stopped by addition of 0.5 ml of 0.1 M K_{2}HPO_{4}. Optical densities at 420 nm were recorded, and Miller units were calculated as described elsewhere (22). To measure catalase activity, \textit{V. cholerae} cells were grown in MOPS-G minimal medium containing 2 mM or 0.1 mM inorganic phosphate, and samples (5 ml) were collected at different times. Cells were collected and lysed by 0.2 ml of Celllytic B cell lysis reagent (Sigma Chemical Co.). Catalase activity was determined with a catalase assay kit (Sigma Chemical Co.) and was expressed in micromoles of H_{2}O_{2} decomposed per minute per milligram of protein.

**Measurement of ATP biosynthesis.** ATP biosynthesis was measured using the firefly luciferase-based BacTiter-Glo microbial cell viability assay (Promega Corporation). Light production was standardized on the basis of the number of CFU in the reaction mixture.

**Growth curves and survival assays.** Growth curves were constructed by measuring OD_{600} and dilution plating in LB agar. Long-term survival experiments were performed by dilution plating \textit{V. cholerae} cells after prolonged incubation at 37°C in LB medium or artificial seawater (ASW) basal salts. For pH stress, salinity stress, and oxidative stress, wild-type and mutant \textit{V. cholerae} strains were grown for 24 h in LB or MOPS-G medium containing 2 mM phosphate. The cells were centrifuged, washed, and resuspended in 1 volume of the appropriate medium in the presence of specific environmental stressors. At different times, viability was assessed by dilution plating on LB agar.

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RESULTS

Construction of a *V. cholerae* Δppk deletion mutant. A standard approach was used to generate *ppk* mutant AJB37 from strain C6709-1. Southern hybridization showed that AJB37 harbored a deletion that removed a unique EcoRI site located within the *ppk* ORF (Fig. 1, left panel). To confirm deletion of the *ppk* gene, we cloned a DNA fragment from AJB37 using primers flanking the *ppk* locus. Sequencing of the cloned DNA confirmed that 1,839 bp was deleted, which removed 87% of the *ppk* ORF. Figure 1 (right panel) shows that the *ppk* mutant was defective in poly-P biosynthesis. In order to confirm that the material which remained at the origin of the TLC plate was poly-P, a plasmid overexpressing PPK (pBC29) (3) (obtained from C. Fraley, Stanford University School of Medicine) was electroporated into strain AJB37. The complemented strain produced a large amount of poly-P that was visible as a strong signal at the TLC plate origin (Fig. 1).

*V. cholerae* Δppk mutant is fully virulent. In an initial study of our *ppk* mutant we examined production of cholera toxin, motility, biofilm formation, HA/protease production, and colonization of the suckling mouse intestine. Strain AJB37 produced levels of cholera toxin (2.1 μg/ml/unit of OD_{600}) similar to the levels produced by its wild type precursor (1.5 μg/ml/unit of OD_{600}). No difference in the abilities of the wild type and the mutant to swarm away from the inoculation site was demonstrated by the motility assay (data not shown). Consistent with the observation that both strains were motile, there were not significant differences in biofilm formation (expressed as OD_{570}/OD_{660}) between the *ppk* mutant (3.0 ± 0.1) and its wild-type precursor (3.9 ± 0.6) at 24 h. Furthermore, there was no difference in production of RpoS-dependent HA/protease between the *ppk* mutant (17.4 ± 0.6 azocasein units/unit of OD_{600}) and its wild-type precursor (16.5 ± 0.5 azocasein units/unit of OD_{600}). Finally, the growth of strain C6709-1 (*lacZ* positive) in the suckling mouse intestine and the growth of strain AJB37 (*lacZ* positive) in the suckling mouse intestine were compared to the growth of strain AC-W66 (*lacZ* negative) in the suckling mouse intestine. Strains C6709-1 and AJB37 showed competitive indices of 1 and 1.9, respectively, compared to AC-W66. These results indicate that poly-P stores are not required for the expression of virulence and for colonization of the suckling mouse intestine.

*V. cholerae* ppk mutant AJB37 is not impaired in starvation survival. There were no differences in the specific growth rate between the wild-type strain and its *ppk* mutant in LB medium or MOPS-G minimal medium containing 2 mM phosphate (data not shown). However, overexpression of poly-P from plasmid pBC29 significantly reduced the growth rates of C6709-1 and AJB37 (data not shown), suggesting that increasing the level of poly-P above its already high levels in *V. cholerae* could be deleterious. Additionally, we studied the survival of the *ppk* mutant in LB medium and ASW basal salts (see Fig. S1 in the supplemental material). The *ppk* mutant did not exhibit increased sensitivity to prolonged incubation in LB medium or ASW basal salts.

In *E. coli*, starvation conditions can also induce the stringent response characterized by increased levels of ppGpp. Considering the capacity of PPK to synthesize ppGpp from GDP and poly-P (19), we examined our *ppk* mutant to determine whether it had a *relA* (relaxed) phenotype. *V. cholerae* relA mutants are sensitive to the histidine analog 1,2,4-triazole due to their inability to derepress histidine biosynthetic genes (33). Strain C6709-1 and *ppk* mutant AJB37 were resistant to 1,2,4-triazole compared to the isogenic *relA* mutant AJB41 (see Fig. S2 in the supplemental material). This result indicates that a lack of PPK activity does not significantly impact the ability of *V. cholerae* to synthesize ppGpp.

Sensitivity of *ppk* mutant to environmental stressors in different culture media. Next, we studied the sensitivities of the wild-type strain and its isogenic *ppk* mutant to low pH, high salinity, and H₂O₂ in different culture media. As shown in Fig. 2, no differences between the wild type and the mutant were found in LB medium or MOPS-G medium containing 2 mM phosphate. In striking contrast, the *ppk* mutant was significantly more sensitive to all these stresses in MOPS-G medium at a low phosphate concentration (0.1 mM) (Fig. 2). This result suggests that poly-P plays an important role in stress resistance under phosphate limitation conditions. Strain AJB37 containing the *ppk* gene on a plasmid grew poorly in minimal medium (data not shown), suggesting that overexpression of poly-P is deleterious. This prevented us from testing if the *ppk* gene provided in trans restored stress resistance in low-phosphate medium.
Mechanism of poly-P-mediated stress resistance. To explore the mechanism by which poly-P could enhance survival to stress in low-phosphate medium, we first examined whether the *ppk* mutant had a growth defect in MOPS-G medium at a low phosphate concentration. We found that there was no difference between the growth rates of strains C6709-1 and AJB37 (*ppk*) (see Fig. S3 in the supplemental material). The *V. cholerae* PhoBR regulatory system has recently been shown to work like the system in *E. coli* by controlling numerous genes involved in phosphate acquisition (35, 36). Since a previously constructed *ppk* mutant showed a slightly lower rate of phosphate uptake (25), we examined whether the phosphate incorporation of the wild type and the phosphate incorporation of our mutant differed. Again, no significant differences were
both strains are capable of inducing the levels of PhoA (alkaline phosphatase) activity, indicating that in addition, the wild type and the mutant expressed similar mental conditions (see Fig. S3 in the supplemental material). Observed for inorganic phosphate uptake under our experimental conditions (see Fig. S3 in the supplemental material).

In E. coli, phosphate starvation induces expression of rpoS by a mechanism different from the mechanism observed with carbon and nitrogen starvation (27). Therefore, it was conceivable that our ppk mutant could induce sufficient RpoS expression to survive starvation in LB medium or ASW salts but could not produce enough RpoS in low-phosphate medium. To test this possibility, we first confirmed expression of RpoS in MOPS-G medium by using the reporter strain AJB2 (32). Expression of the hapa-lacZ fusion in AJB2 is strictly dependent on RpoS (32). When cultures of AJB2 were diluted in MOPS-G medium containing 2 and 0.1 mM phosphate, the β-galactosidase activity steadily increased from 5 to 20 Miller units in both media over 4 h of incubation, indicating that there was RpoS expression. Next, we performed a Western blot experiment to compare production of RpoS in low-phosphate medium by strain C6709-1 and production of RpoS in low-phosphate medium by strain AJB37. As shown in Fig. 3, the production of RpoS protein by ppk mutant AJB37 in MOPS-G medium containing 0.1 mM phosphate was not affected. In fact, strain AJB37 appeared to make slightly more RpoS protein than the wild type made. In order to examine the possibility that the ppk mutant exhibited lower RpoS activity in phosphate-limited medium, we measured the relative increase in catalase activity after a phosphate downshift. It has been shown that V. cholerae RpoS induces catalase production (38). As shown in Fig. 4, transfer of wild-type strain C6709-1 to low-phosphate minimal medium resulted in a sharp increase in catalase specific activity. In contrast, significantly lower induction was observed for the ppk mutant (Fig. 4). These results suggest that the increased sensitivity of the ppk mutant to environmental stressors in phosphorus-limited medium could be a consequence of lower RpoS activity.

Since poly-P can be used to make ATP (1, 2), we tested the possibility that the ppk mutant had a defect in maintaining an adequate energy charge to cope with environmental stresses under conditions in which ATP could be derived mostly from intracellular reservoirs rather than from externally supplied phosphate. To this end, C6709-1 and AJB37 were grown to stationary phase in MOPS-G medium containing 2 mM phosphate, and the cells were resuspended in fresh MOPS-G medium containing either 2 mM or 0.1 mM phosphate. When C6709-1 was refreshed in minimal media containing the two phosphate concentrations, a peak level of ATP per cell was observed at 2 h, followed by a decrease to the initial basal level (Fig. 5). As in the stress response experiments (Fig. 2), the ppk mutant behaved like the wild type with 2 mM phosphate but the ATP levels did not increase with 0.1 mM phosphate (Fig. 5), conditions in which it exhibited increased sensitivity to low pH, salinity, and H₂O₂ (Fig. 2). This result suggests that under phosphate limitation conditions, a significant fraction of ATP is derived from poly-P stores. The ppk mutant appears to be incapable of maintaining an adequate energy charge to overcome environmental stresses in a low-phosphate environment.

**DISCUSSION**

The ability to make poly-P does not appear to affect the expression of RpoS in V. cholerae grown in rich or high-phosphate minimal medium. We reached this conclusion based on the findings that our ppk mutant made similar amounts of RpoS-dependent HA/protease and did not exhibit rpoS phenotypes unless it was grown in phosphate-limited medium. These results are in close agreement with a previous report showing that a V. cholerae ppk mutant did not exhibit phenotypes predicted from E. coli studies (25). In a previous study the motility of and biofilm formation by a V. cholerae ppk mutant were found to be slightly affected (25). We did not observe these effects in our ppk mutant, which was isolated from a V. cholerae El Tor biotype strain belonging to a different
The paucity of ppk-related phenotypes in \textit{V. cholerae} suggests that large amounts of poly-P are made in this bacterium only to be used under very specific conditions. Why has \textit{V. cholerae} evolved to make such large amounts of poly-P? Clearly, poly-P is not required for the expression of virulence and intestinal colonization. However, it has been proposed that \textit{V. cholerae} can survive outside the human host in estuaries and brackish waters (9–11, 29). It is well established that phosphorus and nitrogen play crucial roles in the ecology of aquatic ecosystems (6, 7). Phosphorus has been proposed to be the most common cause of eutrophication in freshwater lakes, reservoirs, streams, and the headwaters of estuaries, while nitrogen is believed to be the key mineral nutrient controlling primary production in the ocean (6, 7). Depending on the specific aquatic environment, both nitrogen and phosphorus could become limiting nutrients simultaneously or in a cyclic manner (6, 7). It is likely that in an environment in which phosphorus is limiting, bacteria capable of synthesizing large poly-P stores could have a competitive advantage. Salinity gradients have been recognized to be an important environmental stressor in aquatic ecosystems. Poly-P-defective mutant AJB37 was found to be remarkably sensitive to high NaCl concentrations (Fig. 2). It will be of interest to examine if more widespread inhabitants of aquatic ecosystems (e.g., \textit{V. cholerae} non-O1 and non-O139 and other members of the genus \textit{Vibrio}) have similarly large poly-P stores.

The major differences in poly-P metabolism between \textit{E. coli} and \textit{V. cholerae} could reflect the evolutionary adaptation of these organisms to different habitats. While \textit{E. coli} is a normal inhabitant of the lower gastrointestinal tract of humans and animals, long-term human carriage of \textit{V. cholerae} is very unusual. Consequently, \textit{V. cholerae} could have evolved to make more poly-P to resist longer exposure to phosphate-limited conditions outside the gastrointestinal tract.

In summary, many mechanisms that potentially enhance the survival of \textit{V. cholerae} outside the human host have been proposed. These mechanisms include the general stress response, formation of biofilm communities, association with phytoplankton and zooplankton, and a viable but not culturable
stage. In this paper we describe a novel mechanism: synthesis of large poly-P stores for ATP biosynthesis. Our results show that availability of a large poly-P high-energy phosphate deposition enhances the capacity of \textit{V. cholerae} to survive environmental stresses in a low-phosphate environment.

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