Microbial helpers allow cyanobacteria to thrive in ferruginous waters

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Running title: Metal-reducing bacteria improve cyanobacterial fitness in early oceans

Abstract

The Great Oxidation Event (GOE) was a rapid accumulation of oxygen (O_2) in the atmosphere as a result of the photosynthetic activity of cyanobacteria. This accumulation reflected the pervasiveness of O_2 on the planet's surface, indicating that cyanobacteria had become ecologically successful. The GOE must have required the emergence of several metabolic innovations, in particular those used for protection from reactive O_2 species (ROS), including superoxides (O·) and hydroperoxides (H_2O_2), which are byproducts of oxygenic photosynthesis. In Archean oceans, near-millimolar levels of Fe^{2+} would have reacted with H_2O_2, exacerbating oxidative damage further. It is thought that cyanobacteria must have not endured the ROS toxicity of Fe^{2+}-rich oceans. Yet, cyanobacteria colonized Archean oceans extensively enough to oxygenate the atmosphere. In modern oceans, catalase-lacking cyanobacteria can be protected by catalase-producing heterotrophs. Here we show experimentally that facultative metal-reducing helper heterotrophs increase the fitness of *Synechococcus* in high Fe^{2+} concentrations, expanding the upper bound for tolerable Fe^{2+} concentrations for cyanobacteria in the ocean. These results suggest that helper bacteria could have been important for the colonization of ancient ferruginous oceans and imply that analyses considering multiple evolutionary lineages may reveal metabolic strategies that were shared by co-evolving microbial populations.

Introduction

Earth's first biogeochemical cycles were driven by anaerobic microorganisms (Martin, Bryant, & Beatty, 2018; Weiss et al., 2016). At around 2.3 billion years ago (Ga), the Great Oxidation Event (GOE) resulted in the initial oxygenation of the atmosphere and surficial biosphere, which ultimately led to the modern dominance of aerobic organisms on Earth's surface (Bar-On, Phillips, & Milo, 2018; Luo et al., 2016). Although biological O_2 production by cyanobacteria was a prerequisite for the GOE (Haqq-Misra, Kasting, & Lee, 2011; Holland, 2002; Lee et al., 2016), oxygenic photosynthesis may have emerged hundreds of millions of years prior to the GOE, during the early Archean (Cardona, Sánchez-Baracaldo, Rutherford, & Larkum, 2019; Lalonde & Konhauser, 2015; Ossa Ossa et al., 2018; Planavsky et al., 2014). The delay between the emergence of biologically-produced O_2 and its accumulation in the atmosphere may have been modulated by geophysical drivers (Catling, Zahnle, & McKay, 2001; Holland, 2009; Lee et al., 2016), but may also reflect the time required for the emergence of several metabolic innovations and the ecological success of cyanobacteria (Blank & Sanchez-Baracaldo, 2010; Johnston, Wolfe-Simon, Pearson, & Knoll, 2009; Lyons, Reinhard, & Planavsky, 2014; Ozaki, Thompson, Simister, Crowe, & Reinhard, 2019). Understanding how cyanobacteria adapted to changing environmental conditions to colonize most of the Earth's surface is essential to understand the microbial ecology of the GOE.

Although the sunlit Archean oceans would have provided cyanobacteria with a large surface area for water oxidation compared to land (Chisholm, 2017; Lyons et al., 2014), high Fe^{2+} concentrations would have created a significant challenge due to its reaction with reactive O_2 species (ROS). Oxygenic photosynthesis produces ROS, namely superoxides (O·) and hydroperoxides (H_2O_2) (Mishra & Imlay, 2012). Hydrogen peroxide can penetrate cellular membranes more easily than O· and subsequently inflict widespread damage inside the cell (Klotz & Loewen, 2003). In Archean oceans, elevated levels of Fe^{2+} (Song et al., 2017) would

have reacted with H_2O_2 to produce highly toxic hydroxyl radicals, further exacerbating oxidative damage and delaying cyanobacterial colonization of the oceans (Mishra & Imlay, 2012; Shcolnick, Summerfield, Reytman, Sherman, & Keren, 2009; Swanner et al., 2015). If cyanobacteria were not equipped to resist ROS from oxygenic photosynthesis, what allowed them to colonize the ocean and ultimately oxygenate the atmosphere?

Cooperation between different microbial organisms may explain the apparent evolutionary conundrum of the absence of an essential trait in a population (Morris, Johnson, Szul, Keller, & Zinser, 2011; Morris, Lenski, & Zinser, 2012). Cooperation occurs when members of a community produce metabolically costly molecules ("public goods") that become available to neighboring members (Özkaya, Xavier, Dionisio, & Balbontín, 2017). Cooperation is common among cyanobacteria and proteobacteria (Christie-Oleza, Sousoni, Lloyd, Armengaud, & Scanlan, 2017; Zinser, 2018), whose intimate relationship is also evidenced by extensive horizontal gene transfer between the two lineages (Ben Said & Or, 2017; Braakman, Follows, & Chisholm, 2017; Cantine & Fournier, 2017; Cavalier-Smith, 2006a, 2006b; Christie-Oleza et al., 2017; Goldenfeld & Woese, 2011). Cooperation by "helper" organisms providing ROS protection may have also allowed early cyanobacteria to overcome ROS toxicity in Fe^{2+}-rich oceans (**Fig. 1A**). At the time of the GOE, several potential helper lineages had already diversified (Battistuzzi, Feijao, & Hedges, 2004), including proteobacteria (Cavalier-Smith, 2006a, 2006b), which would have provided many opportunities for cooperation.

Aerobic microbes use a variety of hydroperoxidases to inactivate H_2O_2 by reduction or dismutation to water (Mishra & Imlay, 2012; Zamocky, Furtmuller, & Obinger, 2008), the most widespread of which are catalases. Catalases first evolved in bacteria independently as heme and non-heme catalases, possibly before ~3.5 Ga (Klotz & Loewen, 2003; Zamocky et al., 2008). Among the heme catalases, monofunctional KatEs originated as clade 2 KatEs and later formed clade 1 and clade 3 (Klotz & Loewen, 2003). Modern proteobacteria can enhance the

growth of cyanobacteria by providing ROS protection by secreting catalase (Morris et al., 2011; Zinser, 2018). Extracellular catalases have mostly been found to be of the clade 3 KatE type (Hanaoka et al., 2013; Naclerio, Baccigalupi, Caruso, De Felice, & Ricca, 1995; Pezzoni, Pizarro, & Costa, 2014). We hypothesize that a similar microbial cooperation, in which a "helper" organism consumed O_2 or scavenged hydroperoxides, protected cyanobacteria from ROS toxicity in early, Fe^{2+}-rich oceans (**Fig. 1A**). Our hypothesis predicts that cyanobacteria in co-culture with proteobacteria should show increased fitness under ferruginous conditions compared to a cyanobacterial monoculture, and that proteobacteria with higher hydroperoxidase activity will have a more protective effect than those with lower activity.

Synthetic communities are a useful method to study ecological and geochemical dynamics of individual species within a community because the initial conditions can be precisely controlled (Widder et al., 2016). Synthetic communities are particularly useful for studies of phototrophic-driven consortia in simulated pre-GOE conditions that do not exist on Earth today (illuminated, eukaryotic-free, Fe^{2+}-rich, and anoxic). To test our hypothesis, we used synthetic consortia of model bacterial species consisting of one KatE-lacking cyanobacterium *Synechococcus* sp. PCC7002 (hereafter *Synechococcus*) and one of six different KatE-containing gammaproteobacterial helpers (metal-reducing *Shewanella*). *Synechococcus* sp. PCC7002 has been used as a model cyanobacteria to understand modern and past phototrophic metabolisms (Alexander S. Beliaev et al., 2014; Bryant & Frigaard, 2006; Swanner et al., 2015). *Shewanella* spp. are facultative anaerobes that can survive O_2 intrusions in the presence of high Fe^{2+} concentrations (Sekar, Shin, & DiChristina, 2016) and contain variable numbers and types of KatEs. Co-cultures were grown under parameters simulating a pre-GOE, Fe^{2+}-rich shallow continental margin overlain by a CO_2- and H_2-rich anoxic atmosphere (**Fig. 1**). The relative fitness of cyanobacterial cultures was compared with and without the inclusion of gammaproteobacterial helpers. We found that some *Shewanella* species allowed

Synechococcus to grow in Fe^{2+}-rich conditions that precluded growth of *Synechococcus* monocultures. Our findings stress the importance of considering microbial cooperation and complementary genetic repertoires in models of early Earth ecology leading to the GOE.

Results

Impaired growth of KatE-lacking cyanobacteria in high Fe^{2+} is restored in the presence of KatE-containing proteobacteria. *Synechococcus* was grown with 25 μM (low Fe^{2+}), 500 μM Fe^{2+}, or 1000 μM Fe^{2+} (high iron) under an anoxic atmosphere**.** *Synechococcus* reached highest cell densities (~2.7×10^7 cells mL^{-1}) at 25 μM Fe^{2+} **(Fig. 2)**. At 500 μM Fe^{2+}, *Synechococcus* showed an increased lag period (2 days, **Fig. S1B**) and similar growth rates **(Fig. S2)**. Growth was significantly impaired at 1,000 μM Fe^{2+} (~20% of the yields under low-Fe^{2+} conditions**; Fig. 2**). When *Synechococcus* was grown in co-culture with different *Shewanella* strains at 1000 μM Fe^{2+}, growth yields increased 30-100% compared to yields from co-cultures with low Fe^{2+} (**Fig. 2**). At low levels of Fe^{2+}, the presence of *Shewanella* generally hindered *Synechococcus* growth, as evidenced by a longer lag phase of 2-4 days in co-culture with 25 μM Fe^{2+} compared to 0.5 days in monoculture (**Fig. S1A),** and 3-4 days in co-culture with 500 μM Fe^{2+} compared to 2 days in monoculture (**Fig. S1B**). Among the strains tested, *Shewanella algae* MN-01 and *Shewanella baltica* OS155 had the most positive effect on the growth rates and yields of *Synechococcus*. *Shewanella oneidensis* MR-1 had a lesser positive effect. By the end of all of the incubations, *Synechococcus* was the only strain remaining in the cultures (**Fig. S3**). Iron levels remained close to initial concentrations only in co-cultures and decreased by the second day in *Synechococcus* monocultures (**Fig. S4**).

Proteobacteria with higher catalase activity are better cyanobacterial helpers. Higher growth yields and stable Fe^{2+} levels in co-cultures with high Fe^{2+} suggested that the mechanism for protection could be due to O_2 or H_2O_2 scavenging. In order to test whether *Shewanella* partners protected cyanobacteria by scavenging O_2 or H_2O_2, we measured the maximum O_2

respiration rate and H_2O_2 scavenging rate in cell suspensions of the most and least "helpful" helper strains. *S. algae* MN-01 and *Shewanella* sp. BrY had higher O_2 respiration rates than *S. baltica* and *S. oneidensis* MR-1 (**Fig. 3A**). *S. algae* MN-01 showed the highest H_2O_2 scavenging capacity, followed by *S. baltica*, while *Shewanella* sp. BrY and *S. oneidensis* MR-1 showed similar lower rates (25-40% of the other two strains; **Fig. 3B**). These differences were also reflected in the different growth rates of *Shewanella* strains upon exposure to increasing concentrations of H_2O_2 (**Fig. S5**). No differences were detected at the amino acid level that would explain these differences (**Fig. S6**). Final growth yields in *Synechococcus* co-cultures were proportional to the H_2O_2 scavenging activity of each *Shewanella* strain (**Fig. 3C**).

Distribution of KatE catalases in Cyanobacteria suggests link with cell morphology and salinity. The well-known extracellular role of KatE on scavenging extracellular H_2O_2 (Naclerio et al., 1995; Shin, Choi, & Cho, 2008), the presence of KatE in metal-reducing strains, and the link between higher rates of extracellular H_2O_2 scavenging and growth of cyanobacteria in pre-GOE conditions is suggestive of an important ecological role of KatE in catalase-based interactions, which are thought to be prevalent in the environment (Zinser, 2018). KatE was absent in our model species, *Synechococcus* spp. PCC7002. Out of 2,886 cyanobacterial genomes, only 135 (~5%) contained a KatE homolog. Of those with assigned taxonomy, the vast majority were Nostocales from freshwater or soils (**Table S1**). A small minority were Synechococcales, Chroococcales, or Oscillatoriales from marine or high-salt environments.

We created a phylogeny of bacterial KatEs to determine the evolutionary history of this gene in cyanobacteria. Almost all of the cyanobacterial clade 3 KatEs grouped together and branched off of gammaproteobacterial KatEs (**Fig. 4**). Multiple phylogenetic models confirmed this result (not shown). The tree topology of KatE is incongruent with the previously established history of bacteria, where Cyanobacteria are ancestral to Proteobacteria (Gupta, 2000; Marin,

Battistuzzi, Brown, & Hedges, 2017), suggesting later horizontal gene transfer of *katE* genes to cyanobacteria.

Among the gammaproteobacterial KatE genes, KatEs formed three distinct groups. The first group branched off earlier than the rest of the gammaproteobacterial KatEs and grouped more closely to KatEs from Flavobacteriia, Deinococci, and Clostridia. This group contained KatEs experimentally verified to be extracellular from *Bacillus subtilis*, *P. aeruginosa* PAO1, *Exiguobacterium oxidotolerans*, and *Alteromonas macleodii* (Hanaoka et al., 2013; Morris et al., 2011; Naclerio et al., 1995; Pezzoni et al., 2014). The second group contained other Alteromonadales, including iron-reducing *Shewanella* and *Ferrimonas* species. The third group contained the majority of the sequences analyzed and also grouped together with proteobacterial KatEs of the alpha, beta, and delta classes. Unlike the fragmented distribution of KatEs among Gammaproteobacteria, the rest of the KatEs from other taxonomic groups clustered together.

Discussion

The fitness of cyanobacteria increases in the presence of helper proteobacteria under pre-GOE conditions*.* In our experiments, axenic growth of *Synechococcus* was impaired at high Fe^{2+} concentrations but was reestablished in the presence of *Shewanella*. These results provide empirical evidence that ecological interactions between members of different species with phenotypes relevant to the onset of the GOE can provide means to overcome Archean levels of Fe^{2+}-induced ROS stress. Our findings underscore the need to consider ecological interactions between members of different species in the reconstruction of our microbial past and Earth's biogeochemical cycles.

Reconstructions of the evolutionary history of oxygenic phototrophs have revealed a variety of functional and morphological innovations that likely contributed to the GOE (Blank & Sanchez-Baracaldo, 2010; Chrismas, Barker, Anesio, & Sánchez-Baracaldo, 2016; Di Cesare et

al., 2018; Hohmann-Marriott & Blankenship, 2011; Sanchez-Baracaldo, Bianchini, Di Cesare, Callieri, & Chrismas, 2019). Expanding analyses to include organisms other than cyanobacterial lineages, but important to their ecological success, can provide new explanations for the interpretation of the geochemical record. For instance, lack of cyanobacteria in modern environments containing above ~80 μM Fe^{2+} and lack of cyanobacterial growth in laboratory culture above ~200 μM Fe^{2+} have contributed to the idea that Fe^{2+}-rich oceans would have slowed down the colonization of the ocean surface due to toxicity, possibly delaying global oxygenation (Swanner et al., 2015). However, based on our data, catalase-producing, iron-reducing bacteria could have augmented the ability of cyanobacteria to respond to Fe^{2+} toxicity while keeping Fe^{2+} levels constant, presumably due to a combination of ROS scavenging and O_2 respiration. Including facultative metal-reducing bacteria in models would attribute the delay in oxygenation to cryptic oxygen cycling (Garcia-Robledo et al., 2017) and reduced levels of Fe^{2+}-catalyzed ROS, conditions that would have allowed cyanobacteria to grow in a largely anoxic ocean during the GOE's "lag phase".

Under our experimental conditions, the beneficial role of *Shewanella* to *Synechococcus* was ultimately transient. This may be a natural consequence of the dynamics of public goods in cooperative interactions between microbial groups (Corno, Villiger, & Pernthaler, 2013; Rodríguez Amor & Dal Bello, 2019), when the members of the population that provide the extracellular "public good" are at a fitness disadvantage by providing a costly product that nonproducers use and benefit from (Cremer et al., 2019; Özkaya et al., 2017). In our system, *Synechococcus* gained the fitness advantage of ROS protection at the expense of *Shewanella*, whose population was eliminated from the system possibly due to limiting organic carbon (A. S. Beliaev et al., 2014), as *Synechococcus* grew in numbers.

The transient partnership between *Synechococcus* and *Shewanella* in our cultures may reflect that the conditions in which cyanobacteria and proteobacteria grow once overlapped (illuminated, anoxic, and Fe^{2+}-rich), but might have changed as Earth became oxidizing enough

to reduce the pool of free Fe^{2+}, leading to spatial separation of these populations. This scenario is consistent with the different ecological niches that *Shewanella* and *Synechococcus* occupy, and the different environments in which they are found. While *Synechococcus* dominates the open ocean surface today (Flombaum et al., 2013), *Shewanella* species have been isolated mostly from dark and organic-rich environments such as sediments or inside the gut of animals (Hau & Gralnick, 2007; Konstantinidis et al., 2009). An association with ROS-scavenging proteobacteria would have been beneficial to cyanobacteria only during the initial stages of the evolution of oxygenic photosynthesis and colonization of the ocean (**Fig. 5**). It is possible that a close interaction could have been sustained in Archean oceans as long as both populations were maintained near our initial concentrations (10^5 cells mL^{-1}).

Differences in fitness effects can be explained by catalase activity and ROS survival capacity of helper partners. Although the six *Shewanella* strains we examined have similar ecological niches, we noted substantial differences in their effect on *Synechococcus'* growth. Strains that scavenged H_2O_2 faster resulted in higher cyanobacterial yields. Genomic sequences of catalase genes, however, did not explain the phenotypic difference observed. These differences may be explained by higher catalase expression levels in strains that are more resistant to ROS stress (Wang et al., 2009). These results support the idea that genomic information alone may not be enough to understand the interplay between microbial community members and their ecological role (M. Lee et al., 2017).

Microbes are thought to have developed resistance to ROS after the GOE, which allowed them to colonize oxic environments (Hamilton, Bryant, & Macalady, 2016). Yet genomic and biochemical evidence suggest that catalases could have evolved earlier, in the Archean (Klotz & Loewen, 2003; Olson et al., 2017). In an Archean ferruginous environment, Fe^{2+}-driven ROS production could have imposed a selection pressure strong enough for the evolution of catalases, much earlier than the oxygenation of the atmosphere and the ecological expansion of

aerobic life. The selection pressure for the evolution of catalases could have been stronger in microbes other than oxygenic cyanobacteria. In fact, the evolution of catalases is thought to have evolved initially in proteobacteria (Klotz & Loewen, 2003). The evolutionary pressure to evolve an ROS scavenging system would have been strong in iron-cycling organisms. Among the metal-reducing bacteria, Gammaproteobacteria are the most widespread facultative aerobes. Due to the high reactivity of Fe^{2+} with hydroperoxides, the iron-rich, multi-heme cytochromes involved in extracellular iron respiration in proteobacteria (Shi et al., 2016) could become targets of extracellular H_2O_2 toxicity. Modern facultative anaerobic metal-reducing bacteria, often found at oxic/anoxic transition zones, may experience strong selection pressure to retain outer membrane or extracellular H_2O_2 scavenging enzymes. The association between catalases and Fe^{2+}-enhanced ROS toxicity is consistent with genomic correlation between the presence of catalase in *Shewanella* species and their ability to reduce metals (**Fig. S7**); for example, *S. denitrificans* is the only *Shewanella* species that lacks *katE* genes and is also the only one unable that reduce metals, due to its lack of heme-rich extracellular electron conduits.

The distribution pattern of clade 3 KatEs in cyanobacteria suggests that extracellular KatEs providing ROS protection may be phylogenetically distinct and adapted to marine conditions. In the modern open ocean, the majority of the nitrogen fixers are filamentous non-heterocyst-forming *Trichodesmium*, heterocyst-forming eukaryotic symbionts *Richelia*, *Calothrix,* and relatives, or unicellular *Crocosphaera* and *Cyanothece,* yet none of the sequenced *Trichodesmium*, *Richelia,* and *Crocosphaera* genomes, and only 9 out of the 66 *Calothrix* genomes, contain KatE (**Table S2**). *Trichodesmium* is usually found in association with *A. macleodii,* which provides protection from ROS (M. Lee et al., 2017). *Trichodesmium* epibionts also include Piscirickettsiaceae (Gradoville, Crump, Letelier, Church, & White, 2017) whose KatEs grouped in the second gammaproteobacterial clade together with Alteromonadales and separate from most other Gammaproteobacteria (**Fig. 4**). Similarly, *A. macleodii* provides catalase as a “public good” to marine *Prochlorococcus* (Morris et al., 2011)

and their KatEs grouped in the first cluster. Alongside nutrient scarcity (Christie-Oleza et al., 2017; Gradoville et al., 2017), protection from toxic byproducts such as ROS toxicity can thus also be an important cooperative ecological mechanism between cyanobacteria and non-phototrophic helpers in modern marine environments (Zinser, 2018), or oceans on early Earth.

The *Prochlorococcus-Alteromonas* interaction via catalase has been used as a case study to explain reductive evolution (Morris et al., 2012). The history of KatE, however, might have had a different evolutionary path. The modern pattern of low KatE incidence in cyanobacteria can be explained by clade 3 KatE originating in proteobacteria and later transferred to a few cyanobacterial lineages (Klotz & Loewen, 2003; Zamocky et al., 2008) supporting a model in which a mutualistic association between cyanobacteria and proteobacteria based on ROS protection originated in the Archean under anoxic conditions (Olson et al., 2017).

This study expands the upper bound for tolerable Fe^{2+} concentrations for cyanobacteria in oceans. For around two billion years, the anoxic conditions of early Earth sustained abundant soluble Fe^{2+} in Archean seawater (Anbar et al., 2007; Hazen & Ferry, 2010; Holland, 2006; Johnson, Beard, & Roden, 2008; Reinhard et al., 2017). Fe^{2+} buffered by carbonates (siderite, $FeCO_3$) or phosphates (vivianite, $Fe_3(PO_4)_2 \bullet 8H_2O$) would have been present at up to ~100 uM to ~1 mM or more (Derry, 2015; Drever, 1974; Holland, 1973). Under these conditions, cyanobacteria might have not survived alone (Swanner et al., 2015). Recent metagenomic analyses suggest that oxygenic photosynthesis preceded aerobic respiration in cyanobacteria, which was acquired later by horizontal gene transfer (Cavalier-Smith, 2006b; Soo, Hemp, Parks, Fischer, & Hugenholtz, 2017), pointing to a close association with other microbial lineages that had already developed ROS resistance and respiration capacity, such as proteobacteria. Based on our study, the presence of other microbial lineages would have allowed cyanobacteria to

grow in Fe^{2+} concentrations close to the most recent estimates of Archean seawater levels (Derry, 2015).

Our results suggest that while the metabolic repertoire of different microbial lineages often provides us with patterns that reveal how life navigated alternative environmental landscapes, metabolic strategies can be shared among closely interacting microbial populations and thus critical evolutionary information may be stored in separate microbial members of a community. Therefore, analyses focused on only one evolutionary lineage may obscure metabolic strategies that were shared by co-evolving microbial populations. Considering microbial interactions in the establishment of novel biochemical pathways can open new windows through which to understand how the physiology of microbes evolved.

Materials and Methods

Strains used in this study. *Synechococcus spp.* PCC7002 was obtained from the Paris Culture Collection. *S. putrefaciens* strain 200, *S. oneidensis* MR-1, *Shewanella spp.* strain BrY were kind gifts from the lab of Dr. Thomas DiChristina (Georgia Institute of Technology). *S. denitrificans* and *S. loihica* were a kind gift from Dr. Jeffrey Gralnick (University of Minnesota). All strains were grown in lysogeny broth (LB, 10 g L^{-1} NaCl, 10 g L^{-1} tryptone, 5 g L^{-1} yeast extract, Sigma-Aldrich) or a modified A+ medium (Stevens Jr., Patterson, & Myers, 1973) with NH_4^+ (10 mM) instead of NO_3^- as carbon source and no iron(III), and additional amino acids L-serine, L-arginine and L-glutamic acid (20 mg L^{-1}) when used to culture *Shewanella* strains. The use of LB or modified A+ is specified in each one of the methods, where appropriate.

GOE-mimicking experimental setup and co-culture growth conditions. *Synechococcus* PCC 7002 was grown on modified A+ medium. To eliminate O_2 as much as possible at the start of the co-culture experiment, *Synechococcus PCC7002* sp. PCC 7002 was incubated in serum

bottles flushed for 10 min with N_2:CO_2 90:10 daily, and once before transferring to the anaerobic chamber. In parallel, *Shewanella* strains were transferred from an overnight culture in LB into anoxic bottles with minimal medium (flushed once with N_2:CO_2 90:10) with lactate as electron donor and fumarate as electron acceptor. After overnight growth, all bottles were opened inside the anoxic chamber (H_2:CO_2:N_2 4:5:91), and mixed at appropriate concentrations based on their OD600 to achieve a 1:1 ratio of OD 0.01 each. Co-cultures were grown in 10 mL in triplicate in 6-well tissue culture plates and homogenized daily by gentle pipetting ~50% of the volume up and down. Light was provided with a fluorescent light in a 12:12 light:dark timer-controlled cycle. $FeCl_2$ was added at 25, 500 or 1000 μM.

Analytical methods during co-culture. Cell numbers were quantified in a LSR Fortessa flow cytometer in a high-throughput manner using the software FACSDiva™ (BD Biosciences, CA) to set the parameters and monitor each quantification. At each time point, 200 μL of sample were loaded into a 96-well plate inside the anoxic chamber, covered in parafilm to minimize iron oxidation, and then transported to the cytometer. Each sample consisted of 10 μL taken for injection after mixing twice (100 μL mixing volume) and run at a rate of 0.5 μL s^{-1}. Cyanobacteria were detected by phycocyanin/chlorophyll autofluorescence using blue (488 nm) and yellow-green lasers (561nm) measured at 655-684 (Hill, Chrisler, Beliaev, & Bernstein, 2017). Optimization and calibration of the quantification parameters were achieved using yellow-green 1 μm microspheres (441/485 ex/em) (Polysciences, PA). Live cyanobacteria were also quantified using Syto9 using FITC filters. Events above the thresholds of PerCP and FITC were considered live cyanobacteria. Propidium iodide could not be used to identify “dead’ cyanobacteria, as the emission spectra overlapped with that of their autofluorescence. *Shewanella* cells could potentially overlap with iron particles in the cytometer, except for their complete absence in the sample. Iron was determined colorimetrically via the ferrozine method (Stookey, 1970), sampling inside the chamber into 0.5 M HCl-filled wells of a 96-well plate and

using a spectrophotometer with plate-reading capacity for quantification (Tecan, Switzerland). Oxygen was monitored using 3 mm tip diameter fiber-optic sensors (Robust Oxygen Sensors; Pyroscience, Germany).

Hydroperoxide resistance assays. Six *Shewanella* strains were incubated in 96-well plates with minimal media and lactate (10 mM) or acetate (10 mM) as electron donor under aerobic conditions. Growth was monitored continuously by OD at 600 nm in a spectrophotometer with plate-reading capacity (Tecan, Switzerland). Hydrogen peroxide was added after an initial period of growth for 4 hours, at a final concentration of 10, 25, 50, 100, or 250 µM.

Whole-cell extracellular hydroperoxide scavenging assays. To compare the abilities of the selected *Shewanella* strains to remove exogenous H_2O_2 from their surrounding environment, strains were first activated from the -80ºC freezer stock by incubating them at 30ºC overnight in lysogeny broth (LB, 10 g L^{-1} NaCl, 10 g L^{-1} tryptone, 5 g L^{-1} yeast extract, Sigma-Aldrich). Activated cultures were streaked onto LB plates (LB in 1.5% w/v agar, Sigma-Aldrich). Cultures were harvested by centrifugation at 3000 rpm, washed and transferred into minimal medium with lactate at an OD_{600} = 0.02. Cells were incubated at 30ºC until harvesting at mid-log phase (OD_{600} = 0.15-0.35), washed twice with minimal medium, then inoculated at OD_{600} = 0.05 into minimal medium amended with various concentrations of exogenous H_2O_2 (3-5000 µM). Samples were collected and analyzed for residual H_2O_2 every 3-5 minutes using the resorufin-horseradish peroxidase colorimetric assay. H_2O_2 disappearance followed an exponential decay [$H_2O_{2(t=n)} = H_2O_{2(t=0)} \times e^{-kt}$] and thus constants were calculated from the estimated curves.

Whole-cell O_2 consumption rates. Each *Shewanella* strain was grown in LB from an overnight tube of the same media until reaching early exponential growth (~0.3). Cells were then washed once and resuspended in LB at an OD at 600nm of 0.1 and flushed with air for 10 minutes. Immediately after, 15 mL of cells were transferred to a screw-top vial with contactless optical

sensors for O_2 to monitor O_2 consumption (TOVIAL20; Pyroscience, Germany). Flushed media was used as the abiotic control.

Catalase phylogeny. All KatE sequences used in this study were downloaded from the PATRIC database searching by taxonomic group. All KatE sequences available were chosen, but only sequences with more than 300 amino acids were kept for analysis. The cyanobacterial sequences types were identified by conserved domain analysis in NCBI. Phylogenies were constructed using MEGA X (Kumar, Stecher, Li, Knyaz, & Tamura, 2018). Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+*G*, parameter = 1.0309). The rate variation model allowed for some sites to be evolutionarily invariable ([+*I*], 8.13% sites). This analysis involved 115 amino acid sequences. All positions containing gaps and missing data were eliminated (complete deletion option). There was a total of 309 positions in the final dataset.

KatE amino acid sequence comparison. KatE amino acid sequences from strains *Shewanella algae* MN-01, *Shewanella* spp. BrY, *Shewanella oneidensis* MR-1, and *S. baltica* OS155 were aligned with MUSCLE in MEGAX (Kumar et al., 2018). Conserved residues in the alignment were color coded using BOXSHADE 3.2.3 (http://sourceforge.net/projects/boxshade/). Conserved sequences of the proteins' heme-binding, NADH-binding and tetramer-binding motifs as determined by NCBI's Conserved Domain search were manually inspected for residue differences.

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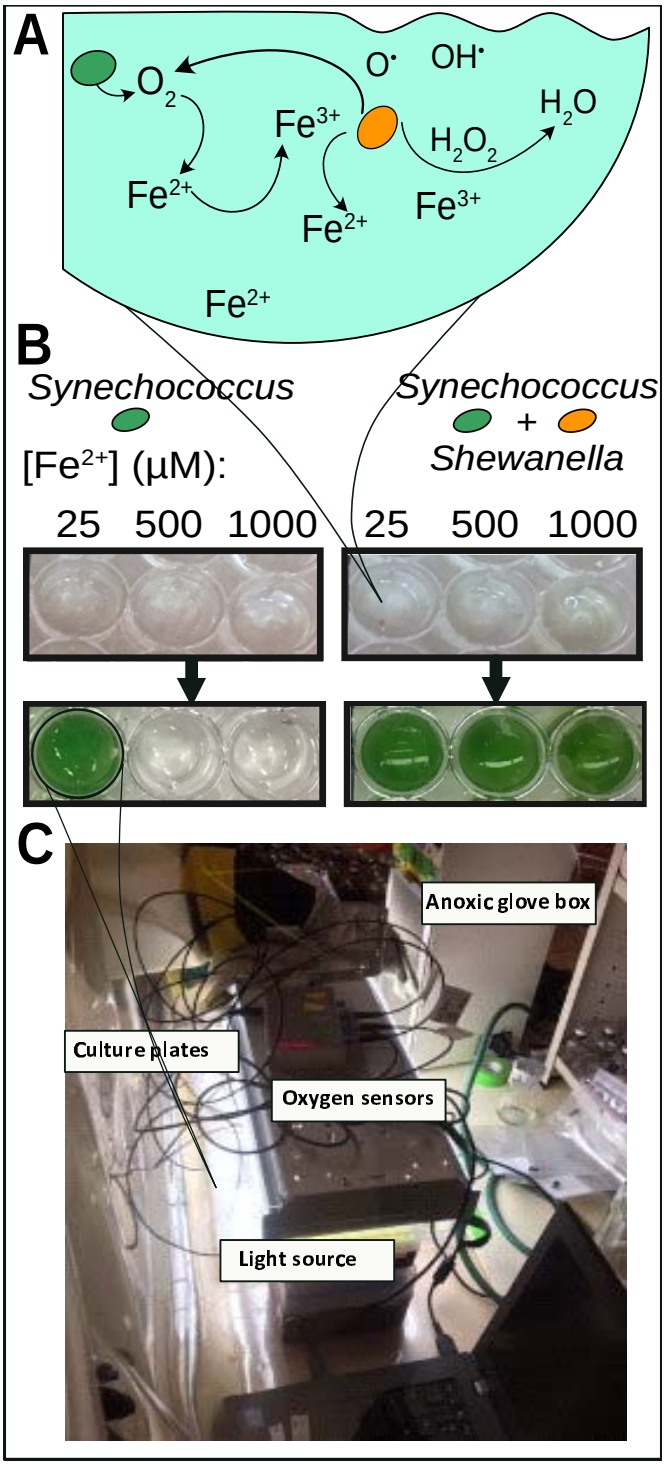

Figure 1. Experimental setup of synthetic communities in pre-GOE environments. A) Conceptual model of potential interactions of metal-reducing proteobacteria and cyanobacteria in Fe^{2+}-rich, anoxic liquid cultures; B) qualitative growth differences in representative culture plates of monocultured *Synechococcus* or co-cultured with *Shewanella;* C) experimental setup mimicking pre-GOE shallow water environments recreated by incubating synthetic communities in small-volume tissue culture wells inside an anoxic chamber.

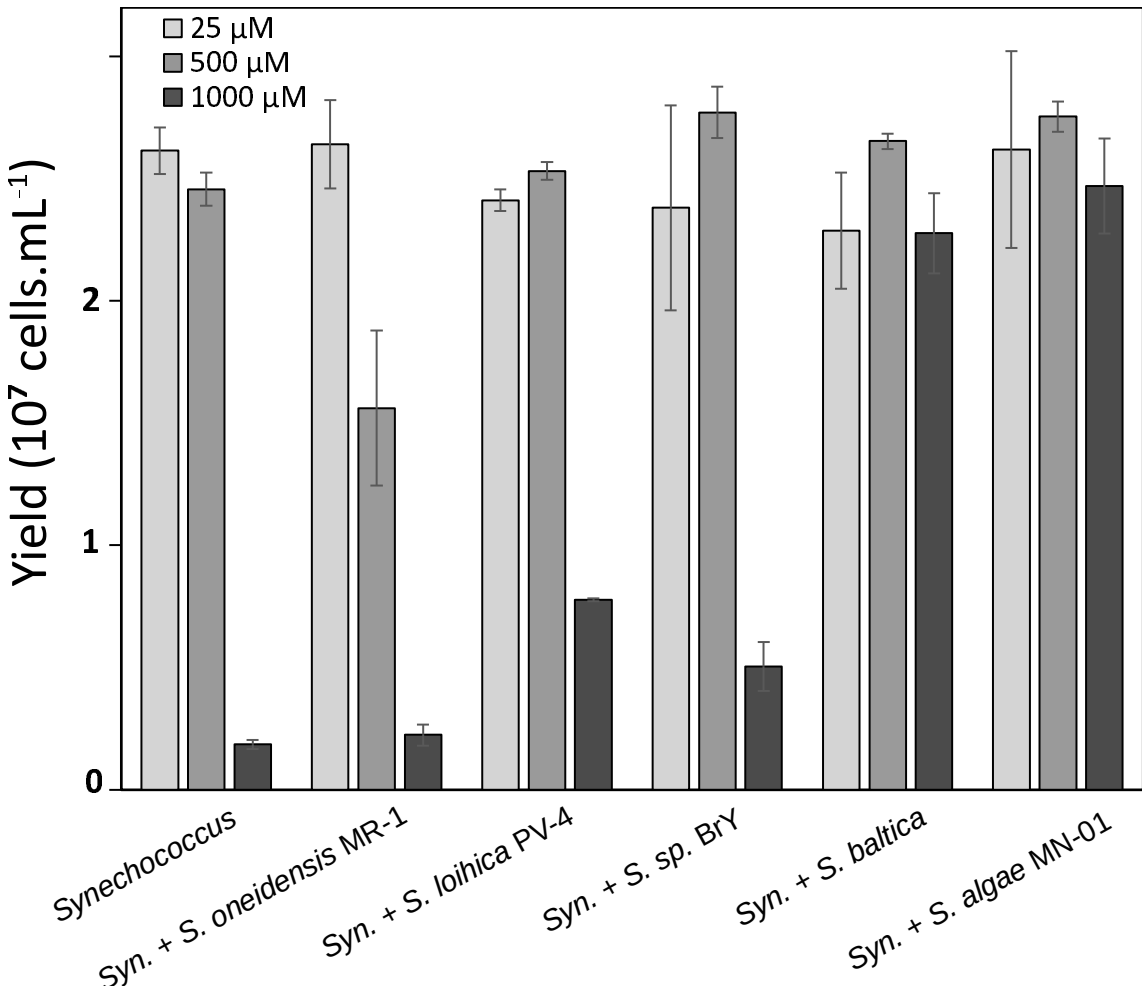

Figure 2. Growth yields of *Synechococcus* in mono- or co-culture in pre-GOE conditions. This bar graph shows the maximum *Synechococcus* growth yields alone or in co-culture with *Shewanella* with 25 µM (light gray), 500 µM (gray) or 1000 µM (dark gray) Fe^{2+}. Yields correspond to the highest number of cells observed during the growth period. Error bars represent the standard deviation of triplicate samples.

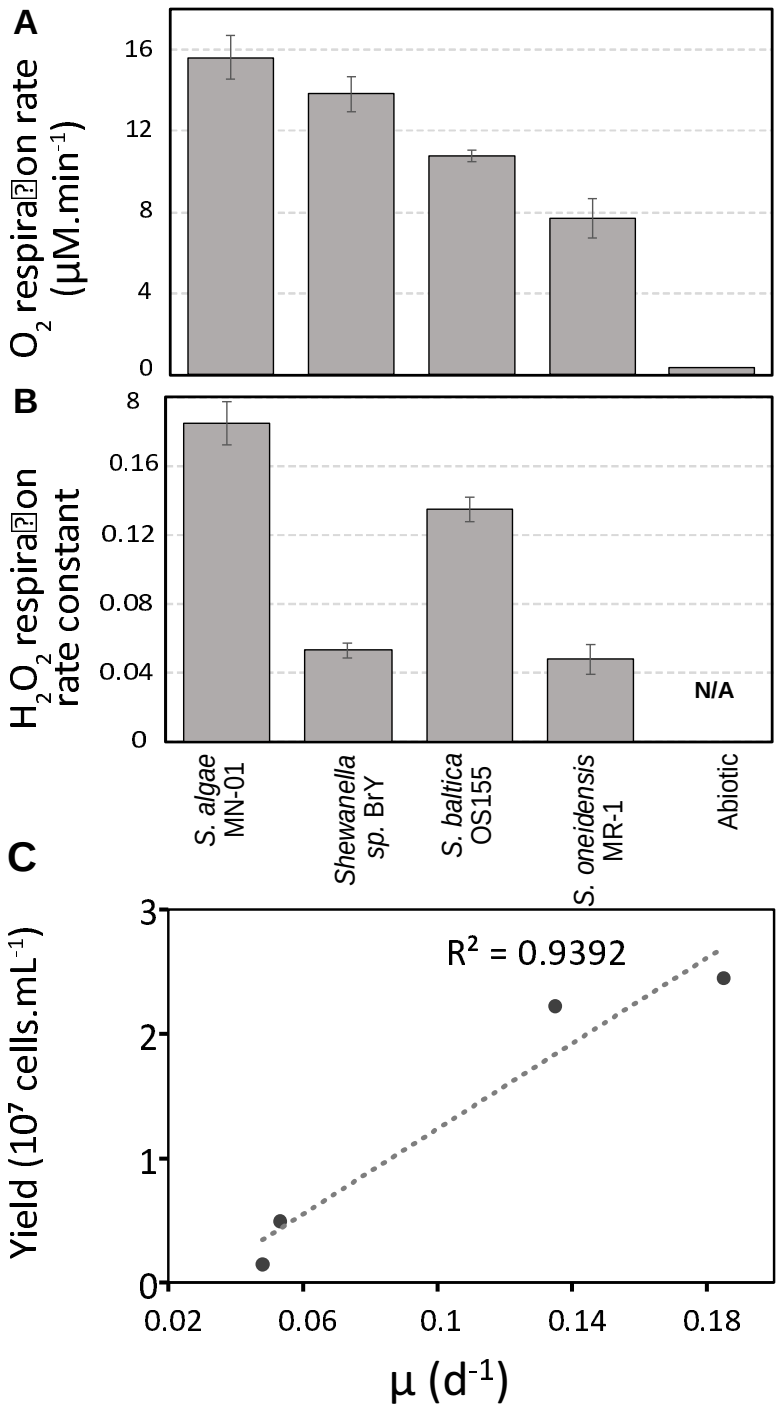

Figure 3. Oxygen and hydrogen peroxide scavenging capacity of *Shewanella* spp. Maximum (A) H_2O_2 scavenging and (B) O_2 utilization rates of *S. oneidensis* MR-1 (MR-1), *Shewanella* sp. BrY (BrY), *S. baltica* OS155 (Baltica), and *Shewanella algae* MN-01 (MN-01). (C) Correlation between the H_2O_2 scavenging rate of each *Shewanella* strain and the growth yield of *Synechococcus* in co-culture when incubated with that strain. Error bars represent standard errors of triplicate measurements. The abiotic control was set up identically but not inoculated.

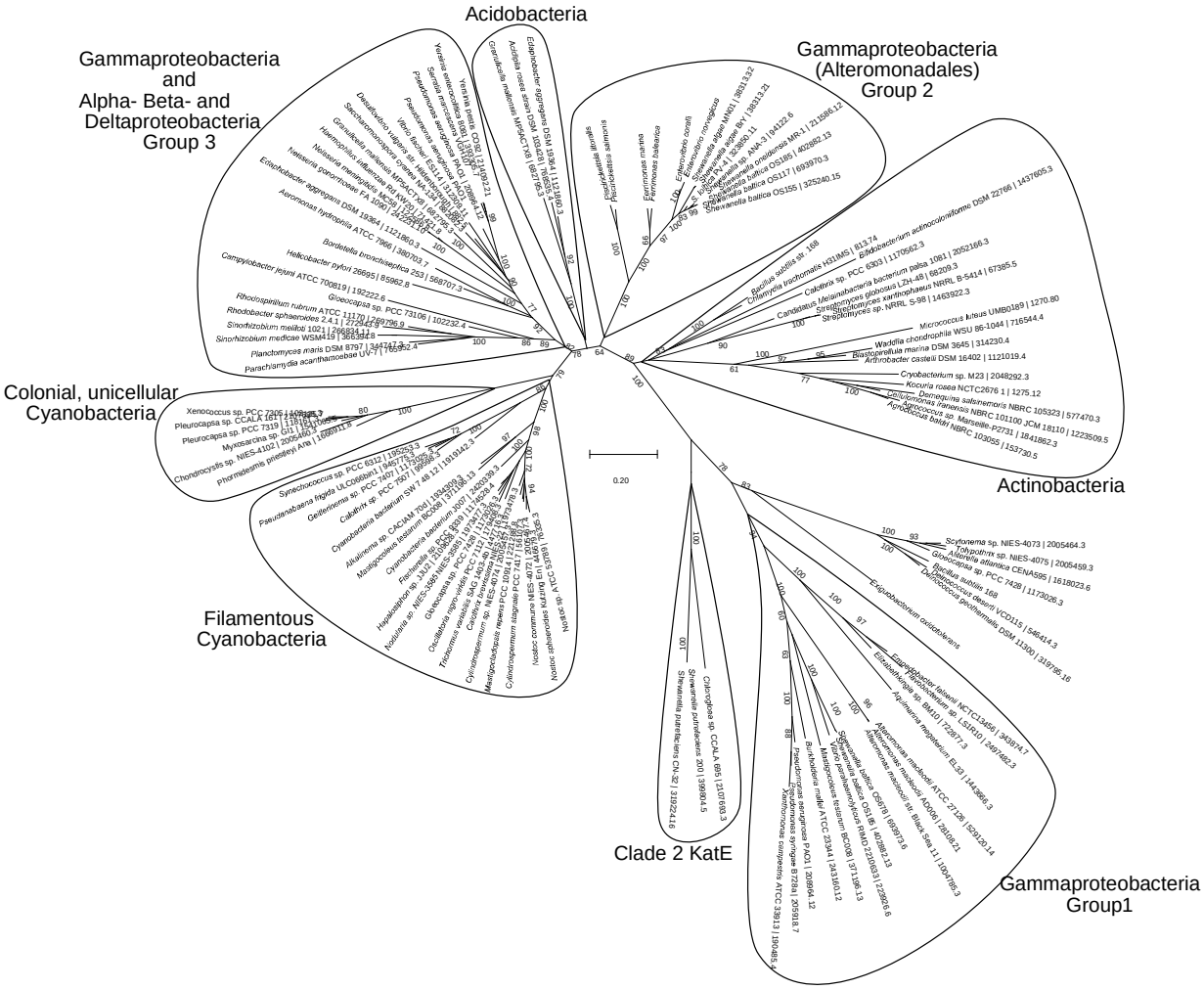

Figure 4. Maximum likelihood phylogenetic inference of clade 3 KatE proteins. All KatE sequences from all sequenced genomes of Cyanobacteria were used to construct a maximum likelihood phylogeny. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 115 amino acid sequences. Clade 2 KatEs were used as the outgroup.

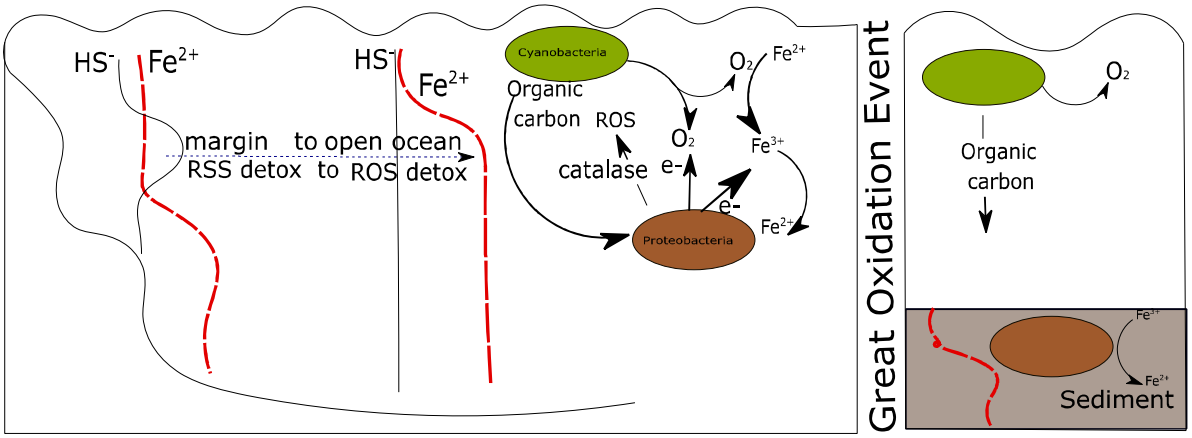

Figure 5. A Proterozoic paleo-eco-evolutionary scenario for Cyanobacteria and Proteobacteria. This schematic is a conceptual model for a potential ecological association between cyanobacteria and proteobacteria coexisting in the Archean ocean. The RSS detoxifying machinery emerging in euxinic margins could have evolved into ROS scavenging proteins, aiding phototrophs. In modern environments, previously coexisting Cyanobacteria and Proteobacteria are now found in separate niches (Cavalier-Smith, 2006b; Hau & Gralnick, 2007; Klotz & Loewen, 2003; Lyons et al., 2014; Olson et al., 2017; Zamocky et al., 2008).

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