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Morphophysiological and transcriptome analysis reveals a multiline defense system enabling cyanobacterium *Leptolyngbya* strain JSC-1 to withstand iron induced oxidative stress

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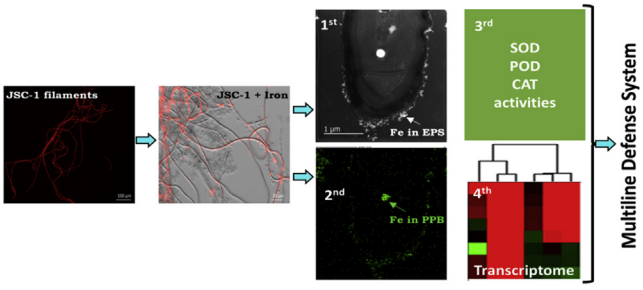
HIGHLIGHTS

- JSC-1 inhabiting hot springs provide insights into the mechanism of iron homeostasis.
- JSC-1 biomineralize extracellular iron in exopolymeric sheath (EPS).
- Intracellular biomineralization of iron takes place in polyphosphate (PP) bodies.
- DEGs and metabolic reprogramming enable JSC-1 to withstand severe oxidative stress.
- JSC-1 can be anticipated for heavy metal polluted waste water bioremediation.

GRAPHICAL ABSTRACT



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ABSTRACT

Iron intoxications induce severe oxidative stress by producing reactive oxygen species (ROS) in cyanobacteria, leading to membrane lipid peroxidation, altered morphology, impaired photosynthesis and other oxidative stress injuries. Given these stresses, mitigation of ROS is a prerequisite for all aerobic organisms. Study of siderophilic cyanobacterium *Leptolyngbya* strain JSC-1 inhabiting iron-rich hot springs may provide insight into the mechanism of iron homeostasis and alleviation of oxidative stress. In this study, we investigated the morphophysiological and molecular mechanisms enabling this cyanobacterium to cope with iron-induced oxidative stress. Strain JSC-1 biomineralized extracellular iron via an exopolymeric sheath (acting as a first line of defense) and intracellular iron via polyphosphate inclusions (second line of defense), thus minimizing the burden of free ferric ions. Physiological parameters, SOD, CAT and POD activities, bacterioferritin and total protein contents fluctuated in response to iron elevation, displaying a third line of defense to mitigate ROS. Differential gene expression analysis of JSC-1 indicated up-regulation of 94 and 125 genes and down-regulation of 89 and 183 genes at low (4 μM) and high (400 μM) iron concentration, respectively. The differentially expressed genes (DEGs) were enriched in 100 KEGG pathways and were found to be involved in lipopolysaccharide and fatty acid biosynthesis, starch, sucrose, chlorophyll and other metabolic pathways. Together with metabolic reprogramming (fourth line of defense), JSC-1 established a unique multiline defense system that allows

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JSC-1 to withstand severe oxidative stress. These findings also provide insight into potential survival strategies of ancient microorganisms inhabiting similar environment present in early earth history.

1. Introduction

Cells are complex molecular machines that employ multiple levels of regulation that enable them to respond to environmental distresses due to either nutrient scarcity or abundance (Ali et al., 2013). Iron is the fourth most abundant element in the earth's crust, however, its low availability to microorganisms at physiological pH can result in stress, while excess iron is also toxic as it catalyzes synthesis of reactive oxygen species (ROS) (Andrews et al., 2003). Almost all living organisms have an absolute requirement for iron to perform basic cellular processes and synthesize high-energy compounds that fuel cell growth, proliferation and metabolism (Rouault, 2003). It has been found that intracellular biochemical reactions catalyzed by iron may have played a critical role in the origination of life (Paerl and Huisman, 2008; Robinson, 2005). Response to environmental fluctuation of nutrient availability, including iron, is critical for growth of photosynthetic organisms, as iron act as a cofactor in cellular processes occurring in chloroplasts and other organelles. Both low and high iron concentrations can have harmful effects on the cells. Being a reactive metal, iron can produce harmful radicals and cause severe damage. Therefore, for maintenance of optimal iron homeostasis, photosynthetic organisms must regulate cellular iron concentrations (Pan et al., 2017; Pattanaik et al., 2014). In addition to cellular growth, iron is essential for many iron-containing proteins that catalyze fundamental reactions in photosynthesis, nitrogen assimilation in the forms of nitrate and nitrite, nitrogen fixation (in the case of cyanobacteria), respiration (electron transport chain), chlorophyll synthesis, nucleic acid synthesis, and a number of other metabolic reactions (Paerl et al., 2001; Rueter and Petersen, 1987).

Photoautotrophic organisms, including cyanobacteria, usually respond to iron scarcity by reduced cell growth, degradation of non-essential proteins and occasionally alterations in the morphology of the cells (Pattanaik et al., 2014). On the other hand, elevated concentration of iron in the medium can cause severe oxidative stress (by producing ROS) in cells that may lead to membrane lipid peroxidation, altered morphology, impaired photosynthesis and other oxidative stress signs (Lee et al., 2017). Mitigation of ROS is a prerequisite for all aerobic organisms, therefore, multiple systems (enzymatic and non-enzymatic) have evolved to maintain cellular homeostasis against various iron-induced oxidants (Brasil et al., 2017; Park et al., 2018). Based on observations, some microorganisms secrete secondary metabolites while others adopt an alternative strategy of making biofilms or mats to cope with nutrient fluctuation and other oxidative stresses, which are associated with ecological competition and survival under stressful conditions (Oliveira et al., 2015). Given the important role of iron as an active component of iron-sulfur (Fe-S) cluster, cytochromes (heme-containing) and non-heme iron (photosynthetic protein complexes) optimum iron availability and homeostasis is important in the regulation as well as function of photosynthetic machinery in these organisms (Shcolnick and Keren, 2006).

Cyanobacteria are distinct among bacteria in performing oxygen-evolving photosynthesis. Additionally, many are capable of nitrogen fixation while inhabiting a variety of ecosystems. One such N-fixing isolate is the siderophilic cyanobacterium, *Leptolyngbya* strain JSC-1 (JSC-1), isolated from a floating cyanobacterial mat in a water channel fed from two iron depositing hot springs at Yellowstone National Park. JSC-1 is a thermotolerant, filamentous and nonheterocystous cyanobacterium that has proven to be a useful model for studying cyanobacterial acclimation to different growth conditions, such as varying light wavelength, light intensity, fluctuating temperature, Fe deprivation and availability, and other nutrient limiting investigations (Brown et al., 2010; Ferreira and Straus, 1994; Gan et al., 2014b).

The siderophilic cyanobacteria JSC-1 is one of the inhabitants of ferrous iron rich hot springs with circum neutral pH. While inhabiting such extreme conditions, this siderophilic cyanobacterium has apparently acquired special mechanisms for iron homeostasis and oxidative stress mitigation. To date, there are limited studies investigating cyanobacterial physiology under environments approximating primitive earth conditions. Therefore, this work investigates the morphophysiological and molecular mechanisms enabling this cyanobacterial strain to cope with severe oxidative stress caused by elevated iron in its natural environment. The well-developed and multiline defense system of JSC-1 was studied under varying iron regimes as well as under an optimized condition. The gene expression profiles of JSC-1 subjected to low and high iron stresses were monitored for in-depth understanding of iron tolerance, oxidative stress mitigation and phenomena associated with its siderophily.

2. Materials and methods

2.1. Cyanobacterial strain and culture conditions

Leptolyngbya sp. strain JSC-1 (hereafter JSC-1) as experimental organism in this study was provided by Igor I. Brown, a visiting professor, in college of life science and technology, Beijing University of Chemical Technology, Beijing, PR China. JSC-1 was grown in DH medium that was buffered at pH 7–7.5 with 5 mM 4-(2-hydroxyethyl)-1piperazineethanesulfonic acid (HEPES). The culture conditions were optimized using different intensities of light, temperature and iron (see results). Optimum temperature and light conditions were then used to incubate JSC-1 with different iron concentrations (4-800 μM). Ferric chloride hexahydrate ($FeCl_3.6H_2O$) was used as the iron source in the current study. The growth was monitored by measuring the optical density (OD) of gently homogenized liquid culture at 730 nm (Brown et al., 2010; Gan et al., 2014b).

2.2. Scanning transmission electron microscope (STEM) examination and elemental mapping

To visualize the extracellular and intracellular iron mineralization, ultrathin sections of JSC-1 filaments were analyzed by scanning transmission electron microscope (STEM) and energy dispersive X-ray spectroscopy (EDS). The samples were prepared using the same method as previously described with slight modifications (Brown et al., 2010; Sumner, 2015). In brief, glutaraldehyde was used initially for fixation of cells and then dehydration was done at room temperature in a graded series of ethanol (starting at 30% then going to 50, 70, 95 and 100%) for 20 min each