Applied Microbiology and Biotechnology
https://doi.org/10.1007/s00253-018-8938-0

APPLIED MICROBIAL AND CELL PHYSIOLOGY

Involvement of organic acids and amino acids in ameliorating Ni(II) toxicity induced cell cycle dysregulation in *Caulobacter crescentus*: a metabolomics analysis

Abhishek Jain[1,2] · **Wei Ning Chen**[3]

Received: 26 October 2017 / Revised: 13 March 2018 / Accepted: 13 March 2018

Abstract
Nickel (Ni(II)) toxicity is addressed by many different bacteria, but bacterial responses to nickel stress are still unclear. Therefore, we studied the effect of Ni(II) toxicity on cell proliferation of α-proteobacterium *Caulobacter crescentus*. Next, we showed the mechanism that allows *C. crescentus* to survive in Ni(II) stress condition. Our results revealed that the growth of *C. crescentus* is severely affected when the bacterium was exposed to different Ni(II) concentrations, 0.003 mM slightly affected the growth, 0.008 mM reduced the growth by 50%, and growth was completely inhibited at 0.015 mM. It was further shown that Ni(II) toxicity induced mislocalization of major regulatory proteins such as MipZ, FtsZ, ParB, and MreB, resulting in dysregulation of the cell cycle. GC-MS metabolomics analysis of Ni(II) stressed *C. crescentus* showed an increased level of nine important metabolites including TCA cycle intermediates and amino acids. This indicates that changes in central carbon metabolism and nitrogen metabolism are linked with the disruption of cell division process. Addition of malic acid, citric acid, alanine, proline, and glutamine to 0.015 mM Ni(II)-treated *C. crescentus* restored its growth. Thus, the present work shows a protective effect of these organic acids and amino acids on Ni(II) toxicity. Metabolic stimulation through the PutA/GlnA pathway, accelerated degradation of CtrA, and Ni-chelation by organic acids or amino acids are some of the possible mechanisms suggested to be involved in enhancing *C. crescentus*'s tolerance. Our results shed light on the mechanism of increased Ni(II) tolerance in *C. crescentus* which may be useful in bioremediation strategies and synthetic biology applications such as the development of whole cell biosensor.

Keywords *Caulobacter crescentus* · Nickel stress · Metabolomics · Amino acids · Organic acids · Cell cycle

Introduction

Bacteria are often exposed to heavy metal ions in their environment. Human activities, such as industrial production, agriculture, mining, and smelting operations, significantly contribute to the elevated level of heavy metals in the environment (Tchounwou et al. 2012; He et al. 2005; Herawati et al. 2000). The US Environmental Protection Agency has placed 13 heavy metals, including nickel (Ni), on its priority pollutants list of 126 compounds (EPA 2014). Nickel toxicity in plants, animals, and humans has been widely studied (Yusuf et al. 2011; Das et al. 2008; Denkhaus and Salnikow 2002). Nickel toxicity has received a lot of attention due to its role as a human carcinogen. Conversely, the importance of nickel toxicosis in microorganisms is less well studied (Macomber and Hausinger 2011). However, the nickel defense system (RcnA) in *E. coli* and detection of putative homologs in archaea and throughout bacteria suggests that excessive nickel is a routine physiological concern (Rodrigue et al. 2005). Therefore, it becomes important to understand the effect of nickel exposure to bacteria. Nickel may support bacterial

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00253-018-8938-0) contains supplementary material, which is available to authorized users.

✉ Wei Ning Chen
wnchen@ntu.edu.sg

[1] Interdisciplinary Graduate School, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798, Singapore

[2] Advanced Environmental Biotechnology Centre, Nanyang Environment and Water Research Institute, Nanyang Technological University, 1 CleanTech Loop, Singapore 637141, Singapore

[3] School of Chemical and Biomedical Engineering, Nanyang Technological University, 62 Nanyang Drive, Singapore 637459, Singapore

Published online: 03 April 2018

growth when present in trace amounts, but at higher concentrations, this metal causes toxic effects to bacteria by inhibiting iron metalloenzymes like taurine/αKG dioxygenase (TauD) in *E. coli* (Kalliri et al. 2005), zinc metalloenzymes like *Trypanosoma cruzi* metallocarboxypeptidase 1 (TcMCP-1) (Niemirowicz et al. 2007) or some other metalloenzymes such as nitrous oxide reductase from *Rhodobacter sphaeroides* (Sato et al. 1998), Cobaltochelatase from *Pseudomonas denitrificans* (Debussche et al. 1992). Nickel is also known to inhibit non-metal enzymes, for example, Uricase (urate oxidase) from *Bacillus fastidiosus* (Bongaerts et al. 1978) and N-carbamoyl-D-amino acid amidohydrolase from *Agrobacterium* lose activity in the presence of nickel (Louwrier and Knowles 1996). Hence, it is essential for cells to maintain homeostasis by tightly regulating the cellular concentration of nickel. When the metal concentration is insufficient inside the cell, bacteria express metal import systems, such as NikABCDE which is a part of the ABC transporter subfamily found in eubacteria and archaea (Navarro et al. 1993; Mulrooney and Hausinger 2003). Conversely, if the cellular concentration of metal is more than the required limit, bacteria use several types of metal transport system, such as the RcnA efflux pump in *E. coli* (Rodrigue et al. 2005), the RND transport system in *C. metallidurans*, *A. xylosoxidans*, and *H. pylori* to remove the excess metal ions (Stähler et al. 2006; Macomber and Hausinger 2011) .

It is known that bacteria employ different molecular mechanisms to survive in often unknown and fluctuating nickel stressed environmental conditions. However, despite years of research, it is still not fully understood how microbes detect and cope with nickel stress in their surroundings. Furthermore, external environmental conditions (e.g., nickel stress) can influence the cellular physiology of microorganisms, but it is not entirely clear how the different external signals or cues impinge on fundamental biological processes, like the cell cycle, in bacteria. Cell cycle regulation has been studied in several model bacteria. One prominent example is the asymmetrically dividing alpha proteobacterium *Caulobacter crescentus*. *C. crescentus* has been found in many different environments, including wastewater, seawater, soil, and deep subsurface goldmines (Hughes et al. 2012). It is particularly noted for its ability to survive in low nutrient conditions or in habitats that contain harmful levels of metal contamination. In particular, scientists have found that *C. crescentus* can tolerate at least up to 2–3 times more uranium stress than *E. coli*. *C. crescentus* could survive at 1 mM of uranium while *E. coli* was completely non-viable at 0.5 mM (Hillson et al. 2007). In addition, *C. crescentus* is a model organism for understanding the cell cycle regulation, asymmetric cell division, and cellular differentiation (Jensen et al. 2002; Curtis and Brun 2010). The cell cycle mechanism of *C. crescentus* has been studied mostly in ideal laboratory conditions, but it is still not understood how natural environmental stresses (e.g., heavy metal stress) affect the complex regulatory cascade of the cell cycle. A deep understanding of bacterial stress responses is important for the development of antibiotics or other antimicrobial strategies as well as for synthetic biology applications such as the development of whole cell biosensors. Previous studies have also shown that *C. crescentus* has applications in uranium biosensor and cadmium bioremediation (Hillson et al. 2007; Patel et al. 2010).

In this paper, we seek to (1) investigate how Ni(II) stress in the environment can affect the proliferation of microorganisms, such as *C. crescentus*, and (2) identify the metabolites that enhance or reduce *C. crescentus*'s ability to survive in Ni(II) stress condition. This study began with the systematic analysis of the effect of Ni(II) stress on *C. crescentus* growth and survival. Our analysis revealed that certain Ni(II) concentration severely affect both cell morphology and cell cycle of *C. crescentus*. The diameter of Ni(II) stressed *C. crescentus* cells was increased due to mislocalization of important cytoskeleton protein MreB. We also found that mislocalization of the cell division proteins such as FtsZ, MipZ, and ParB was responsible for dysregulation in chromosome replication and cell division process. Next, GC-MS metabolomics analysis revealed nine important metabolites which were increased in Ni(II) stressed *C. crescentus*. The restoration of growth of 0.015 mM Ni(II)-treated cells upon supplementation of malic acid, citric acid, glutamine, alanine, and proline confirmed their role in ameliorating Ni(II) toxicity in *C. crescentus*.

Materials and methods

Bacterial strains, media, and growth

The *Caulobacter crescentus* strain CB15N (ATCC 19089) and its derivatives cultures were maintained on PYE (peptone-yeast extract) agar plates containing 0.2% (wt/vol) bacto peptone (Difco), 0.1% (wt/ vol) yeast extract (Difco), 1 mM $MgSO_4$, 0.5 mM $CaCl_2$, and 3% (wt/vol) agar (Difco) supplemented with appropriate combinations of antibiotics. Broth cultures used for growth curve, qPCR, and microscopic studies were PYE and M2G Minimal media contains per liter [50 mL of 20X M2 salts (34.8 g Na_2HPO4, 21.2 g KH_2PO4, 10 g NH_4Cl per liter), 0.5 mM $MgSO_4$, 10 mL of 20% glucose, 1 mL 10 mM $FeSO_4$, and 10 mM EDTA (Sigma #F-0518), 0.5 mM $CaCl_2$]. The overnight culture was inoculated with colonies from PYE plates, grown at 30 °C, and shaken continuously at 225 rpm (Hu et al. 2005).

Toxic metal effect on growth and survival

One molar, 0.1 M, and 10 mM stock solutions of $Nicl_2{\cdot}6H_2O$, $CoSO_4{\cdot}7H_2O$, K_2CrO_4, $CdSO_4$, Na_2SeO_3, and $CuSO_4$

(Sigma-Aldrich) were prepared by dissolving the metal compounds in MilliQ water. All metal stocks were sterilized by filtration through a 0.2-μm membrane. Similarly, 0.5 and 0.25 M stock solutions of glutamine, proline, alanine, valine, glutamic acid, malic acid, and citric acid (Sigma-Aldrich) were prepared.

Five-milliliter culture of *C. crescentus* was grown in PYE overnight. The PYE culture is diluted 1:50 in minimal media and grew until bacteria reached mid-log phase. The mid-log phase culture then diluted again to OD 0.03–0.04 in fresh minimal media and 5 mL of culture were aliquot into different 14-mL snap cap tubes already containing heavy metals. A control was also prepared with no heavy metal. The OD was measured at 660 nm using the spectrophotometer. For spot dilution test, OD values were normalized for all the cultures after 24 h, and tenfold serial dilutions were prepared for each. Four microliters of each dilution was spotted on PYE agar plates. Plates were kept in 30 °C incubator for 40–48 h; then, images were taken using Gel doc system (Bio-Rad).

To test the effect of organic acids and amino acids on growth of 0.015 mM Ni(II)-treated cells,1 or 10 mM of each amino acid or organic acid exogenously added to *C. crescentus* culture in M2G minimal media containing 0.015 mM Ni(II).

RNA extraction

The overnight *C. crescentus* culture in PYE was diluted in minimal media and grew until bacteria reached mid-log phase. The mid-log phase culture in minimal media was diluted again to OD 0.03–0.04 in fresh minimal media. When the bacteria reached exponential phase (OD 0.25–0.30), 10 mL of culture were aliquot into different 50-mL tubes already containing Ni(II) concentrations and incubated for 30 min. A control was also prepared with no heavy metal. After 30 min of heavy metal shock, samples were centrifuged at 10,000×*g* for 5 min, and the supernatant was removed. The cell pellets were stored at − 80 °C. RNA was first isolated by QiAzol-chloroform extraction. Then, RNeasy mini kit from Qiagen including on-column DNase digestion was used for further extraction steps. RNA concentration was measured using Nanodrop.

Quantitative PCR

Quantitative PCR (qPCR) was performed in 96-well plates in a CFX96 Touch Real-Time PCR Detection System (Bio-Rad). qScript cDNA SuperMix (Quantabio) was used for reverse transcription. For quantitation of transcript levels, PerfeCTa SYBR Green FastMix (Quantabio) was used and thermocycling conditions were as follows: 3 min at 95 °C, and 39 cycles (30 s at 95 °C, 45 s at 55 °C, 30 s at 60 °C) for amplification. Primers used for qPCR are listed in Table S1.

Fluorescence microscopy

The overnight culture in PYE was diluted in minimal media and grew until *C. crescentus* strains reached mid-log phase. This mid-log phase culture in minimal media was diluted again to OD 0.03–0.04 in fresh minimal media, and 5 mL of cultures were aliquot into different 14-mL snap cap tubes already containing heavy metal concentrations to be tested. A control was also prepared with no heavy metal. All the strains were exposed to heavy metal for 12 h. Expression of xylose-inducible genes or vanillate-inducible genes was induced by adding 0.3% xylose (Sigma-Aldrich) or 0.5 mM vanillate (Sigma-Aldrich) for 2–3 h (Heinrich et al. 2016). All samples were imaged on freshly prepared 1% agarose pad onto microscopy slides. Microscopy was performed on a DM6000B (Leica) upright microscope with Leica Application Suite X (LAS X) software. An external light source Leica EL6000 was used for enhanced fluorescence imaging. Cell dimensions were measured using ImageJ (Schindelin et al. 2012). Strains used in this study are listed in Table S2.

Collection of samples and extraction of metabolites

The overnight *C. crescentus* culture in PYE was diluted in minimal media and grew until bacteria reached mid-log phase. This mid-log phase culture in minimal media was diluted again to OD 0.03–0.04 in fresh minimal media. When the bacteria reached mid-log phase (OD 0.25–0.30), 5 mL of culture were aliquot into different 14-mL tubes already containing 0.003, 0.008, and 0.015 mM of Ni(II) concentrations. A control was also prepared with no heavy metal. Three separate sets of samples were collected after 30 min, 2 h, and 4 h of heavy metal shock, respectively. Samples were centrifuged at 10,000×*g* for 5 min, and the supernatant was removed. The cell pellets were stored at − 80 °C. Metabolites were extracted after collecting all the samples. Nine hundred microliters of 2:1 methanol/chloroform solution was added to frozen cell pellets for extraction. Samples were then lysed using glass beads and a bead grinder. Five microliters of 4 mg/mL ribitol dissolved in MilliQ water was added to each sample as an internal standard to correct for any loss of metabolite during the extraction process. After this, 200 μL of water and chloroform solution was added to each sample. Samples were centrifuged for 10 min at 24,000 RPM, and supernatant was transferred to a fresh Eppendorf tube (Booth et al. 2015). The supernatant was air-dried using a heat block set at 37 °C for 24 h. Fifty microliters of 2% methoxyamine HCL in pyridine (ThermoFisher Scientific) was added to all the samples and incubated for 1 h at 37 °C. Next, 100 μL of *N*,O-bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane (BSTFA+1%TMCS, Sigma-Aldrich) was added to all samples and incubated for 30 min at 70 °C. Samples were centrifuged for 1 h at room temperature and then transferred to GC-MS glass vials (Wang et al. 2010).

GC-MS analysis

The samples were analyzed using an Agilent Technologies 7890B GC and 5977A GC-MSD system. Metabolites were isolated through a HP-5MS capillary 54 column (30 m × 0.250 mm i.d.; 0.25-μm film thickness; Agilent J&W Scientific). One microliter of each sample was injected into the system by autosampler and separated using the column in splitless mode. Helium was used as carrier gas with a flow rate of 1.1 mL/min. Temperatures for inlets and MS source were set at 250 and 230 °C, respectively. The oven temperature was kept at 75 °C for 4 min and increased to 280 °C with a rate of 4 °C/min then held for 1.56 min. Mass spectrum was recorded from 40 to 600 *m*/*z* with a scan time of 0.2 s.

Metabolites detection and quantification

Acquisition of the total ion chromatogram and identification of mass spectra were done by GC-MS solution software (GC/MSD Chemstation Data Analysis, Agilent). NIST14 mass spectral library (National Institute of Standards and Technology) was used to identify the detected metabolite peaks. Peak area of compounds was compared to internal control for normalization. Peaks with a similarity index of more than 80% were extracted to name the candidate compounds. Partial least square discriminant analysis using MetaboAnalyst 3.0(Xia et al. 2015) was performed to see the difference between metabolite profiles of untreated *Caulobacter* and *Caulobacter* cells treated with different concentrations of Ni(II).

Results

Ni(II) toxicity inhibits cell growth and proliferation of *C. crescentus*

The effect of Ni(II) toxicity on cell growth was compared with five other heavy metals (Co(II), Cr(VI), Cu(II), Se(IV), Cd(II)) which are listed as priority pollutants (EPA 2014). IC_{50} values of all the heavy metals are presented in Table 1. IC_{50} value refers to the concentration of heavy metal responsible for 50% reduction in the growth of bacteria during mid-log phase.

The results obtained show that relatively lower concentrations of $NiCl_2$ and $CdCl_2$ were sufficient to inhibit the growth of *C. crescentus* (Table 1, Figs. 1a and S1). We found firstly that 0.003 mM slightly affected the cell growth, secondly, that 0.008 mM reduced the cell growth by 50%, and thirdly that 0.015 mM of Ni(II) completely inhibited the growth. Next, spot dilution test results confirmed that the cells did not only stop proliferating but were also killed by 0.015 mM of Ni(II) (Fig. 1b). This is in contrast to the effect seen using 0.008 mM concentration of Ni(II), whereby cells only stopped proliferation but were still viable (Fig. 1b). On the other hand, growth reduced by half at 0.004 mM Cd(II), and the cells stopped proliferating when treated with 0.1 mM Cd(II) (Fig. S1). However, spot dilution test results showed that cells were viable even after exposure to 0.2 mM Cd(II) concentration (Fig. S1). Thus, our results showed that out of all six metals tested, Ni(II) produced the most toxic effect on cell growth and survival.

Table 1 IC_{50} (mM) values of heavy metals

Metal	Ni(II)	Cd(II)	Cu(II)	Co(II)	Cr(VI)	Se(IV)
$IC_{50\ (mM)}$	0.008	0.004	0.02	0.02	0.04	0.08

Mislocalization of MreB leads to a severe cell shape defect in Ni(II)-treated *C. crescentus*

The morphology of Ni(II)-treated *C. crescentus* cells was severely affected. It has been shown that different stress conditions affect the *C. crescentus* morphology in different ways. The cell size of *C. crescentus* was largely maintained during carbon starvation, growth in stationary phase and acute heat shock (Jonas et al. 2013; Boutte et al. 2012; Leslie et al. 2015). In another study, *C. crescentus* cells grew into long filaments after treatment with 100 mM NaCl or 4% ethanol (Heinrich et al. 2016). However, we observed a different cell morphology when *C. crescentus* treated with Ni(II). The diameter of 0.015 mM Ni(II)-stressed cells was significantly increased at the mid cell as compared to untreated or 0.003 mM Ni(II)-treated cells (Fig. 2a). The cells showed different morphologies when treated with 0.008 and 0.015 mM concentrations of Ni(II), as shown in Fig. 2a. The expanded predivisional cells with constriction at mid-cell were dominant in the presence of 0.008 mM Ni(II), whereas large lemon-shaped cells were observed at 0.015 mM concentration of Ni(II) (Fig. 2a). The cells were also found to be increased in length in both the cases. A comparison of cell size under different Ni(II) stress conditions is presented in Table 2.

The bacterial actin homolog MreB is required to maintain the rod shape of the cell in *C. crescentus*, *E. coli*, and *Bacillus* (Gitai et al. 2004). The lemon shape or expanded predivisional morphology of Ni(II)-treated cell was similar to what observed in the *C. crescentus* cell with malfunctioning MreB protein (Figge et al. 2004). This led us to examine the localization of MreB using fluorescence microscopy. We found that Ni(II) stress disrupted the localization pattern of MreB in *C. crescentus*. In the case of untreated or 0.003 mM Ni(II)-treated cells, MreB bands were concentrated to the mid-cell region, whereas at 0.008 and 0.015 mM concentrations of Ni(II), MreB was distributed throughout the cells or sometimes accumulated at the poles (Fig. 2b).

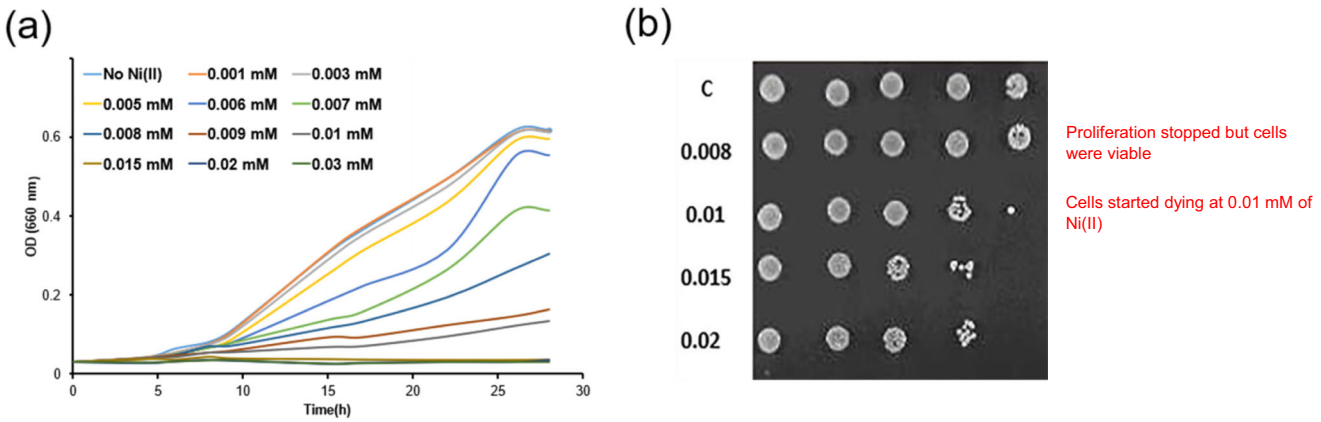

Fig. 1 Ni(II) stress severely affected growth and survival of *C. crescentus* CB15N. **a** *C. crescentus* was grown in M2G minimal media in presence of 0.005 to 0.03 mM Ni(II), and the effect on growth with respect to control (without metal) is measured in terms of cell optical density at 660 nm. **b** Spot dilution test confirms the detrimental effect of Ni(II) on the growth of *C. crescentus* . The results are shown in the presence of 0.008 to 0.02 mM Ni(II) .All the results are representative of at least three experiments. *C control without metal

In the present study, we have also compared the effects of five other metals with Ni(II) stress on the morphology of *C. crescentus* (Fig. S2). *C. crescentus* cells had shown different morphological patterns when exposed to various heavy metals. Cells were found to be slightly longer and more crescent shaped when exposed to 0.02 mM Co(II) and 0.05 mM Cr(VI), while 0.1 mM Cd(II) stress produced longer cells. An increase in width and length of the cells was observed in the presence of 0.02 mM Cu(II). On the other hand, Se(IV) did not affect the morphology of *C. crescentus* cells and were similar to the untreated cells. It can be concluded that the lemon-shaped or expanded predivisional phenotype of cells under Ni(II) stress condition is not a common pattern under heavy metal stress condition but a unique effect specifically produced in Ni(II)-treated cells.

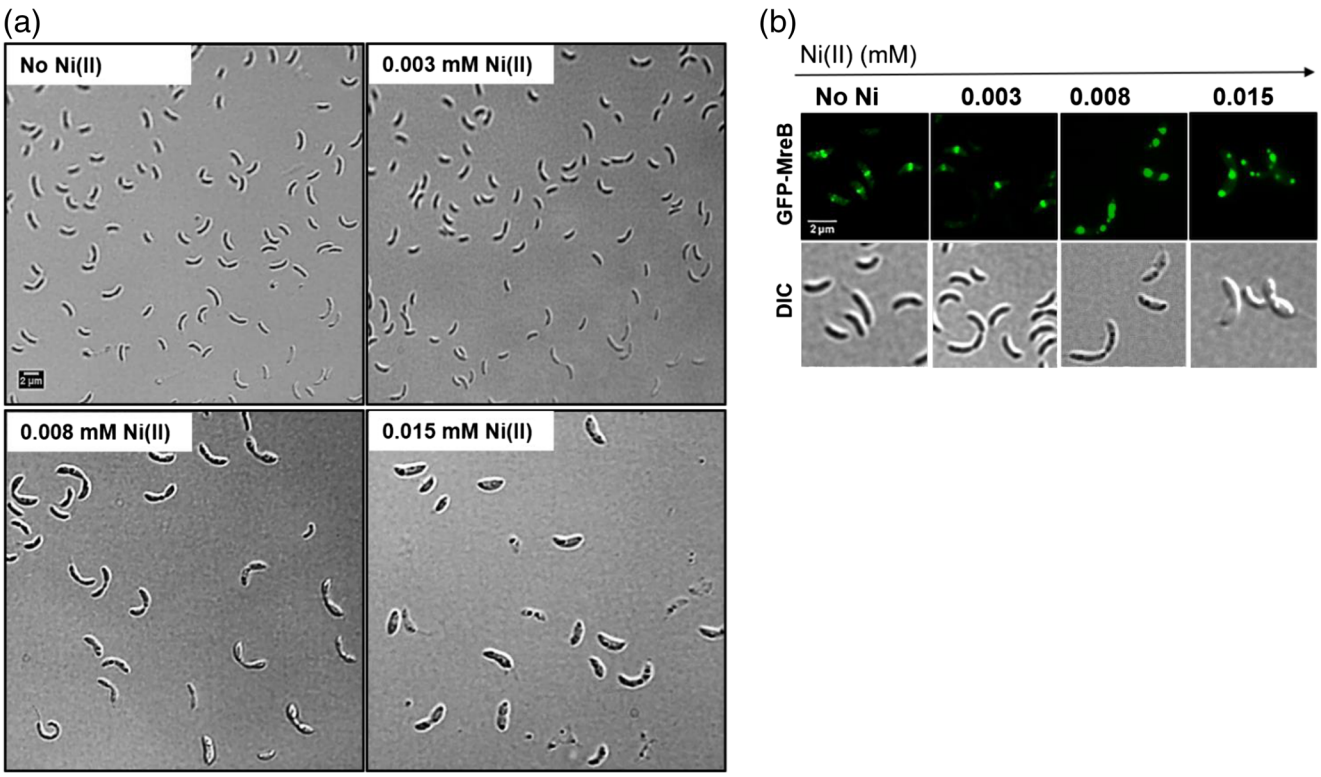

Fig. 2 Mislocalization of MreB affected cell morphology in Ni(II)-treated *C. crescentus*. **a** Differential interference contrast (DIC) microscopy images of *C. crescentus* cells treated with different Ni(II) concentrations. **b** Representative DIC and fluorescence microscopy images from cell expressing MreB-GFP. Shown are untreated cells, cells treated with 0.003, 0.008, or 0.015 mM Ni(II)

Table 2 Cell size under different Ni(II) stress conditions

Ni(II) (mM)	Length (μm)	Width (μm)
No Ni(II)	2–2.2	0.45–0.49
0.003	2–2.3	0.45–0.49
0.008	3.7–4.3	1–1.2
0.015	2.8–3.3	0.85–1.1

Ni(II)-stressed *C. crescentus* cells show altered expression of master cell cycle regulatory genes and abnormal localization of major cell division proteins

It was previously observed that the cell cycle master regulator CtrA was differentially expressed upon exposure of *C. crescentus* to ethanol and salt stress condition, but FtsZ expression or localization pattern was found to be normal (Heinrich et al. 2016). In another study, carbon starvation in *C. crescentus* altered expression of important cell cycle proteins such as CtrA, DnaA, GcrA, and cell division protein FtsZ (Britos et al. 2011). Therefore, it was of interest to investigate if the expression of genes regulating the cell cycle is affected in Ni(II)-stressed *C. crescentus* cells. qPCR results showed that the cell cycle regulatory genes *dnaA*, *gcrA*, and *ctrA* and gene responsible for cytokinesis, *ftsZ*, were upregulated in all three concentrations (0.003,0.008, 0.015 mM) of Ni(II)-treated *C. crescentus* cells (Fig. 3). The altered expression of *ctrA*, *dnaA*, *gcrA*, and *ftsZ* is consistent with the slowing down of the cell cycle in presence of Ni(II). The transcription of *ccrM* gene is dependent on CtrA protein, but interestingly, *ccrM* expression level was not found to be affected in Ni(II)-stressed *C. crescentus* cells. CtrA regulates the expression of *ccrM* only in phosphorylated state and phosphorylation of CtrA depends on hybrid membrane kinase CckA and the histidine phosphotransferase ChpT (Mohapatra et al. 2014). It could be hypothesized that dysregulation in CtrA phosphorylation pathway results in unchanged expression of *ccrM*.

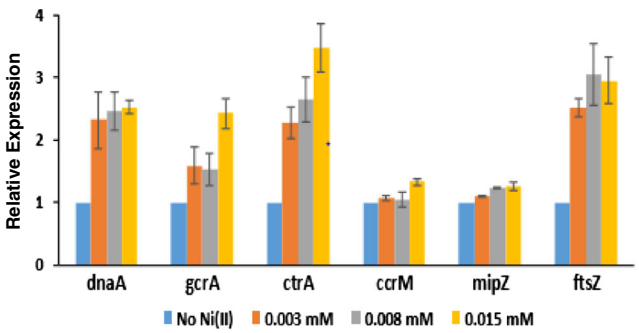

Fig. 3 Ni(II) stress altered expression of major cell cycle regulatory genes in *C. crescentus*: relative expression levels of four key master regulatory genes (*dnaA*, *gcrA*, *ctrA*, *ccrM*) and cell division genes (*ftsZ*, *mipZ*), under different Ni(II) stress conditions. The error bars indicate the SD of three biological replicates

In our previous experiments, we have observed that Ni(II) affected cell growth and morphology. The difference in *C. crescentus* cell morphology in 0.008 and 0.015 mM Ni(II)-stressed cells suggests that different concentrations of Ni(II) affected the cell cycle at different stages. At 0.008 mM Ni(II) treatment, most of the *C. crescentus* cells were at the predivisional stage which shows that the cells were capable of replicating initially but were arrested at the G2 phase later and unable to divide. On the other hand, 0.015 mM Ni(II)-treated *C. crescentus* cell phenotype, lemon-shaped cells without mid-cell septa, suggested that both replication and cell division process were affected. This led us to hypothesize that the cell cycle is dysregulated. To better understand the underlying molecular mechanism, we proceeded to test the localization pattern of key proteins implicated in cell division like MipZ, FtsZ, and chromosome partitioning protein ParB in the presence of Ni(II).

The localization of FtsZ-mCherry, MipZ-YFP, and ParB-mCherry fusion proteins was examined in Ni(II)-treated *C. crescentus* cells (Fig. 4). It was found that at Ni(II) concentrations of 0.003 and 0.008 mM, the localization pattern of these proteins was similar to untreated cells. But in the case of 0.015 mM Ni(II)-treated cells multiple 3–4 abnormal MipZ, FtsZ, and ParB foci were observed (Fig. 4). Multiple MipZ and ParB foci suggest that there are multiple origins of replication. We confirmed by using a fluorescent repressor-operator system (FROS), which marks the origins of replication (Viollier et al. 2004), that 0.015 mM Ni(II)-treated lemon-shaped cells contained multiple three to four well-segregated origins (Fig. S3).

The FtsZ-mCherry and MipZ-YFP localization patterns were also analyzed in the other five heavy metals stress. It was found that the FtsZ and MipZ had normal localization. However, mislocalization of MipZ was observed in 0.02 mM Cu(II)-treated cells (data not shown). These results confirmed that different heavy metal stresses affected the cell cycle of *C. crescentus* at different stages. Additionally, 0.015 mM Ni(II) stress produced the most severe effect by disturbing the localization of important cell division protein such as FtsZ, MipZ, and ParB.

Fluorescence microscopy has shed light onto the mechanism of how Ni(II) toxicity affects cell morphology and cell cycle process in *C. crescentus*. Now, as the second part of this paper, we aspire to unveil the cellular metabolism involved in alleviating the Ni(II) toxicity in *C. crescentus*. Therefore, in our next section, we have described the GC-MS metabolomics performed on *C. crescentus* cells to identify the important metabolites which enhance or reduce its capability to survive in Ni(II) stress condition.

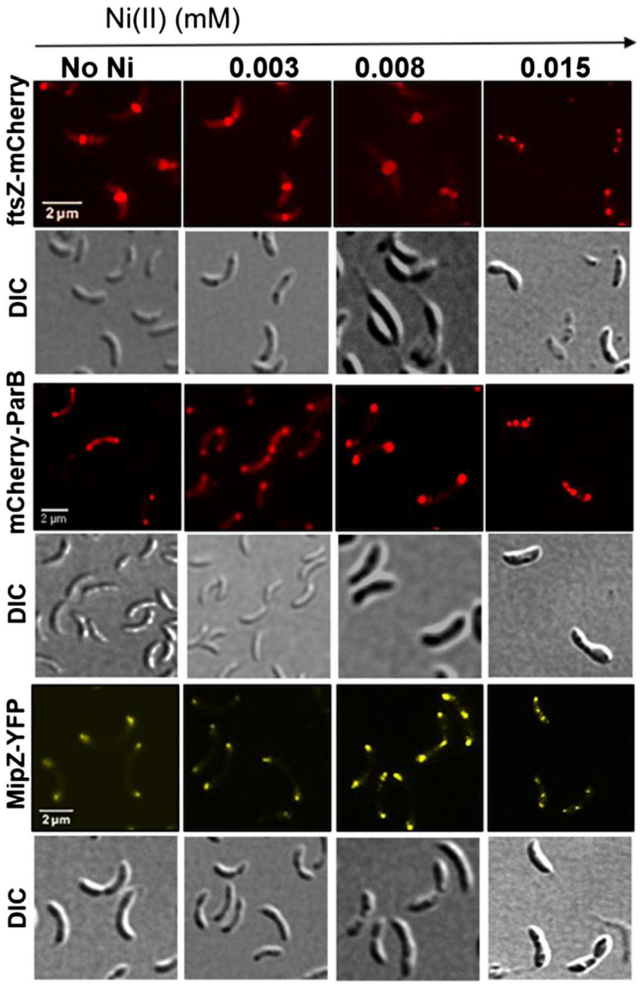

Fig. 4 Ni(II) stress induced abnormal localization pattern of FtsZ, MipZ, and ParB: representative DIC and fluorescence microscopy images from cell expressing ftsZ-mCherry, mCherry-ParB, and MipZ-YFP. Shown are untreated cells, cells treated with 0.003, 0.008, or 0.015 mM Ni(II)

Comparison of metabolite profiles of *C. crescentus* treated with different Ni(II) concentrations

The role of amino acids and central metabolism in cell growth and division has been explored (Ron et al. 1977; Monahan et al. 2014). The toxic effect on cell growth and division induced by Ni(II) stress suggests that Ni(II) might affect the *C. crescentus* metabolome. To investigate this possibility, we performed untargeted GC-MS metabolomics to compare untreated *C. crescentus* with *C. crescentus* cells treated with three different NI(II) concentrations. The changes in metabolome would help shed light on the mechanism for *C. crescentus's* ability to cope with Ni(II) toxicity. In total, 42 metabolites including amino acids, sugar, and organic acids were identified (Table S3). The GC-MS spectra of untreated and 0.015 mM Ni(II)-treated *C. crescentus* was overlaid, as illustrated in Fig. 5. The visual inspection shows the differences in metabolite profiles between untreated and 0.015 mM Ni(II)-treated cells. Multivariate analysis using partial least square discriminant analysis was used to accurately describe the variations between GC-MS metabolite profiles. As shown in Fig. 6, PLS-DA analysis clearly separated untreated *C. crescentus* cells from Ni(II)-treated cells. Ni(II)-treated cells (0.003 and 0.008 mM) were grouped close to each other and clearly separated apart from 0.015 mM Ni(II) cells. Nine important differential metabolites were identified and the changes in their levels are shown in Fig. 7. Citric acid and glutamine could be detected only when cells were exposed to 0.015 mM of Ni(II) for 4 h. The metabolites can be classified into amino acids (alanine, proline, glutamic acid, glutamine, valine, aminomalonic acid), TCA cycle intermediates (malic acid, citric acid), and 3,4,5-trihydroxypentanoic acid. We mapped the metabolomic changes in relation to biochemical pathways (Fig. 8). This gives a view of metabolite changes in presence of different Ni(II) concentrations in comparison to untreated *C. crescentus* cells which help us understand (1) the connection of carbon and nitrogen metabolism with cell cycle dysregulation and (2) the cellular metabolism to ameliorate Ni(II) toxicity.

Exogenous addition of malic acid, citric acid, proline, glutamine, and alanine enhanced the Ni(II) tolerance of *C. crescentus*

GC-MS metabolomics revealed nine differential metabolites in Ni(II) stressed *C. crescentus* cells. Aminomalonic acid and 3,4,5-trihydroxypentanoic acid are generated as a product of stress damage. We have added 1 or 10 mM concentration of the remaining six metabolites (malic acid, citric acid, alanine, proline, glutamic acid, glutamine, valine) to 0.015 mM Ni(II)-treated cells to test whether they have a direct role in Ni(II) tolerance in *C. crescentus*. Interestingly, we found that the growth of 0.015 mM Ni(II)-stressed cells was significantly improved after the addition of 10 mM of malic acid or proline or alanine or glutamine or 1 mM of citric acid (Fig. 9). The growth was slightly improved in presence of 1 mM of malic acid or proline. There was no restoration of growth observed when glutamic acid or valine added to 0.015 mM Ni(II)-treated *C. crescentus* cells (Fig. 9). The observation from this experiment and GC-MS metabolomics combinedly confirmed the role of malic acid, citric acid, proline, glutamine, and alanine in conferring the resistance against Ni(II) toxicity in *C. crescentus*.

Discussion

Comparison of the effect of different Ni(II) concentrations on *C. crescentus* cell cycle

MipZ, a member of ParA superfamily of P loop ATPases, is an essential protein for chromosome segregation and cell

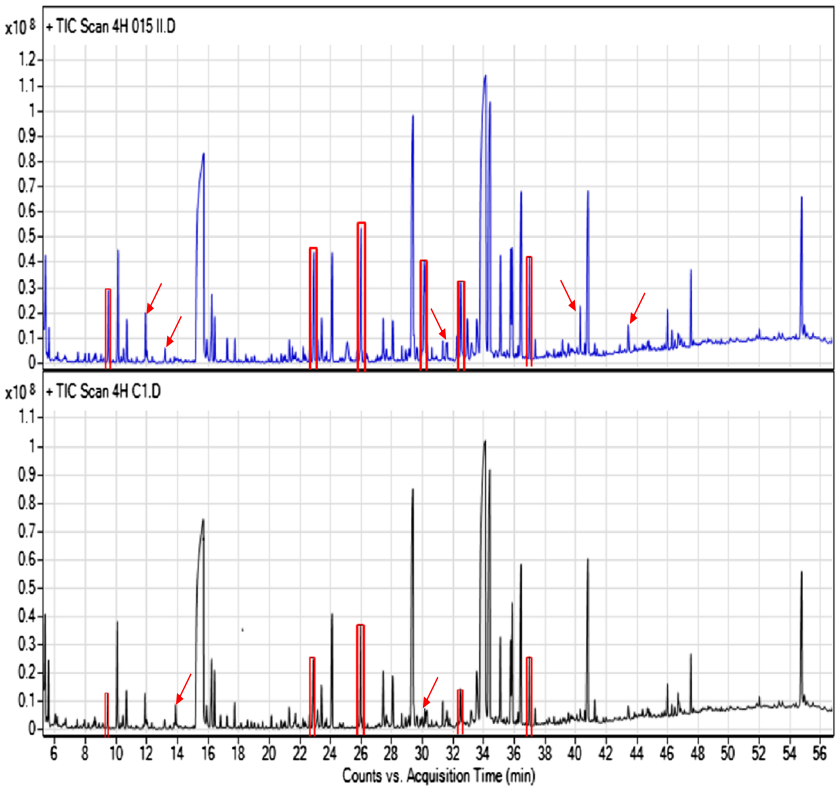

Fig. 5 The GC-MS spectra of untreated (black) and 0.015 mM Ni(II)-treated (blue) *C. crescentus*. The observable differences are shown with red blocks or arrows

division in *C. crescentus*. The localization of MipZ is parallel to the origin of replication during the cell cycle (Thanbichler and Shapiro 2006). The MipZ protein interacts with the DNA partitioning protein, ParB. The MipZ-ParB complex and the origin of replication occupy the same position in the cell and share a similar pattern of localization over the cell cycle (Figge et al. 2003; Mohl et al. 2001). Another protein FtsZ is responsible for cytokinesis process in *C. crescentus*. FtsZ localization is MipZ dependent and restricted to subcellular locations with the lowest concentration of MipZ. The MipZ-ParB-origin complex displaces FtsZ towards the mid-cell, where MipZ concentration is lowest (Thanbichler and Shapiro 2006; Goley et al. 2011). Multiple mislocalized foci of MipZ, FtsZ, and ParB in 0.015 mM Ni(II)-treated *C. crescentus* cells suggest that replication occurred multiple times. Cells did not increase in length and accumulated only three to four origin of replication in 15 h. It could be inferred that cell growth and DNA replication proceeded for only one to two doubling times without division then later cells got arrested at the G1 stage. These observations recommend that chromosome replication and segregations process has gone awry in 0.015 mM Ni(II)-treated *C. crescentus*. Past investigations have also detailed that stress conditions such as carbon starvation, growth in stationary phase, and acute heat shock caused DNA replication block and G1 arrest in *C. crescentus* (Jonas et al. 2013; Boutte et al. 2012; Leslie et al. 2015). All these cell cycle proteins showed the normal pattern of localization in 0.003 and 0.008 mM Ni(II)-treated *C. crescentus* cells (Fig. 4). It implies that 0.008 mM of Ni(II) impaired cell proliferation; however, DNA replication process remain unaffected. The morphology of 0.008 mM Ni(II)-treated cells also suggest that a large portion of the cells are captured at the predivisional stage (Fig. 2a) after completing replication process normally. The morphological manifestations of cell polarity are essential for the life cycle of *C. crescentus* (Lam et al. 2005). The bacterial actin homolog, MreB, is involved in generating cell polarity in *C. crescentus* and *E. coli* (Carballido-López 2006; Gitai et al. 2004). We speculate that 0.008 mM Ni(II)-treated *C. crescentus* cells were unable to establish correct polarity due to the abnormal localization of MreB. Because of mislocalization of MreB, cells were arrested at predivisional stage without further division. In contrast to Ni(II) stress, upon treatment with 100 mM NaCl or 4% ethanol cells grew into long filaments and accumulated seven to eight chromosomes within 8 h. It demonstrated that cells continued to undergo DNA replication, chromosome segregation, and cellular growth; however, cell division was ceased (Heinrich et al. 2016).

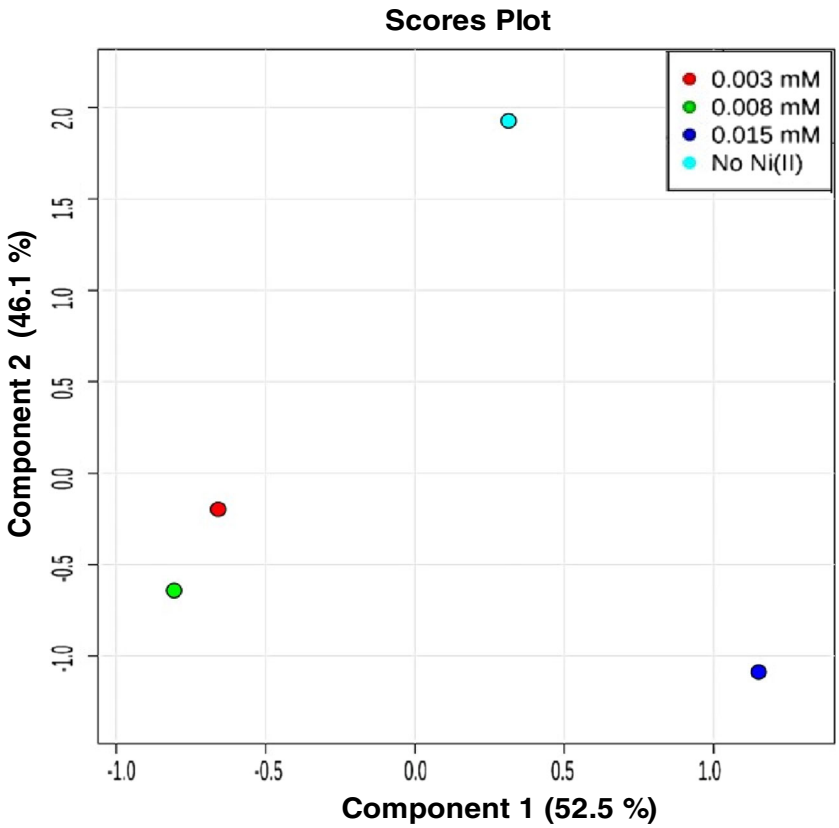

Fig. 6 Ni(II) stress affected *C. crescentus* metabolome. Partial least square discriminant analysis of GC-MS metabolite profiles of untreated cells (light blue) and cells treated with 0.003 mM (red), 0.008 mM (green), or 0.015 mM (dark blue) Ni(II) for 4 h

Role of organic acids and amino acids in Ni(II) stress response of *C. crescentus*

Metabolomic profiling can be useful to gain insight into the mechanism of cell cycle dysregulation in Ni(II)-stressed *C. crescentus*. Aminomalonic (Copley et al. 1992) and 3,4,5-trihydroxypentanoic acid (Kroeger et al. 2003) have been reported as a product of stress damage. We could interpret that the increased level of aminomalonic acid is associated with errors in protein

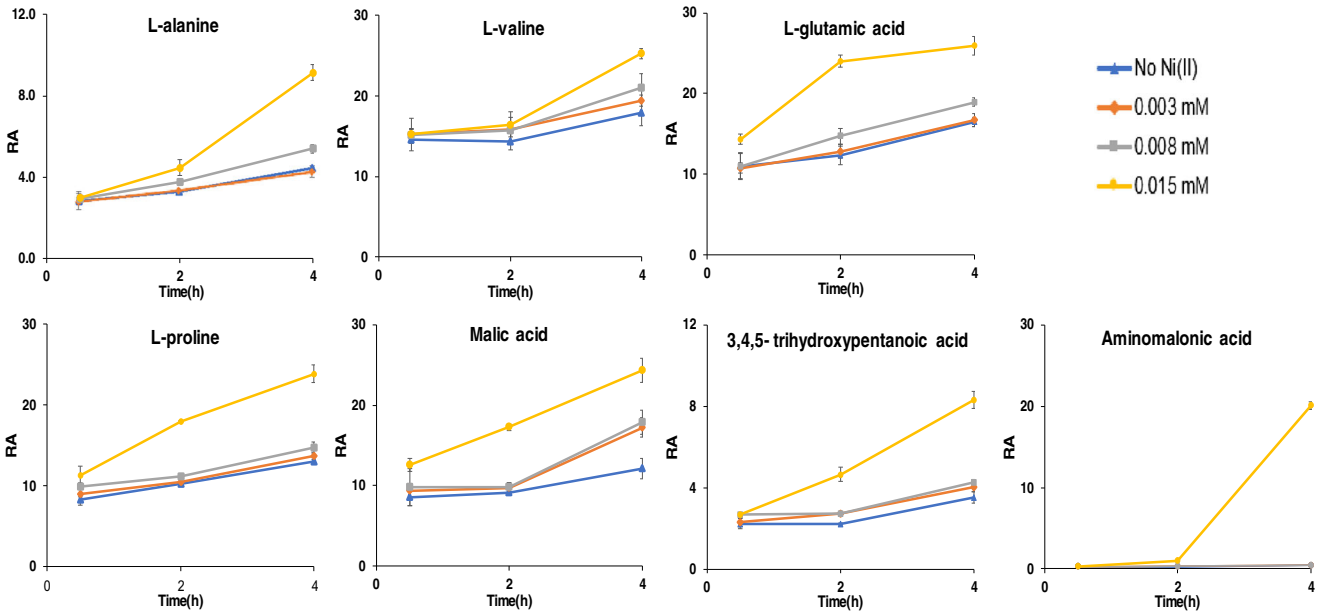

Fig. 7 Relative abundance of metabolites in 0.003, 0.008, and 0.015 mM Ni(II)-treated *C. crescentus* cells as compared to untreated cells: changes in the TCA cycle metabolite malic acid, amino acid metabolites (alanine, proline, glutamic acid, valine, aminomalonic acid), and levels of 3,4,5-trihydroxypentanoic acid. The error bars indicate the SD of three biological replicates

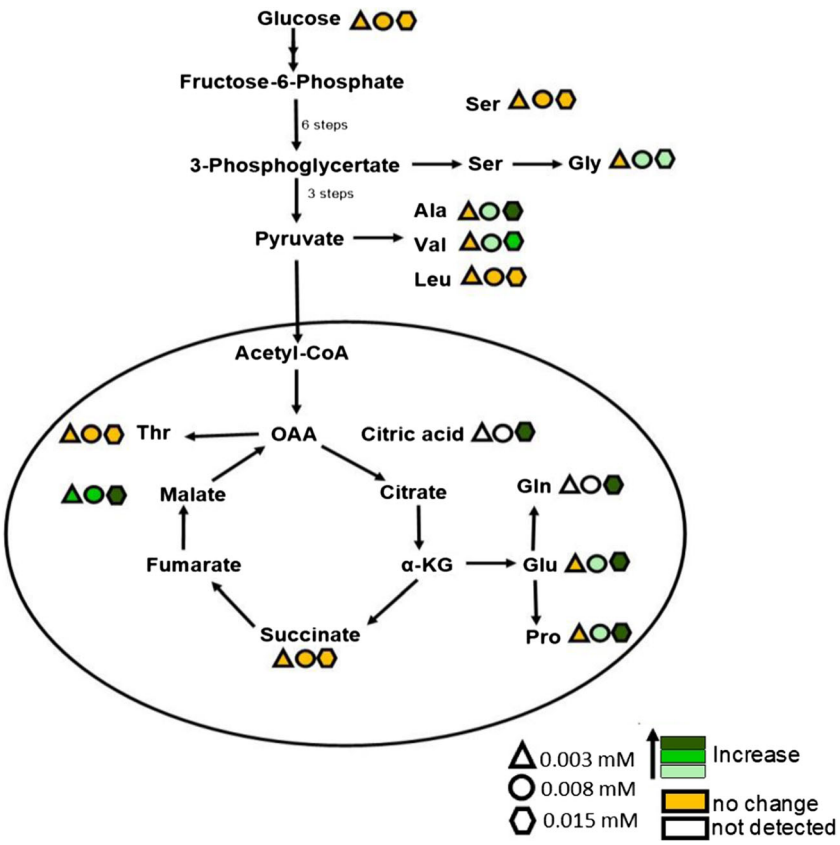

Fig. 8 Metabolic changes in *C. crescentus* exposed to 0.003, 0.008, and 0.015 mM Ni(II) for 4 h mapped onto metabolic pathways. Colored symbols represent an increase or no change in the metabolite level at each Ni(II) concentration as compared to untreated *C. crescentus*. Open symbol represents metabolites below detectable level

synthesis and oxidative damage to amino acid residues in proteins whereas 3,4,5-trihydroxypentanoic acid production is related with DNA damage process in Ni(II)-stressed *C. crescentus*.

Increased levels of amino acids and organic acids have previously been associated with increased tolerance to various stress conditions (Jespersen et al. 2017; Singh et al. 2016; Sharma and Dietz 2005; Lee et al. 1978). The higher accumulation of amino acids and organic acids may be a result of a major shift in metabolism and activation of defense mechanism in Ni(II)-stressed *C. crescentus*. The restoration of growth upon exogenous addition of proline, glutamine, alanine, malic acid, and citric acid in 0.015 mM Ni(II)-stressed *C. crescentus* cells affirmed their role in enhancing Ni(II)

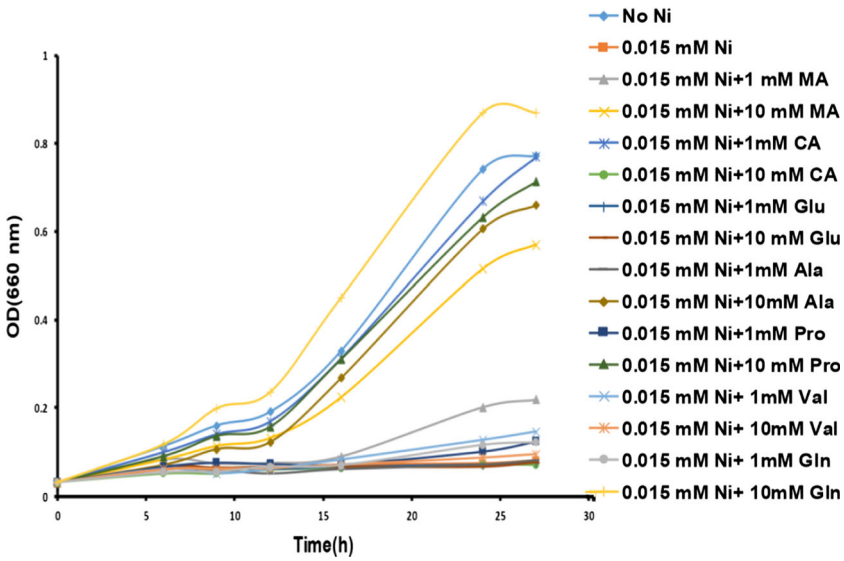

Fig. 9 Effect of exogenous addition of 1 or 10 mM of malic acid or citric acid or proline or alanine or glutamic acid or glutamine or valine on the growth of 0.015 mM Ni(II) stressed *C. crescentus* Cells. Cell optical density was monitored to represent cell Ni(II) tolerance. The results shown are representative of at least three experiments

tolerance (Fig. 9). While the role of these metabolites is very well documented in plant stress conditions, there is still a dearth of literature in the case of bacteria. Previous studies on Ni-accumulating plants have demonstrated nickel's tendency to form stable complexes with amino acids and organic acids as a mechanism of detoxification (Kersten et al. 1980; Montargès-Pelletier et al. 2008; Homer et al. 1995; Sharma and Dietz 2005). Elevated levels of organic and amino acids in *C. crescentus* may involve in Ni-chelation mechanism to protect against Ni(II) toxicity-related damages. Increased levels of alanine, citric acid, and malic acid have previously shown to contribute to nickel complexation to alleviate toxicity in *Stackhousia tryonii* (Bhatia et al. 2005)

Proline is very well known for its role as an osmoprotectant in bacteria and plants (Kawahara et al. 1989; Csonka 1989; Liang et al. 2013). Furthermore, as compared to other amino acids, the proline accumulation under heavy metal stress is a more prevalent phenomenon (Talanova et al. 2000; Yusuf et al. 2002; Liang et al. 2013; Sharma and Dietz 2005). Proline is involved in the synthesis of glutathione, an important antioxidant, which has been associated with presenting resistance against heavy metal stress including Ni(II). (Siripornadulsil et al. 2002; Freeman et al. 2004; Sharma and Dietz 2009; Hoque et al. 2008). Elevated level of proline may help *C. crescentus* undergo osmotic adjustment and enhance antioxidant metabolism to secure against Ni(II) toxicity-related damages. We also propose that 0.015 mM Ni(II) induced *putA* and *glnA* expression may be a reason of improved growth in the presence of glutamine and proline. *C. crescentus* imported exogenous proline and PutA converted this proline into glutamate (Zhou et al. 2008). Glutamate is converted by glutamate dehydrogenase to alpha-ketoglutarate, an intermediate of TCA cycle, which can subsequently generate ATP via oxidative phosphorylation. GlnA (glutamine synthetase) catalyzes the reaction between ammonia and glutamate to produce glutamine which is a key nitrogen donor in nitrogen metabolism process (Harth et al. 2005). It could be conceived that *C. crescentus* can take up exogenous proline and glutamine but not glutamic acid. In this manner, proline and glutamine encouraged metabolic stimulation through PutA/GlnA pathway may be an explanation behind improved resilience of *C. crescentus* against Ni(II). Cheng et al. (2014) reported another role of proline and glutamine in accelerating degradation of DNA-binding protein CtrA in alpha proteobacterium *E. chaffeensis*. Ni(II) treatment has significantly increased the expression of *ctrA* in *C. crescentus* (Fig. 3a). The chromosome replication is tightly controlled by CtrA, and it must be deactivated to start the process (Reisenauer and Shapiro 2002). We speculate that like *E. chaffeensis*, increased level of glutamine and proline might be associated with the accelerated degradation of CtrA to facilitate replication process in 0.015 mM Ni(II)-stressed *C. crescentus*.

The connection of carbon and nitrogen metabolism with cell growth and division has been established (Ron et al. 1977; Monahan et al. 2014; Beaufay et al. 2015). We perceive that changes in amino acids and TCA cycle intermediates are linked with the cell cycle dysregulation in Ni(II)-stressed *C. crescentus*. Elevated level of malic acid and citric acid might affect FtsZ assembly. Our argument could be supported by a recently highlighted connection between central carbon metabolism and cell division in *B. subtilis* (Monahan et al. 2014). *B. subtilis* Z-ring assembly is regulated by pyruvate; thus, it was a key metabolite in the coordination of bacterial growth with cell division. In another study, glutamate dehydrogenase(GdhZ) was involved in the molecular mechanism that coordinates central metabolism with cell division in *C. crescentus* (Beaufay et al. 2015). GdhZ also acts as a bridging molecule between nitrogen cycle and TCA cycle. GdhZ directly interferes with FtsZ polymerization and its catabolic activity is mainly controlled by glutamate. It is conceivable that change in glutamic acid concentration affect GdhZ activity which may lead to mislocalization of FtsZ in Ni(II)-treated *C. crescentus*. Furthermore, Beaufay et al. (2015) reported that alanine suppressed the growth and morphology defect in ΔgdhZ *C. crescentus* cells which concurs with the protective effect of alanine on Ni(II) toxicity.

In summary, Ni(II) toxicity severely affected growth, morphology, and cell cycle of *C. crescentus*. The use of GC-MS metabolomics has enabled us to understand Ni(II)-induced changes in metabolism and biochemical pathways of *C. crescentus*. The results suggest that changes in carbon and nitrogen metabolism are linked with the dysregulation of the cell cycle. Also, metabolite profiling helped us identify the role of amino acids and organic acids in ameliorating Ni(II) toxicity. However, more studies to uncover the exact mechanism of protective effect of amino acids and organic acid on Ni(II) toxicity is required. For example, gene manipulations or reporter gene assay to check the role of central metabolism and PutA/GlnA pathway, pulse chase experiment to calculate the half-life of CtrA. Knowledge from this study could aid future work to understand bacterial responses to environmental stress conditions.

Acknowledgements The authors would like to thank the Nanyang Environment and Water Research Institute (NEWRI), Singapore, and the Interdisciplinary Graduate School (IGS), Nanyang Technological University, Singapore, for the award of research scholarship to Abhishek Jain and the support for this research. We would also like to thank Asst. Prof. M.H. Tan (Nanyang Technological University) for supplying us strains.

Compliance with ethical standards

Competing interests The authors declare that they have no conflicts of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

Beaufay F, Coppine J, Mayard A, Laloux G, De Bolle X, Hallez R (2015) A NAD-dependent glutamate dehydrogenase coordinates metabolism with cell division in *Caulobacter crescentus*. EMBO J 34(13): 1786–1800. https://doi.org/10.15252/embj.201490730

Bhatia NP, Walsh KB, Baker AJ (2005) Detection and quantification of ligands involved in nickel detoxificationn in a herbaceous Ni hyperaccumulator *Stackhousia tryonii* Bailey. J Exp Bot 56(415): 1343–1349

Bongaerts G, Uitzetter J, Brouns R, Vogels G (1978) Uricase of *Bacillus fastidiosus* properties and regulation of synthesis. Biochim Biophys Acta 527(2):348–358

Booth SC, Weljie AM, Turner RJ (2015) Metabolomics reveals differences of metal toxicity in cultures of *Pseudomonas pseudoalcaligenes* KF707 grown on different carbon sources. Front Microbiol 6(827). https://doi.org/10.3389/fmicb.2015.00827

Boutte CC, Henry JT, Crosson S (2012) ppGpp and polyphosphate modulate cell cycle progression in *Caulobacter crescentus*. J Bacteriol 194(1):28–35. https://doi.org/10.1128/JB.05932-11

Britos L, Abeliuk E, Taverner T, Lipton M, McAdams H, Shapiro L (2011) Regulatory response to carbon starvation in *Caulobacter crescentus*. PLoS One 6(4):e18179. https://doi.org/10.1371/journal.pone.0018179

Carballido-López R (2006) The bacterial actin-like cytoskeleton. Microbiol Mol Biol Rev 70(4):888–909. https://doi.org/10.1128/MMBR.00014-06

Cheng Z, Lin M, Rikihisa Y (2014) *Ehrlichia chaffeensis* proliferation begins with NtrY/NtrX and PutA/GlnA upregulation and CtrA degradation induced by proline and glutamine uptake. mBio 5(6): e02141-14. https://doi.org/10.1128/mBio.02141-14

Copley SD, Frank E, Kirsch WM, Koch TH (1992) Detection and possible origins of aminomalonic acid in protein hydrolysates. Anal Biochem 201(1):152–157. https://doi.org/10.1016/0003-2697(92)90188-D

Csonka LN (1989) Physiological and genetic responses of bacteria to osmotic stress. Microbiol Rev 53(1):121–147

Curtis PD, Brun YV (2010) Getting in the loop: regulation of development in *Caulobacter crescentus*. Microbiol Mol Biol Rev 74(1):13–41. https://doi.org/10.1128/MMBR.00040-09

Das KK, Das SN, Dhundasi SA (2008) Nickel, its adverse health effects & oxidative stress. Indian J Med Res 128(4):412–425

Debussche L, Couder M, Thibaut D, Cameron B, Crouzet J, Blanche F (1992) Assay, purification, and characterization of cobaltochelatase, a unique complex enzyme catalyzing cobalt insertion in hydrogenobyrinic acid a,c-diamide during coenzyme B12 biosynthesis in *Pseudomonas denitrificans*. J Bacteriol 174(22):7445–7451

Denkhaus E, Salnikow K (2002) Nickel essentiality, toxicity, and carcinogenicity. Crit Rev Oncol Hematol 42(1):35–56

Figge RM, Easter J, Gober JW (2003) Productive interaction between the chromosome partitioning proteins, ParA and ParB, is required for the progression of the cell cycle in *Caulobacter crescentus*. Mol Microbiol 47(5):1225–1237. https://doi.org/10.1046/j.1365-2958.2003.03367.x

Figge RM, Divakaruni AV, Gober JW (2004) MreB, the cell shape-determining bacterial actin homologue, co-ordinates cell wall morphogenesis in *Caulobacter crescentus*. Mol Microbiol 51(5):1321–1332. https://doi.org/10.1111/j.1365-2958.2003.03936.x

Freeman JL, Persans MW, Nieman K, Albrecht C, Peer W, Pickering IJ, Salt DE (2004) Increased glutathione biosynthesis plays a role in nickel tolerance in Thlaspi nickel hyperaccumulators. Plant Cell 16(8):2176–2191. https://doi.org/10.1105/tpc.104.023036

Gitai Z, Dye N, Shapiro L (2004) An actin-like gene can determine cell polarity in bacteria. Proc Natl Acad Sci U S A 101(23):8643–8648. https://doi.org/10.1073/pnas.0402638101

Goley ED, Yeh YC, Hong SH, Fero MJ, Abeliuk E, McAdams HH, Shapiro L (2011) Assembly of the *Caulobacter* cell division machine. Mol Microbiol 80(6):1680–1698. https://doi.org/10.1111/j.1365-2958.2011.07677.x

Harth G, Maslesa-Galic S, Tullius MV, Horwitz MA (2005) All four *Mycobacterium tuberculosis glnA* genes encode glutamine synthetase activities but only GlnA1 is abundantly expressed and essential for bacterial homeostasis. Mol Microbiol 58(4):1157–1172. https://doi.org/10.1111/j.1365-2958.2005.04899.x

He ZL, Yang XE, Stoffella PJ (2005) Trace elements in agroecosystems and impacts on the environment. J Trace Elem Med Biol 19(2–3): 125–140. https://doi.org/10.1016/j.jtemb.2005.02.010

Heinrich K, Sobetzko P, Jonas K (2016) A kinase-phosphatase switch transduces environmental information into a bacterial cell cycle circuit. PLoS Genet 12(12):e1006522. https://doi.org/10.1371/journal.pgen.1006522

Herawati N, Suzuki S, Hayashi K, Rivai IF, Koyama H (2000) Cadmium, copper and zinc levels in rice and soil of Japan, Indonesia and China by soil type. Bull Environ Contam Toxicol 64(1):33–39. https://doi.org/10.1007/s001289910006

Hillson NJ, Hu P, Andersen GL, Shapiro L (2007) *Caulobacter crescentus* as a whole-cell uranium biosensor. Appl Environ Microbiol 73(23): 7615–7621. https://doi.org/10.1128/AEM.01566-07

Homer FA, Reeves R, Brooks RR (1995) The possible involvement of amino acids in nickel chelation in some nickel accumulating plants. Curr Top Phytochem 14:31–33

Hoque MA, Banu MN, Nakamura Y, Shimoishi Y, Murata Y (2008) Proline and glycinebetaine enhance antioxidant defense and methylglyoxal detoxification systems and reduce NaCl-induced damage in cultured tobacco cells. J Plant Physiol 165(8):813–824. https://doi.org/10.1016/j.jplph.2007.07.013

Hu P, Brodie EL, Suzuki Y, McAdams HH, Andersen GL (2005) Whole-genome transcriptional analysis of heavy metal stresses in *Caulobacter crescentus*. J Bacteriol 187(24):8437–8449. https://doi.org/10.1128/JB.187.24.8437-8449.2005

Hughes V, Jiang C, Brun Y (2012) *Caulobacter crescentus*. Curr Biol 22(13):507–509

Jensen RB, Wang SC, Shapiro L (2002) Dynamic localization of proteins and DNA during a bacterial cell cycle. Nat Rev Mol Cell Biol 3(3): 167–176. https://doi.org/10.1038/nrm758

Jespersen D, Yu J, Huang B (2017) Metabolic effects of acibenzolar-*S*-methyl for improving heat or drought stress in creeping bentgrass. Front Plant Sci 8(1224). https://doi.org/10.3389/fpls.2017.01224

Jonas K, Liu J, Chien P, Laub MT (2013) Proteotoxic stress induces a cell cycle arrest by stimulating Lon to degrade the replication initiator DnaA. Cell 154(3):623–636. https://doi.org/10.1016/j.cell.2013.06.034

Kalliri E, Grzyska PK, Hausinger RP (2005) Kinetic and spectroscopic investigation of CoII, NiII, and N-oxalylglycine inhibition of the FeII/alpha-ketoglutarate dioxygenase, TauD. Biochem Biophys Res Commun 338(1):191–197. https://doi.org/10.1016/j.bbrc.2005.08.223

Kawahara Y, Ohsumi T, Yoshihara Y, Ikeda S (1989) Proline in the osmoregulation of *Brevibacterium lactofermentum*. Agric Biol Chem 53(9):2475–2479

Kersten WJ, Brooks RR, Reeves RD, Jaffré A (1980) Nature of nickel complexes in *Psychotria douarrei* and other nickel-accumulating plants. Phytochemistry 19(9):1963–1965. https://doi.org/10.1016/0031-9422(80)83013-5

Kroeger KM, Hashimoto M, Kow YW, Greenberg MM (2003) Cross-linking of 2-deoxyribonolactone and its beta-elimination product by base excision repair enzymes. Biochemistry 42(8):2449–2455. https://doi.org/10.1021/bi027168c

Lam H, Schofield WB, Jacobs-Wagner C (2005) A landmark protein essential for establishing and perpetuating the polarity of a bacterial cell. Cell 124(5):1011–1023. https://doi.org/10.1016/j.cell.2005.12.040

Lee J, Reeves RD, Brooks RR, Jaffré T (1978) The relation between nickel and citric acid in some nickel-accumulating plants. Phytochemistry 17(6):1033–1035. https://doi.org/10.1016/S0031-9422(00)94274-2

Leslie DJ, Heinen C, Schramm FD, Thüring M, Aakre CD, Murray SM, Laub MT, Jonas K (2015) Nutritional control of DNA replication initiation through the proteolysis and regulated translation of DnaA. PLoS Genet 11(7):e1005342. https://doi.org/10.1371/journal.pgen.100534

Liang X, Zhang L, Natarajan SK, Becker DF (2013) Proline mechanisms of stress survival. Antioxid Redox Signal 19(9):998–1011. https://doi.org/10.1089/ars.2012.5074

Louwrier A, Knowles CJ (1996) The purification and characterization of a novel D-specific carbamoylase enzyme from an *Agrobacterium* sp. Enzym Microb Technol 19(8):562–571. https://doi.org/10.1016/0141-0229(95)00044-5

Macomber L, Hausinger RP (2011) Mechanisms of nickel toxicity in microorganisms. Metallomics 3(11):1153–1162. https://doi.org/10.1039/c1mt00063b

Mohapatra SS, Fioravanti A, Biondi EG (2014) DNA methylation in *Caulobacter* and other *Alphaproteobacteria* during cell cycle progression. Trends Microbiol 22(9):528–535. https://doi.org/10.1016/j.tim.2014.05.003

Mohl DA, Easter J, Gober JW (2001) The chromosome partitioning protein, ParB, is required for cytokinesis in *Caulobacter crescentus*. Mol Microbiol 42(3):741–755. https://doi.org/10.1046/j.1365-2958.2001.02643.x

Monahan LG, Hajduk IV, Blaber SP, Charles IG, Harry EJ (2014) Coordinating bacterial cell division with nutrient availability: a role for glycolysis. mBio 5(3):e00935-14. https://doi.org/10.1128/mBio.00935-14

Montargès-Pelletier E, Chardot V, Echevarria G, Michot LJ, Bauer A, Morel JL (2008) Identification of nickel chelators in three hyperaccumulating plants: an X-ray spectroscopic study. Phytochemistry 69(8):1695–1709. https://doi.org/10.1016/j.phytochem.2008.02.009

Mulrooney SB, Hausinger RP (2003) Nickel uptake and utilization by microorganisms. FEMS Microbiol Rev 27(2–3):239–261. https://doi.org/10.1016/S0168-6445(03)00042-1

Navarro C, Wu LF, Mandrand-Berthelot MA (1993) The nik operon of *Escherichia coli* encodes a periplasmic binding-protein-dependent transport system for nickel. Mol Microbiol 9(6):1181–1191. https://doi.org/10.1111/j.1365-2958.1993.tb01247.x

Niemirowicz G, Parussini F, Agüero F, Cazzulo JJ (2007) Two metallocarboxypeptidases from the protozoan *Trypanosoma cruzi* belong to the M32 family, found so far only in prokaryotes. Biochem J 401(2):399–410. https://doi.org/10.1042/BJ20060973

Patel J, Zhang Q, McKay RM, Vincent R, Xu Z (2010) Genetic engineering of *Caulobacter crescentus* for removal of cadmium from water. Appl Biochem Biotechnol 160(1):232–243. https://doi.org/10.1007/s12010-009-8540

Reisenauer A, Shapiro L (2002) DNA methylation affects the cell cycle transcription of the CtrA global regulator in *Caulobacter*. EMBO J 21(18):4969–4977. https://doi.org/10.1093/emboj/cdf490

Rodrigue A, Effantin G, Mandrand-Berthelot MA (2005) Identification of *rcnA* (*yohM*), a nickel and cobalt resistance gene in *Escherichia coli*. J Bacteriol 187(8):2912–2916. https://doi.org/10.1128/JB.187.8.2912-2916.2005

Ron E, Grossman N, Helmstetter CE (1977) Control of cell division in *Escherichia coli*: effect of amino acid starvation. J Bacteriol 129(2):569–573

Sato K, Okubo A, Yamazaki S (1998) Characterization of a multi-copper enzyme, nitrous oxide reductase, from *Rhodobacter sphaeroides* f. sp. *denitrificans*. J Biochem 124(1):51–54. https://doi.org/10.1093/oxfordjournals.jbchem.a022096

Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, Preibisch S, Rueden C, Saalfeld S, Schmid B, Tinevez JY, White DJ, Hartenstein V, Eliceiri K, Tomancak P, Cardona A (2012) Fiji: an open-source platform for biological-image analysis. Nat Methods 9(7):676–682. https://doi.org/10.1038/nmeth.2019

Sharma SS, Dietz KJ (2005) The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. J Exp Bot 57(4):711–726. https://doi.org/10.1093/jxb/erj073

Sharma SS, Dietz KJ (2009) The relationship between metal toxicity and cellular redox imbalance. Trends Plant Sci 14(1):43–50. https://doi.org/10.1016/j.tplants.2008.10.007

Singh S, Parihar P, Singh R, Singh VP, Prasad SM (2016) Heavy metal tolerance in plants: role of transcriptomics, proteomics, metabolomics, and ionomics. Front Plant Sci 6(1143). https://doi.org/10.3389/fpls.2015.01143

Siripornadulsil S, Traina S, Verma DP, Sayre RT (2002) Molecular mechanisms of proline-mediated tolerance to toxic heavy metals in transgenic microalgae. Plant Cell 14(11):2837–2847. https://doi.org/10.1105/tpc.004853

Stähler FN, Odenbreit S, Haas R, Wilrich J, Van Vliet AH, Kusters JG, Kist M, Bereswill S (2006) The novel *Helicobacter pylori* CznABC metal efflux pump is required for cadmium, zinc, and nickel resistance, urease modulation, and gastric colonization. Infect Immun 74(7):3845–3852. https://doi.org/10.1128/IAI.02025-05

Talanova V, Titov A, Boeva N (2000) Effect of increasing concentration of lead and cadmium on cucumber seedlings. Biol Plant 43(3):441–444. https://doi.org/10.1023/A:1026735603890

Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ (2012) Heavy metals toxicity and the environment. EXS 101:133–164. https://doi.org/10.1007/978-3-7643-8340-4_6

Thanbichler M, Shapiro L (2006) MipZ, a spatial regulator coordinating chromosome segregation with cell division in *Caulobacter*. Cell 126(1):147–162. https://doi.org/10.1016/j.cell.2006.05.038

US EPA (2014) Priority Pollutant List. https://www.epa.gov/sites/production/files/2015-09/documents/priority-pollutant-list-epa.pdf

Viollier PH, Thanbichler M, McGrath PT, West L, Meewan M, McAdams HH, Shapiro L (2004) Rapid and sequential movement of individual chromosomal loci to specific subcellular locations during bacterial DNA replication. Proc Natl Acad Sci U S A 101(25):9257–9262

Wang M, Bai J, Chen WN, Ching CB (2010) Metabolomic profiling of cellular responses to carvedilol enantiomers in vascular smooth muscle cells. PLoS One 5(11):e15441. https://doi.org/10.1371/journal.pone.0015441

Xia J, Sinelnikov IV, Han B, Wishart DS (2015) MetaboAnalyst 3.0—making metabolomics more meaningful. Nucleic Acids Res 43(W1):W251–W257. https://doi.org/10.1093/nar/gkv380

Yusuf M, Fariduddin Q, Ahmad A (2002) 24-Epibrassinolide modulates growth, nodulation, antioxidant system, and osmolyte in tolerant and sensitive varieties of Vigna radiata under different levels of nickel: a shotgun approach. Plant Physiol Biochem 57:143–153. https://doi.org/10.1016/j.plaphy.2012.05.004

Yusuf M, Fariduddun Q, Hayat S, Ahmad A (2011) Nickel: an overview of uptake, essentiality and toxicity in plants. Bull Environ Contam Toxicol 86(1):1–17. https://doi.org/10.1007/s00128-010-0171-1

Zhou Y, Larson JD, Bottoms CA, Arturo EC, Henzl MT, Jenkins JL, Nix JC, Becker DF, Tanner JJ (2008) Structural basis of the transcriptional regulation of the proline utilization regulon by multifunctional PutA. J Mol Biol 381(1):174–188. https://doi.org/10.1016/j.jmb.2008.05.084