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Synechococcus elongatus PCC 7942 is more tolerant to chromate as compared to *Synechocystis* sp. PCC 6803

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Abstract Two unicellular cyanobacteria *Synechocystis* sp. PCC 6803 and *Synechococcus elongatus* PCC 7942 showed contrasting responses to chromate stress with EC_{50} of 12 ± 2 and 150 ± 15 μM potassium dichromate respectively. There was no depletion of chromate in growth medium in both the cases. Using labeled chromate, very low accumulation (<1 nmol/10^8 cells) was observed in *Synechocystis* after incubation for 24 h in light. No accumulation of chromate could be observed in *Synechococcus* under these conditions. Chromate oxyanion is known to enter the cells using sulfate uptake channels. Therefore, inhibition of sulfate uptake caused by chromate was monitored using ^{35}S labeled sulfate. IC_{50} values of chromate for ^{35}sulfate uptake were higher in *Synechococcus* as compared to *Synechocystis*. The results suggested that the sulfate transporters in *Synechococcus* have lower affinity to chromate than those from *Synechocystis* possibly due to differences in affinity of sulfate receptors for chromate. Bioinformatic analyses revealed presence of sulfate and chromate transporters with considerable similarity; however, minor differences in these may play a role in their differential response to chromate. In both cases the IC_{50} values decreased when sulfate concentration was reduced in the medium indicating competitive inhibition of sulfate uptake by chromate. Interestingly, *Synechococcus* showed stimulation of growth at concentrations of chromate less than 100 μM, which affected its cell size without disturbing the ultrastructure and thylakoid organization. In *Synechocystis,* growth with 12 μM potassium dichromate damaged the ultrastructure and thylakoid organization with slight elongation of the cells. The results suggested that *Synechococcus* possesses efficient strategies to prevent entry and to remove chromate from the cell as compared to *Synechocystis*. This is the first time a differential response of *Synechococcus* 7942 and *Synechocystis* 6803 to chromate is reported. The contrasting characteristics observed in the two cyanobacteria will be useful in understanding the basis of resistance or susceptibility to chromate.

Keywords Chromate-resistance · Cyanobacteria · IC_{50} chromate · Sulfate-uptake · *Synechococcus* PCC 7942 · *Synechocystis* PCC 6803

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Abbreviations

EC_{50} of chromate	Concentration of potassium dichromate at which number of cells ml^{-1} was 50 % as compared to control
IC_{50} of chromate	Concentration of chromate required for 50 % reduction in uptake of sulfate as compared to control
OD	Optical density

Introduction

Chromium is a member of transition metals and exhibits various oxidation states from +2 to +6, of which +3 and +6 are predominant in chromium compounds. Hexavalent chromium (Cr^{6+}) is highly soluble and hence toxic; it usually exists as oxyanions such as chromate (CrO_4^{2-}) and dichromate ($Cr_2O_7^{2-}$) whereas the trivalent form (Cr^{3+}) is less soluble, less toxic and is found in the form of oxides, hydroxides or sulfates (Cheung and Gu 2007). The hexavalent form is released in the environment as aqueous waste from leather, paints, electroplating and other industries. Chromate is highly mobile and hence available, resulting in biological toxicity mainly due to oxidative damage to biomolecules (Cervantes et al. 2001). Microorganisms have developed different strategies to thrive in the presence of chromate in the aquatic environment. One of these strategies deals with reduction of chromate to the less toxic chromium(III) by chromate reductase identified in diverse bacterial species (Cervantes et al. 2001). Another strategy in prokaryotes uses chromate efflux system using a plasmid encoded gene, *chrA*. ChrA belongs to a small family of proteins (CHR), which occur in bacteria and archaea and represents a novel kind of prokaryotic proton motive force driven chromate transporters. Several members of CHR superfamily have been shown to confer resistance to chromate (Nies et al. 1998; Alvarez et al. 1999; Rami'rez-Diaz et al. 2008).

Among photoautotrophs, cyanobacteria and algae isolated from chromate contaminated sites or some mutants are able to tolerate chromate to varied extent (Garnham and Green 1995; Khattar et al. 2004; Anjana et al. 2007; Yewalkar et al. 2007; Kiran et al. 2007, 2008; Ozturk et al. 2009). Some of these adsorb chromate on the surface or reduce chromate to chromium(III). Recently an investigation into mechanism of chromate resistance in *Synechococcus elongatus* showed that it possesses a homologue of chromate transporter gene *srpC* on pANL plasmid which harbors genes of sulfur metabolism (Aguilar-Barajas et al. 2012). Although overexpression of *srpC* conferred chromate resistance to *E. coli* by reducing chromate uptake, it did not complement *E. coli cysA* sulfate uptake mutant, suggesting that *srpC* is not sulfate transporter. Since chromate oxyanion is structurally related to sulfate, chromate actively crosses biological membranes by means of the sulfate uptake pathway (Rami'rez-Diaz et al. 2008; Aguilar-Barajas et al. 2011). This results in sulfate deficiency as well as in creating oxidative stress in cells. Thus a complex relation is known to exist between sulfate and chromate transporters vis a vis the effect of chromate in prokaryotes.

Cyanobacteria are some of the oldest organisms living on the earth and have evolved mechanisms to combat various environmental stresses. The comparative study on the relative responses of different cyanobacteria to variety of stresses is an interesting field of research. The standard cultures of two taxonomically related cyanobacteria *Synechococcus* PCC 7942 and *Synechocystis* sp. PCC 6803 are known to differ in their responses to salt, light and oxidative stress (Fulda et al. 1999; Stork et al. 2005). We observed these two standard strains also showed contrasting tolerance to chromate, *Synechococcus* being more tolerant than *Synechocystis.* In this paper we report differential responses of these two cyanobacteria to chromate with respect to its effect on growth, ultrastructure, chromate and sulfate uptake. The bioinformatic analysis of chromate and sulfate transporters is also presented.

Materials and methods

Analysis of chromate toxicity

The two strains of unicellular cyanobacteria, *S. elongatus* PCC 7942 and *Synechocystis* sp. PCC 6803 (referred to as *Synechococcus* and *Synechocystis* respectively hereafter) were inoculated in 50 ml of BG-11 medium (Rippka et al. 1979) and grown at 30 °C under continuous white light of intensity

21 W m^{-2}. To study the effect of chromate on growth, $\sim 10^8$ cells of log phase culture were inoculated in BG-11 medium containing $K_2Cr_2O_7$ ranging from 0 to 200 μM and growth was monitored periodically as cell density and expressed as number of cell ml^{-1} using Neubauer's hemacytometer in the Zeiss Axio imager digital microscope. Growth was monitored as OD_{730} up to 30 days. EC_{50} value represents the concentration of potassium dichromate at which number of cells ml^{-1} was 50 % as compared to control.

Determination of ^{51}chromate accumulation

Chromate uptake was determined by using ^{51}chromium labeled chromate as a tracer. Log phase cultures of *Synechococcus* and *Synechocystis* were washed and incubated (10^8 cells ml^{-1}) in light (21 W m^{-2}) for 24 h at room temperature in fresh BG-11 medium containing 10 or 100 μM $K_2Cr_2O_7$ with 20 μCi ^{51}Cr labeled sodium chromate (0.24 μCi $nmol^{-1}$). Cells were washed with BG-11 medium followed by 1 mM EDTA, resuspended in 90 % methanol and mixed with 175 μl of Perkin-Elmer's Hidex aqualight cocktail. ^{51}Chromate in the cells was estimated by counting β emission by using liquid scintillation counter.

Determination of IC_{50} of chromate for ^{35}sulfate uptake

Inhibitory concentration (IC_{50}) of chromate for sulfate uptake was determined by monitoring uptake of ^{35}S labeled sodium sulfate. Log phase cultures of *Synechococcus* and *Synechocystis* were washed and suspended in fresh BG-11 medium (10^8 cells ml^{-1}) containing 30 or 300 μM sulfate along with 40 μCi ^{35}sodium sulfate (28.6 μCi μmol^{-1}) and increasing concentration of chromate (0–10 mM) for 2 h in light at room temperature. Cell pellets were washed with BG-11 medium followed by 1 mM EDTA. The washed cells were resuspended in 90 % methanol and mixed with 175 μl of Perkin-Elmer's Hidex aqualight cocktail. ^{35}Sulfate in the cells was measured by liquid scintillation counting.

Transmission electron microscopy

Synechococcus and *Synechocystis* cells were grown with different concentrations of potassium dichromate for 9 days as indicated in figures. The cells were harvested and washed with sodium phosphate buffer (100 mM, pH 7.4), fixed with 0.5 % glutaraldehyde—2 % paraformaldehyde for 2 h at room temperature followed by washing with water. Serial dehydration was carried out using 35, 50, 75 and 100 % ethanol for 30 min each. Ethanol was removed by incubation with propylene oxide for 3 h followed by further incubation with 3:1, 1:1, 1:3 (v/v propylene oxide:araldite) for 2 h each. The samples were infiltrated with Araldite for 16 h and embedded in it by incubation at 60 °C for 72 h. Thin (70 nm) sections were contrasted for 15 min with 10 % uranyl acetate in 50 % methanol followed by staining with lead citrate (Reynolds 1963) for 2 min. The sections were viewed under Libra 120 keV transmission electron microscope.

Sequence comparison of sulfate and chromate transporters

Amino acid sequences of sulfate and chromate transporters from *Synechococcus* and *Synechocystis* were downloaded from cyanobase (http://genome.kazusa.or.jp/cyanobase) and compared with similar well characterized genes from other bacteria. Alignment and analyses of proteins were performed using BioEdit v7.0.8. Conserved domains were obtained using conserved domain database (CDD) available at http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml with CDDv2.3.CDD) and simple modular architecture research tool (SMART) available at http://smart.embl-heidelberg.de.

Results

Differential effect of chromate on growth of *Synechococcus* and *Synechocystis*

When log phase culture of *Synechococcus* and *Synechocystis* were inoculated in 50 ml of BG-11 medium, the exponential growth phase started after 4 days and lasted for about 30 days in untreated *Synechococcus* where as in case of untreated *Synechocystis* the log phase lasted for 15 days after which slow decline in growth was observed under similar growth conditions. The two organisms showed comparable growth rates in absence of chromate with doubling time in the range of 40–50 h till 15 days period indicating that both were growing normally

under the conditions used for their growth. Growth was monitored as OD_{730} and also by measuring number of cells ml^{-1}. *Synechococcus* cultures showed over twice the number of cells at equivalent OD_{730} as compared to *Synechocystis* culture suggesting difference in scattering properties of the cells.

To measure the effect of chromate on cell survival and growth, $\sim 10^8$ cells were inoculated in BG-11 medium containing different concentrations of chromate and growth was monitored by cell count.

In case of *Synechocystis* survival was reduced in the presence of potassium dichromate at concentration as low as 10–20 μM. On the contrary in case of *Synechococcus*, cells could grow even in presence of 200 μM potassium dichromate (Fig. 1). In *Synechococcus* inclusion of potassium dichromate up to 100 μM in growth medium stimulated growth; highest stimulation was observed with 70–80 μM potassium dichromate. Concentration of potassium dichromate required to inhibit growth completely in *Synechococcus* was 300 μM. In case of *Synechocystis*, survival declined with increasing concentration of chromate and was completely inhibited in presence of 30 μM potassium dichromate. Since the growth rates of *Synechocystis* and *Synechococcus* were comparable under the experimental conditions, the growth inhibition seen in *Synechocystis* in presence of chromate was due to chromate toxicity rather than difference in growth characteristics between the two under normal conditions.

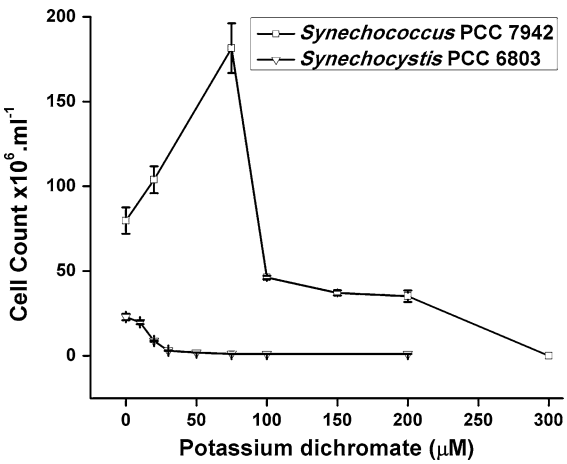

Fig. 1 Tolerance of *Synechococcus* and *Synechocystis* to potassium dichromate. BG11 medium containing increasing concentration of potassium dichromate was inoculated with log phase cultures of *Synechococcus* and *Synechocystis* containing $\sim 10^8$ cells ml^{-1}. Growth was monitored as number of cells ml^{-1}. EC_{50} for potassium dichromate was calculated after growth for 9 days. The *points* represent average of four experiments and *bars* represent SE

Since both cultures showed exponential growth from 4 to 15 days, the concentration of potassium dichromate at which number of cells ml^{-1} was 50 % as compared to control was monitored after 9 days of inoculation and was used to compare the tolerance of *Synechococcus* and *Synechocystis* to chromium stress. The 9 days EC_{50} of potassium dichromate was 12 ± 2 μM for *Synechocystis* and 150 ± 15 μM for *Synechococcus* (Fig. 1). The EC_{50} values increased with time after inoculation as well as with increase in the size of inoculum (Table 1 on line resource; Fig. 1 on line resource).

Differential accumulation of chromate by *Synechococcus* and *Synechocystis*

Synechococcus and *Synechocystis* were grown in the presence of increasing concentration of potassium dichromate. Cell free medium from each culture was obtained and chromate concentration in the medium was determined using di phenyl carbazide (DPC) reduction assay as described by Urone (1955). There was no change in chromate concentration in the supernatant of the growth medium indicating that chromate was not absorbed or reduced by both the cyanobacteria (data not shown). Chromate accumulation was also monitored using radiolabeled chromate as a tracer. In *Synechocystis* after 24 h of incubation in light, the chromate accumulation up to 0.04–0.05 nmol 10^8 $cells^{-1}$ was observed when extracellular concentration of chromate was 10 μM. This increased to 0.4 nmol 10^8 $cells^{-1}$ when chromate concentration in the medium was increased to 100 μM (Fig. 2). In contrast, there was no accumulation of chromate in *Synechococcus* cells under these conditions (Fig. 2). In *Synechocystis* there was no accumulation of chromate when the cells were boiled in the medium or when they were incubated in dark (data not shown).

Differential effect of chromate on uptake of sulfate by *Synechococcus* and *Synechocystis*

Sulfate uptake using ^{35}S labeled sodium sulfate in the presence of varying concentrations of chromate was monitored in *Synechococcus* (Fig. 3a, b) and *Synechocystis* (Fig. 3c, d) incubated in medium

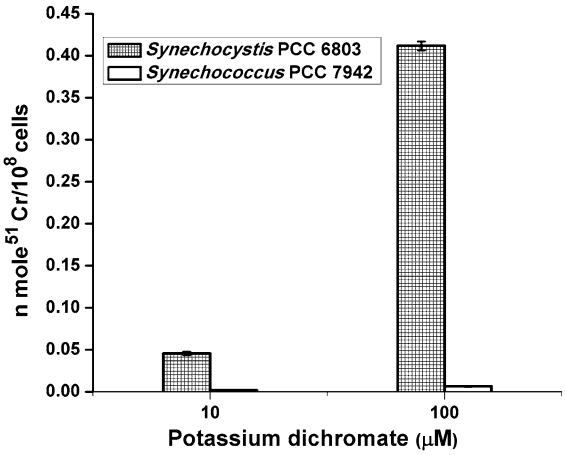

Fig. 2 Uptake of ^{51}chromate by *Synechococcus* and *Synechocystis* in light. Log phase cultures of *Synechococcus* and *Synechocystis* (10^8 cells ml^{-1}) were incubated in BG11 medium containing 10 or 100 μM of potassium dichromate with ^{51}Cr as tracer. The intracellular ^{51}Cr was measured after incubation for 24 h in light at room temperature

containing 30 and 300 μM sodium sulfate. Chromate was found to decrease the uptake of sulfate in *Synechococcus* and *Synechocystis*. When concentration of sulfate was increased from 30 to 300 μM the concentration of chromate required for IC_{50} was increased from 30.35 μM to 1.7 mM in case of *Synechococcus.* In *Synechocystis w*hen concentration of sulfate was increased from 30 to 300 μM the concentration of chromate required for IC_{50} was increased from 8.6 to 99 μM.

Differential effect of chromate on ultrastructure of *Synechococcus* and *Synechocystis*

Ultrastructural changes in *Synechococcus* and Sy*nechocystis* grown with EC_{50} concentration of potassium dichromate were observed by transmission electron microscopy. The length of cells in case of *Synechococcus* was reduced whereas there was slight elongation of cells in case of *Synechocystis* while the cell width was not affected. In *Synechococcus* the cells grown with chromate did not show extensive damage to ultrastructure (Fig. 4a, b, c). The cell wall, thylakoids and carboxysomes were not affected by the presence of dichromate in medium. However, growth in the presence of chromate resulted in reduction of length to breadth (L/B) ratio (Fig. 4f). In *Synechocystis* growth in the presence of chromate resulted in distortions in thylakoid membranes and damage to cell wall in addition to slight elongation of regular spherical shape with increase in L/B ratio (Fig. 4d, e, f).

Bioinformatic analysis of sulfate and chromate transporters in *Synechococcus elongatus* PCC 7942 *and Synechocystis* sp. PCC 6803

To investigate if the differences in the IC_{50} of chromate for sulfate uptake in the two organisms could be attributed to the differences in primary structure of the sulfate and chromate transporters, a comparative bioinformatic analysis of amino acid sequences of these proteins from *Synechococcus* and *Synechocystis* was carried out (Tables 1, 2); (Figs. 2, 3, 4, 5, 6 on line resource). The analysis included SulT permease constituting the permease, membrane proteins and ATPase; and chromate transporter ChrA. Their sequences were also compared with the sequences of well characterized sulfate and chromate uptake related proteins from other prokaryotic organisms.

Sulfate-thiosulfate transporters

Sulfate-thiosulfate (SulT) permease complex from some bacteria is well characterized and typically consists of sulfate or thiosulfate binding protein Sbp or CysP, and the proteins of ABC transporter viz CysA, ATPase, CysW and CysT. These were used as reference to identify differences among the corresponding proteins in the two organisms under study. Ten and nine genes for sulfate transporters are identified in *Synechococcus* and *Synechocystis* respectively in cyanobase (Tables 1, 2). The sulfate binding proteins (SbpA) in *Synechococcus* and *Synechocystis* have ~58.9 % identity. SbpA from these organisms showed 99 conserved amino acid residues with SbpA from *Salmonella typhimurium* (Fig. 2 on line resource). Cys A, the ATPase subunit of the SulT permease of *Synechococcus* and *Synechocystis* have ~63 % identity (Tables 1, 2). Figure 3 (on line resource) shows that there were 148 conserved amino acid residues among them and the CysA from *Pseudomonas syringae*. Bacterial CysT and CysW constitute the transport channel of the SulT permease typically with six transmembrane helices which are also present in CysT and CysW from *Synechocystis*. *Synechococcus* CysT and CysW show ~46–64 % identities with homologs from *Synechocystis*. All of

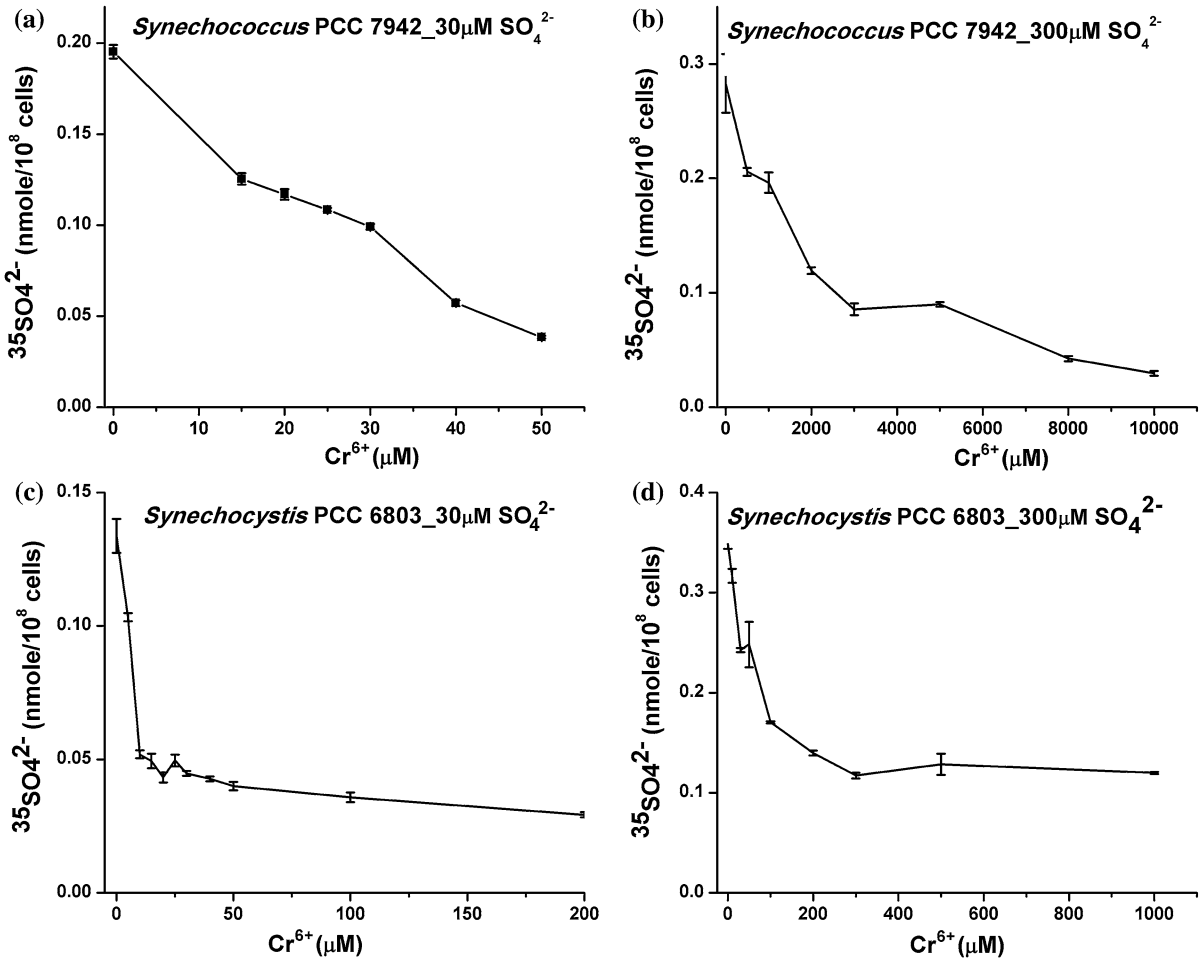

Fig. 3 Effect of chromate on uptake of ^{35}S labeled sulfate by *Synechococcus* and *Synechocystis*. Log phase cultures of *Synechococcus* and *Synechocystis* (10^8 cells ml^{-1}) were incubated in BG11 medium containing 30 or 300 μM sodium sulfate with ^{35}S labeled sodium sulfate as tracer and different concentrations of potassium dichromate as mentioned in figure. Uptake of ^{35}sulfate was monitored after 2 h. The average of four estimations is shown. *Bars* represent SE. **a** *Synechococcus* incubated in medium containing 30 μM sodium sulfate. **b** *Synechococcus* incubated in medium containing 300 μM sodium sulfate. **c** *Synechocystis* incubated in medium containing 30 μM sodium sulfate and **d** *Synechocystis* incubated in medium containing 300 μM sodium sulfate

these have six transmembrane helices except Synpcc7942_1687 (Cys T) which has seven transmembrane helices. *Synechocystis* and *Synechococcus* CysT and CysW showed 70 and 96 conserved amino acid residues with CysT and CysW from *E. coli*. (Figs. 4, 5 on line resource).

In addition to the conserved SulT components, the genomes of *Synechococcus* PCC 7942 and *Synechocystis* PCC 6803 show presence of some putative sulfate transporters, thiosulfate binding protein, low and high affinity sulfate transporter and sulfate permease (Tables 1, 2). Some of these have transmembrane domains suggesting a possible membrane location for them.

Chromate transporters

Chromate resistance in prokaryotes has been attributed to ChrA which is a chemiosmotic pump responsible for chromate efflux using proton motive pump (Alvarez et al. 1999). Two ORFs have been annotated as chromate transporters in *Synechococcus* and

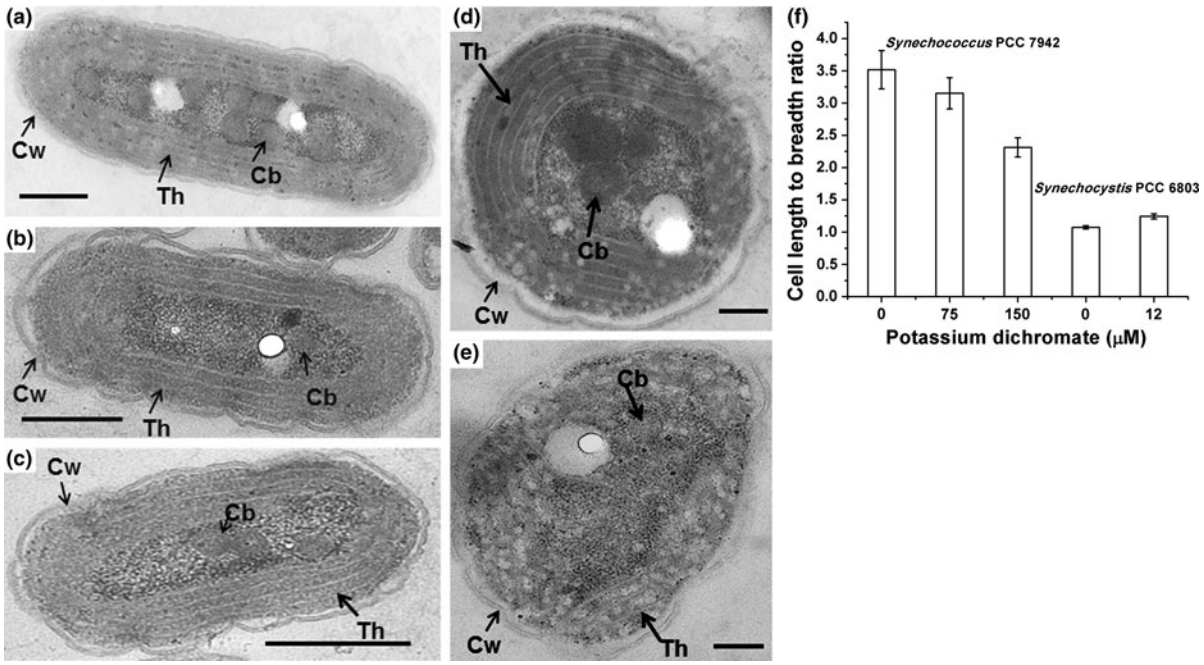

Fig. 4 Ultrastructure of *Synechococcus* and *Synechocystis* cells grown with or without potassium dichromate. Log phase cultures of *Synechococcus* and *Synechocystis* containing 10^8 cells ml^{-1} were inoculated in BG11 medium containing potassium dichromate as mentioned below. Cells were harvested after 9 days of growth and were processed for transmission electron microscopy as described in "Materials and methods" section. **a** *Synechococcus* control. **b** *Synechococcus* grown with 75 μM of potassium dichromate **c** *Synechococcus* grown with 150 μM of potassium dichromate. *Bars* represent 500 nm. **d** *Synechocystis* control. **e** *Synechocystis* grown with 12 μM of potassium dichromate. *Bars* represent 200 nm. **f** Length to breadth ratio of *Synechococcus* and *Synechocystis* grown with above mentioned chromate concentrations in the growth medium. The *values* represent mean ratio obtained from electron micrographs of 15–20 cells and *bars* represent SE. *Th* thylakoids, *Cb* carboxysomes, *Cw* cell wall

Synechocystis, one on plasmid and other on chromosome (Tables 1, 2). These showed ~29–40 % identity, and presence of two CHR domains containing homologous halves with membrane spanning regions which is a feature of long chain CHR family proteins in bacteria. The cyanobacterial chromate transporters showed 37 conserved amino acid residues when compared with chromate transporter from *Pseudomonas aeruginosa* (Fig. 6 on line resource).

Discussion

Chromate toxicity in aquatic organisms is mainly examined by exposing cultures to different concentrations of chromate for a given time, followed by investigations on physiological parameters. We compared two non-nitrogen fixing unicellular aquatic cyanobacteria belonging to order *Chroococcales* for their sensitivity to chromate when included in their growth media. Comparative analysis of chromate tolerance in *S. elongatus* PCC 7942 and *Synechocystis* PCC 6803 revealed that the former showed ~ 12 times higher tolerance to chromate than the latter, with EC_{50} values of 150 ± 15 and 12 ± 2 μM respectively. The EC_{50} values were dependent on the inoculum size in both cases indicating stoichiometric relation between chromate receptors per cell and number of chromate ions available in the growth medium. Thompson et al. (2002) have compared *Synechococcus* PCC 7942 and *Nostoc* PCC 7120 for resistance to chromate under high and low density conditions and have shown that there is general decrease in toxicity in dense cultures. Interestingly, stimulation in growth at less than 100 μM potassium dichromate was observed in *Synechococcus.* Lesser extent of ultrastructural damage in *Synechococcus* supports this argument.

In bacteria, two mechanisms of chromate tolerance are known: reduction of Cr(VI)–Cr(III) and efflux of chromate ions from cytoplasm (Rami'rez-Diaz et al. 2008).

Table 1 Comparative analysis of sulfate and chromate transporter genes in *Synechococcus*

Sulfate transporters in *Synechococcus* PCC 7942							
Sr. no.	ORF ID	Gene symbol	Definition	Aa	TM	Homologue in *Synechocystis*	% Identity
1	Synpcc7942_1681	*sbpA*	Thiosulfate-binding protein	350	Nil	slr1452	58.9
2	Synpcc7942_1680	cysA	Sulfate transport system permease protein 1	338	Nil	slr1455	63.3
3	Synpcc7942_1688	*cysW*	Sulfate ABC transporter, permease protein CysW	286	6	slr1454	51.9
4	Synpcc7942_1685	*cysW*	Sulfate transport system permease protein 2	286	6	slr1454	62.8
5	Synpcc7942_1682	*cysT*	Sulfate transport system permease protein 2	278	6	slr1453	62.5
6	Synpcc7942_1687	*cysT*	Sulfate ABC transporter, permease protein CysT	288	7	slr1453	46.1
7	Synpcc7942_1722		Thiosulfate-binding protein	361	Nil	slr1452	54.5
8	Synpcc7942_1686		Thiosulfate-binding protein	341	Nil	slr1452	42.4
9	Synpcc7942_0366		Putative sulfate transporter	727	11	slr0096	22.9
10	Synpcc7942_1380		Sulfate permease	574	11	slr1776	32.8
Chromate transporters in *Synechococcus* PCC 7942							
1	Synpcc7942_0390		Chromate transporter	383	9	slr1457	40.2
2	Synpcc7942_B2622_ plasmid 1[a]	*srpC*	Probable chromate transport transmembrane protein	393	12	slr1457	29.2

Translated amino acid sequences annotated as sulfate and chromate transporters as given in cyanobase were used for comparative analysis of *Synechococcus* PCC 7942 with *Synechocystis* PCC 6803. The values of amino acid chain length (Aa), number of transmembrane helices (TM) and % identity (as in orthologue search) for individual genes have been obtained from cyanobase

ORF ID open reading frame identity

[a] Gene is located on plasmid

Table 2 Comparative analysis of sulfate and chromate transporter genes in *Synechocystis*

Sulfate transporters in *Synechocystis* PCC 6803							
Sr. no.	ORF ID	Gene symbol	Definition	Aa	TM	Homologue in *Synechococcus*	% Identity
1	slr1452	*sbpA*	Sulfate transport system substrate-binding protein	352	Nil	Synpcc7942_1681	58.9
2	slr1455	*cysA*	Sulfate transport system ATP-binding protein	355	Nil	Synpcc7942_1680	63.3
3	sll1041	*cysA*	Similar to sulfate transport ATP-binding protein CysA	260	Nil	Synpcc7942_0350	53.1
4	slr1454	*cysW*	Sulfate transport system permease protein	276	6	Synpcc7942_1685	62.8
5	slr1453	*cysT*	Sulfate transport system permease protein	286	6	Synpcc7942_1682	62.5
6	slr1229		Sulfate permease	453	9	Synpcc7942_1380	22.7
7	slr0096		Low affinity sulfate transporter	556	11	Synpcc7942_1380	25
8	sll0834		Low affinity sulfate transporter	564	12	Synpcc7942_1380	29.1
9	slr1776		High affinity sulfate transporter	566	11	Synpcc7942_1380	32.9
Chromate transporters in *Synechocystis* PCC 6803							
1	slr1457	*chrA*	Chromate transport protein	399	9	Synpcc7942_0390	40.5
2	slr5038 pSYSM[a]		Chromate transporter	412	11	Synpcc7942_0390	36.4

Translated amino acid sequences annotated as sulfate and chromate transporters as given in cyanobase were used for comparative analysis of *Synechocystis* PCC 6803 with *Synechococcus* PCC 7942. The values of amino acid chain length (Aa), number of transmembrane helices (TM) and % identity (as in orthologue search) for individual genes have been obtained from cyanobase

ORF ID open reading frame identity

[a] Gene is located on plasmid

DPC assays showed that these two cyanobacteria were not chromate reducers. Only nanomolar concentration of chromate accumulation was detected in *Synechocystis* using ^{51}chromate and accumulation was not observed if cells were killed or incubated in dark indicating involvement of an active process in uptake of chromate (data not shown). Although in *Synechococcus*, no chromate accumulation was observed under these conditions, there was stimulation of growth along with reduction of cell length when chromate concentration in medium was less than 100 μM. This entailed that S*ynechococcus* was able to sense the presence of low concentration of chromate in the growth medium. These observations indicated that in *Synechococcus* there are efficient mechanisms for sensing and efflux of chromate. In contrast, *Synechocystis* cells showed deterioration of ultrastructure, reduction in growth and elongation of cells with 12 μM chromate in medium. Recently effects of chromate on cell morphology are reported in case of *Rhodobacter sphaeroides* by Italiano et al. (2012).

It is known that chromate ion enters the cells using sulfate transporters present in cell membrane as chromate ion is nearly identical in size shape and charge as sulfate ion (Riedel 1985). IC_{50} values of chromate in both *Synechococcus* and *Synechocystis* were dependent on sulfate concentration in medium indicating that sulfate uptake was competitively inhibited by chromate in both the cases. The inhibition of sulfate uptake by chromate suggested an interaction of chromate ions with sulfate uptake mechanism. However, the IC_{50} value of chromate was much higher in *Synechococcus* as compared to *Synechocystis* showing that *Synechocystis* has higher affinity for chromate as compared to *Synechococcus*. Sequence analysis showed that both the cyanobacteria have similar type of sulfate uptake channels and also contain similar genes related to efflux of chromate having typical bi-domain structure of LCHR. The differences in IC_{50} value of chromate suggested that the sequence variation in the non-conserved regions of sulfate uptake systems in both these organisms may be contributing to higher affinity of the sulfate uptake system for chromate in *Synechocystis* 6803 as compared to *Synechococcus* 7942. *Synechococcus elongatus* PCC 7942 possesses a plasmid (pANL) that contains a gene (*srpC*/*chrA*) conferring chromate resistance. The higher chromate susceptibility of *Synechocystis* cannot be attributed to absence of pANL as the sequence analysis shows that it has chrA homologues on its chromosome. Whether the location of chrA is responsible for the differential tolerance remains to be explored.

IC_{50} of chromate for sulfate uptake was higher in *Synechococcus* as compared to *Synechocystis* indicating that differential affinity of sulfate transporters for chromate may be contributing to the chromate tolerance in *Synechococcus* 7942 as compared to *Synechocystis* 6803. While IC_{50} for chromate at 10-fold increase in sulfate needed ~50-fold increase in chromate in case of *Synechococcus*, in case of *Synechocystis* with similar increase in sulfate, IC_{50} was attained by only ~12-fold increase in chromate concentration. Thus in *Synechococcus* the sulfate limitation by chromate would be avoided by the its sulfate transporters which have lower affinity to chromate as well as by the efficient chromate efflux systems resulting in higher EC_{50} for chromate. Although bioinformatic comparison of chromate and sulfate transporters revealed identity to a varying extent, the differences in their primary sequences could account for the difference in IC_{50} of chromate. Thus in addition to differences mentioned above, the different putative sulfate permease in these two organisms may play a role in their distinct chromate response. Chromate resistance of *Synechococcus* would give it growth advantage in chromate contaminated sites over *Synechocystis.*

Thus the multifarious interaction of sulfate/chromate transporters could be the basis of contrasting response of *Synechococcus* and *Synechocystis* to chromate.

In case of *Synechococcus* there was stimulation of growth at concentrations of chromate lower than 100 μM. It may be due to hormetic response characterized by low dose stimulation and high dose inhibition resulting in typical inverted U type dose response. Although biochemical mechanism of hormesis is not well understood it is possible that at low doses, the stressor activates repair processes which can repair the damage caused by chromate as well as other accumulated damages. Even though no chromate accumulation was seen in *Synechococcus* at low concentration, the effect on morphology and ultrastructure indicated presence of sensing mechanism and response. Another possibility may be that the reduced sulphate uptake due to chromate and subsequent adjustment of metabolic rate in *Synechococcus* has

effect on growth. Also at low concentration, chromate may interact with growth regulators, a possibility which needs to be addressed in future.

Bioinformatic analysis of sulfate and chromate transporters of *Synechococcus* and *Synechocystis* shows the complexity of these transporters. Chromate resistance is a manifestation of numerous biochemical processes governed by different genes and their homologues. The complexity in composition of sulfate and chromate transporters and subsequent metabolic adjustments to sulfate deficiency in these two strains may be the cause of differential response of these two cyanobacteria. On this back ground, exploring the molecular basis of the contrasting chromate resistance in these two organisms and also to comprehend the phenomenon of stimulation of growth and induction of morphological changes in *Synechococcus* at low concentration of chromate is a challenge in future.

Summary

The standard strains of *S. elongatus* PCC 7942 and *Synechocystis* sp. PCC 6803 showed significant differences in the EC_{50} to chromate. There was difference in uptake of chromate; the resistant *Synechococcus* prevented entry by virtue of lower affinity of its sulfate transporters to chromate (IC_{50} was higher). *Synechococcus* probably also removed chromate from the cells more efficiently as no chromium was detected in the cells. The resistant type sensed chromate at low concentration and possibly brought about changes in metabolism which resulted in stimulation of growth in terms of cell numbers. Bioinformatic analysis showed that chromate transporters are present in both the organisms; however the location of the responsible genes is different. Also, there is 29–40 % identity in the chromate transporter genes. Whether and how much, the differences in location and sequence variations contribute to the functioning of chromate transporter needs to be seen. The components of sulphate transporters from the two organisms also revealed identity in the range of ~40–60 % indicating that there is adequate scope for variation which can account for the observed differences. Although the cell length in the resistant type was reduced, the ultrastructure of the cell was protected in presence of chromate. The results indicate that the large difference in response to chromate by the two organisms was due to a multi-component process. Although the comparison is between two genera, the large difference between the two makes it a suitable system for investigations on the resistance contributing factors and their interplay.

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