

Adaptive responses and arsenic transformation potential of diazotrophic Cyanobacteria isolated from rice fields of arsenic affected Bengal Delta Plain

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Abstract In the Bengal Delta Plains (BDP) of South Asia, there is an increased report of bioaccumulation of arsenic (As) in rice grains and plants which can ultimately result in health hazards in human population consuming rice as a primary staple food. Five abundant cyanobacteria were isolated from the rice fields of BDP and maintained in vitro. The characterized isolates resembled *Leptolyngbya* sp. (isolate LBK), *Nostoc* spp. (isolates NOC and NOK) and *Westiellopsis* spp. (isolates WEC and WEK) based on polyphasic taxonomy. All the five isolates were assessed for biotransformation potential of As vis-à-vis adaptability and survivality under different levels of arsenite compared to control set of experiments. Adaptive changes of cyanobacterial photosynthetic pigments in terms of autofluorescence emission along with nitrogenase activity and exopolysaccharide production were measured for all isolates. The inorganic As absorption in terms of bioconcentration factor (BCF) in dry biomass was found to be highest in NOC (0.201–0.220), followed by NOK (0.147–0.150), WEK (0.071–0.074), WEC (0.051) and LBK (0.014) when exposed in presence of higher (200–400 μ M) to lower (100 μ M) arsenite concentrations respectively for 7 days. The

transformation of arsenite to relatively less toxic arsenate was detected in varying efficiency in all the studied isolates. When treated with 100–400 μ M arsenite, 9.58–78.4 % arsenate was detected in growth medium whereas 33–100 % in dry biomass of cyanobacterial isolates. The cyanobacterial isolates of this study could be potentially applied to reduce bioavailability of As in rice fields of South Asia based on further field trials, thereby ultimately rendering rice grains safe for human consumption.

Keywords Cyanobacteria · Bengal Delta Plain · Rice field · Arsenite oxidation

Introduction

Arsenic (As) is present in the earth crust as a natural element in varying concentration since the origin of this planet (Nordstrom 2002). This metalloid is ranked first as it is considered to be highly hazardous to various organisms including humans (Vaughan 2006). In recent decades, the environmental fate and behaviour of As has received increased attention due to ongoing crisis in parts of South Asia and South-East Asia (Nickson et al. 2000; Bhattacharya et al. 2001). Millions of people in this part of the Asian continent have been exposed to high levels of As due to intake of As-contaminated drinking water (Smedley and Kinniburgh 2002). Bioaccumulation of As has been also reported in rice grains and plants (Williams et al. 2007). This can ultimately affect human populations consuming rice as a primary staple food (Zhu et al. 2008; Meharg et al. 2009). It is now known that the use of As-contaminated ground water for irrigation is the main source behind As bioaccumulation in rice (Zhao et al. 2013; Newbigging et al. 2015). The average contribution to total As intake in human population from drinking water has been

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reported to be 13 % (Ohno et al. 2007) whereas from cooked rice it is 56 % (Zheng et al. 2013), thus clearly highlighting that rice contributes most to daily As intake in this part of the world.

Globally, rice is normally cultivated in flooded agricultural soil. The flooded rice soil ecosystem is characterized by aerobic and anaerobic zones represented in three major ecological layers (Venkataraman 1993). Inorganic As species such as arsenite [As(III)] or arsenate [As(V)] are generally more abundant and toxic than organoarsenic (methyl arsenic) species in the environment (Rossman 2003). The As(III) is most prevalent under reduced conditions (Hasegawa et al. 2010) as observed in flooded rice fields (Boivin et al. 2002; Gao et al. 2002). Arsenite is more mobile than As(V) due to its neutral nature and remains loosely adsorbed to soil particles (Lakshminathiraj et al. 2006). As(V) is found to be more stable in oxic environments (Zhang et al. 2015). Thus As(III) is more bio-available and get transported to the rice plant and ultimately bioaccumulates in rice grains (Zhang et al. 2015).

Microorganisms undertake As cycling in terrestrial ecosystem by oxidation, reduction, methylation and volatilization (Oremland and Stolz 2003; Qin et al. 2006). Oxidation of As(III) to less toxic As(V) is thought to be the most important detoxification mechanism which ultimately leads to methylation (Rahman and Hassler 2014). Oxidation of As(III) by different organisms involves a key enzyme arsenite oxidase. Over the last few decades, numerous studies have described different responses in microorganisms exposed to this metalloid including induction of transport system (Thiel 1988), expression of resistance genes (Jackson and Dugas 2003; López-Maury et al. 2003), phytochelatin production (Pawlik-Skowrońska et al. 2004) and de novo synthesis of thiol and related compounds (Morelli et al. 2005).

In the rice field ecosystem of tropical countries, cyanobacteria are one of the indigenous nitrogen-fixing soil-borne oxygenic photosynthetic prokaryotic microorganisms abundantly present in soil-water interphase and act as natural bio-fertilizers. In this specialized niche, cyanobacteria play a critical role in As transformation, mobilization and transport from soil-water interphase to rice plants (Zhang et al. 2015). Cyanobacteria can take up and concentrate As from the surrounding environment and As-resistant genera such as *Synechocystis*, *Phormidium*, *Oscillatoria*, *Nostoc* and *Anabaena* have been reported as dominant taxa from different As-contaminated areas globally, including rice field environments (Maeda et al. 1998; Shaheen et al. 2007; Banerjee 2008; Yin et al. 2011). Some studies have used natural algal populations from fresh water ecosystems and exposed them to As(V) and these algae showed promising biotransformation potential (Pawlik-Skowrońska et al. 2004; Bahar et al. 2013; Wang et al. 2013). Only a handful of studies have been undertaken with freshwater cyanobacteria which were exposed to

As(III) and showed ability to transform into As(V) followed by methylation (Yin et al. 2011; Wang et al. 2013).

The Bengal Delta Plain (BDP) located in South Asia and formed by the Gangetic alluvial deposits have characteristic topography, geomorphological and geological features. The BDP is drained by three of the major rivers of the world—the Ganges, Brahmaputra and Meghna (also known as the GBM Delta), which originate from the Himalayas. In West Bengal, India, a part of the BDP has been formed by elevated alluvial terraces of the Barind and Madhupur tracts of Pleistocene age overlaid by Holocene flood plain deposits (Alam et al. 1990). The nine As-affected districts in West Bengal have an area of 38,865 km² and a population of 42.7 million. Around six million people living in this region presently consume As-contaminated water with As level of 50 µg L⁻¹. It has been estimated around 300,000 people may be suffering from As-related diseases in the BDP region (Rahman et al. 2001). The source of As in groundwater of this deltaic plain is considered to be geogenic in origin and largely restricted to the Holocene aquifer sediments (Bhattacharya et al. 1997; BGS and DPHE 2001). Several models have been put forward to explain the source and mobilization mechanism of As in groundwater of the BDP, an area of international interest (Nath et al. 2011). Large areas of the BDP are home to rice fields and rice cultivation is entirely dependent on contaminated ground water for the purpose of irrigation which ultimately results in bioaccumulation of As in rice grains and plants (Mondal and Polya 2008a, b; Chatterjee et al. 2010). The rice field ecosystems are home to indigenous microbial communities including cyanobacteria which may have the potential to biotransform different species of As. However, almost no study has been undertaken to date to look at the biotransformation potential of indigenous cyanobacteria present in As-contaminated rice field ecosystems of BDP in the presence of As(III) under in vitro conditions.

The objectives of the present study were the identification of the five most abundant indigenous cyanobacteria isolated from As-contaminated rice fields of BDP using polyphasic taxonomy and subsequent evaluation of their adaptive responses, tolerance, absorption and biotransformation potential in the presence of arsenite (sodium arsenite) in vitro.

Materials and methods

Study area The district of Nadia in West Bengal, India, is situated in the alluvial zone of BDP and designated as one of the As-affected ‘hot spots’ nationally and globally (WHO 2011; Chowdhury et al. 2000; Bhattacharya et al. 2001). In the present study, soil samples were collected from two highly As-affected blocks from Nadia, namely Karimpur II (KMP) and Chakdaha (CHK). In both the sampling areas, As

concentration is much higher in irrigation as well as drinking water exceeding the permissible limits of FAO (1985) and WHO (2011). One shallow tube well-irrigated rice field from each blocks namely Barbakpur of Karimpur II (KMP: N 23° 55.064', E 88° 33.350') and Gontera of Chakdaha (CHK: N 23°41.86', E 87°40.66'), were selected for sampling activities. The Karimpur II site is routinely monitored by our laboratory to study the role of microbial communities in arsenic biogeochemical cycling (Ghosh et al. 2014, 2015). The other site, CHK, was chosen since it has numerous rice fields with very high concentration of As in the soil (Shaheen et al. 2007; Chatterjee et al. 2010; Nath et al. 2011).

Sample collection and storage Soil cores from each site were collected between April and June, 2014. Soil cores of 15 cm in length were collected using a soil auger (6 inches; locally made). Soil from the upper crust (10 mm thick) was used for isolation of cyanobacteria. Soil samples representing a depth of up to 6 inches (excluding upper crust) were separated and air-dried. Subsequently, air-dried soil samples were ground and passed through sieve and homogenized to make a representative sample for subsequent nutrient analyses.

Soil physico-chemical analysis In situ soil temperature was measured in the field using a thermometer. Soil pH was determined by a Sartorius digital pH meter and electrical conductivity (EC) was measured using a Systronics conductivity bridge at 25 °C. Available soil nitrogen was determined by alkaline-KMnO₄ method (Subbaiah and Asija 1956) which primarily measures easily oxidizable nitrogen in a Kjeltec Auto 1030 Analyzer (Tecator, Sweden). Available phosphorus content was estimated based on a previously published methodology (Bary and Kurtz 1945). The percentage of organic carbon (OrC) was estimated following Walkley and Black (1934). For total As determination, digestion of soil samples was undertaken following Bhattacharya et al. (2009) and samples were subsequently analysed by inductively coupled plasma emission mass spectrometer (ICP-MS) (Q-ICP-MS, X Series 2, Thermo Scientific, USA).

Isolation of cyanobacteria Ten gram of finely ground soil was put into 100 mL of distilled water, stirred and allowed to settle. The supernatant was subsequently used as inoculum. Agar plates (1.2 % agar) containing nitrogen-free BG-11 medium (Stanier et al. 1971) were used as growth medium. One mL of soil suspension was put in each plate. Subsequently, agar plates were kept at 25±2 °C in a culture rack receiving 25–30 μmol photons m⁻² s⁻¹ from a cool fluorescent lamp under 20:4-h light/dark (LD) cycle. Visible blue green or brown colonies appeared within 7–10 days of inoculation which were characterized using a stereo zoom microscope. Colonies which were 15–20 days old were subsequently used to establish liquid cultures.

Cyanobacterial isolates and culture condition Pure cultures were maintained in liquid BG11 medium with or without combined nitrogen at 25±2 °C and 25–30 μmol photons m⁻² s⁻¹ with a 20:04-h L:D cycle. All five isolates are presently maintained in algal culture collection in the Department of Biological Sciences, Indian Institute of Science Education and Research Kolkata (IISERK), India.

Morphotaxonomy of isolates Morphological features of isolates grown in culture medium were studied under a light microscope (LM) enabled with a camera and differential interference contrast (DIC) images were taken using CellSens software (BX53, Olympus, Japan). Prior to field emission scanning electron microscopy (FESEM), cyanobacterial biomass was fixed in 2.5 % glutaraldehyde [in 100 mM phosphate buffered saline (PBS), pH-7.4] and left overnight followed by washing with PBS. Dehydration was performed in graded ethanol series (40–100 %). Both treated and untreated samples were drop coated on a 1-cm glass piece and air-dried. The samples were coated by gold-palladium sputtering (Quorum Technologies Ltd., UK) and subsequently imaged by FESEM (SUPRA 55VP, Carl Zeiss, Germany). The cyanobacterial isolates were identified based on available morpho-taxonomic keys (Desikachary 1959; Anagnostidis and Komárek 1990; Komárek and Anagnostidis 2005; Komárek et al. 2014).

Molecular phylogeny Genomic DNA was extracted from 2 mL of cyanobacterial culture representing each isolate using standard method of Böstrom et al. (2004). The yield of extracted genomic DNA was quantified by Nano Drop (Thermo Scientific, USA) prior to PCR amplification. Partial 16S rRNA fragments were amplified from the extracted genomic DNA using cyanobacterial specific forward primer CyaF581 and reverse primer CyaR631 (Mühling et al. 2008). PCR reaction conditions were as per Mühling et al. (2008). The generated PCR products were purified using Qiagen PCR purification kit as per manufacturer's instructions. The purified PCR products were sequenced in an ABI 3730 Genetic Analyzer based on Big Dye Terminator chemistry. Generated chromatograms were checked in BioEdit v7.0 (Hall 1999) for any ambiguity before further analysis. Generated sequences were compared with published cyanobacterial 16S rRNA sequences (GenBank/RDB/EMBL databases) using Blastn tool. The alignment, which included the 16S rRNA sequence (approx. 298 bp) of each isolate generated from this study and published 16S rRNA sequences of representative cultured cyanobacterial strains (GenBank), was generated using Clustal Omega (<http://www.ebi.ac.uk/Tools/msa/clustalO>) and subsequently checked in SEAVIEW. A phylogenetic tree based on Maximum Likelihood method (ML) with GTR model and gamma

distribution was constructed in MEGA v6.0 (Tamura et al. 2013). The ML tree was subsequently bootstrapped in MEGA v6.0 (1000 replicates) to generate consensus tree retaining significant branching of >45 %. The generated partial 16S rRNA sequences representing the five isolates have been submitted to GenBank and their accession numbers are KT361166 (NOC isolate), KT361167 (WEC isolate), KT361168 (NOK isolate) and KT361169 (LBK isolate) KT361170 (WEK isolate).

Determination of inorganic arsenic [As(III)] tolerance and inhibition by cyanobacterial isolates The inorganic salt of sodium arsenite (NaAsO_2 ; Sd Chemicals, India) was used to test cyanobacterial isolates under various concentration (0, 10, 50, 100, 200, 500 and 1000 μM). Medium used to grow each isolate but without sodium arsenite was designated as control. The sodium arsenite solution was filter sterilized and spiked aseptically to each set up of autoclaved culture medium before inoculation. Arsenite tolerance and inhibition in each isolate were determined and expressed in terms of representative concentration of As(III), viz., no observed effective change (NOEC, 10–100 μM); effective concentration 50 (EC_{50} ; growth reduces by 50 %, >200–1000 μM) and concentration lower than EC_{50} or sub-effective concentration (SEC_{50} , growth present above 50 %, 200–400 μM) as compared to control, based on survivality response in terms of chlorophyll-*a* (Chl-*a*) content of 7-day-old cyanobacterial culture (Debnath et al. 2012). The NOEC and SEC_{50} were also calculated from the same growth experiments.

The absorbance of inoculum (homogenized biomass) during inoculation in the liquid medium containing sodium arsenite at 750 nm was 0.2. Inoculation was done aseptically and finally 100 mL of cultures were maintained at 25 ± 2 °C and $25\text{--}30$ $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ under 20:04-h LD cycle conditions. All experiments were conducted under same incubation condition unless mentioned separately.

Physiological characterization Growth, specific growth rate, generation time, pigment content, pigments relative autofluorescence signature, nitrogenase activity and extracellular polymeric substances (EPS) production were characterized for each isolate in this study. Biomass and growth of the isolates were determined in terms of Chl-*a* content using methods described by Mackinny (1941). The growth rate was calculated by using the formula $k = \ln(N_1/N_0)/t$ of Guillard (1973), where N_1 and N_0 are Chl-*a* amount at the end and beginning of experiments in time t days. Specific growth rate (k) and generation time ($24/k$ h) were calculated from Chl-*a* readings taken on every 3rd day up to 15 days.

Relative autofluorescence and emission spectra of pigments for each cyanobacterial isolate were determined using confocal laser scanning microscopy (CLSM). Twenty microlitre of the resultant sample was placed on a glass slide

and sealed with cover slip for observation compared to untreated control set. Filaments or cells were imaged with a Zeiss CLSM 710 with Axioobserver and Zen 2010 software (Carl Zeiss, Germany) using DIC optics with a $40\times/1.3\text{NA}$ oil immersion Plan-Neofluar objective lens or $63\times/1.4\text{NA}$ oil immersion Plan-Apo objective lens. DIC imaging was conducted with excitation by a 488-nm argon laser at 10 % power, using an NT 80/20 filter. Initial autofluorescence settings for chlorophyll [in Photosystem II, (PSII)] detection were based on settings adapted from previously published method (Sinha et al. 2002). To determine adaptability and survivality under As(III) stress in terms of Chl-*a* and phycobiliproteins (PBPs) such as phycocyanin (PC), phycoerythrin (PE) and allophycocyanin (APC) autofluorescence, spectral scanning was initiated for each isolate. Emission scans were collected in ~ 10.7 nm bandwidth with increments ranging from 565 to 724 nm.

Nitrogenase activity of a 7-day-old culture for each isolate was estimated based on an acetylene reduction assay (ARA) method following the principle of Turner and Gibson (1980) by gas chromatography (YL6000, Youngling, Korea). EPS extraction were done by separating cells from culture medium by centrifugation at 12,000 rpm for 30 min. Total carbohydrate content of the supernatant was measured following the protocol of Dubois et al (1956).

Arsenic absorption and transformation in cyanobacterial isolates To investigate the As absorption and transformation in cyanobacterial isolates, sodium arsenite was used at the indicated concentration (NOEC and SEC_{50} of each isolate). Each set of cyanobacterial culture (in 100 mL medium) exposed to As(III) was incubated for 7 days, subsequently harvested, rinsed with de-ionized water and ice-cold phosphate buffer for 10 min to remove apoplastic As. For absorption study, total As was determined from cyanobacterial biomass and culture medium cyanobacterial biomass against a control set using ICP-MS (Q-ICP-MS, X Series 2, Thermo Scientific, USA) and reference standard of 23 element standard (ICP23A20, Reagecon, Ireland). Samples were prepared as mentioned below. The bioconcentration factor (BCF) was estimated to find out the bioabsorption potential for each cyanobacterial isolate. The ratio of total inorganic arsenic in cyanobacterial biomass [dry weight, (C_{BM})] to initial As concentration in growth medium (C_{IN}) was used to calculate BCF following Bahar et al (2013).

To evaluate the biotransformation potential of each cyanobacterial isolate, fractions of inorganic As [As(III) and As(V) fractions] were determined. Approximately 0.02 g (dry weight) of cyanobacterial biomass was put in 50 mL centrifuge tubes and treated in 10 mL of 2 % HNO_3 overnight. The samples were subsequently heated in a microwave based on published protocol (Zhu et al. 2008). The digested extract of each isolate and corresponding 1 % HNO_3 acidified culture

medium was filtered separately through 0.45 μm nitrocellulose filter disc (Rankem, India) and stored for absorption (ICP-MS) and transformation analysis. Inorganic As(III) and As(V) were separated using As speciation cartridge (Metal Soft Center, USA) which retains As(V) through alluminosilicate adsorbent. A Varion AA240 model atomic absorption spectrophotometer (AAS) equipped with vapour generation accessories (model no. VGA77) was used for estimation of total inorganic As and filtrate collected from speciation cartridge following the protocol of Datta et al. (2010) for undertaking transformation study. Reducing agent (Aqua solution of 0.6 % sodium borohydride in 0.5 % sodium hydroxide) and acid (40 % hydrochloric acid) were freshly prepared before use. For reliability of this method, all As samples were previously analysed by ICP-MS for total inorganic As and total As concentration was determined by AAS. A reference standard (ACCU Trace; AA03N-1, USA) and reagent blanks were used to maintain accuracy of the analysis.

Results

Physico-chemical properties of soil samples The physico-chemical characteristics of soil samples collected from sites KMP (Karimpur II) and CHK (Chakdaha) are detailed in Table 1. Briefly, both the soils were slightly alkaline, low in electrical conductivity, moderate in available nitrogen with low available phosphate and organic carbon. Total inorganic As content of both the soils were found to be higher than recommended globally [up to 10 and 20 mg kg^{-1} in normal and agriculture soil, respectively; (Rahaman et al. 2013 and references therein)]. The soils are alluvial, sub humid and representative of megathermal tropical agro climatic zone of South Asia.

Organisms and culture conditions Cyanobacterial colonies appeared within 7–10 days on the agar plates exhibiting various colour, size and shapes. The most abundant colonies were chosen for further study. Three isolates from KMP site and two isolates from CHK site were selected for establishment of pure liquid cultures. Mucilaginous round colony of olive green to brown in colour was most abundant followed by non-mucilaginous radiating blue green colour designated as NOC and WEC respectively isolated from rice field soil of CHK. From KMP soil sample, same colonies (as in CHK)

appeared with maximum abundance and designated as NOK and WEK throughout the study. All these NOC, WEC, NOK and WEK were grown on BG11 agar plates without combined nitrogen. In agar plates with combined nitrogen, no significant colony appeared when inoculated with CHK soil, but very distinct irregular mat-like blue green colony appeared following inoculation with KMP soil. This particular isolate referred as LBK isolate was also selected for further study.

Morphotaxonomy of isolates The five most abundant isolates of cyanobacteria obtained from both sites were chosen for this study. Isolates were assigned to three distinct filamentous genera namely unbranched nonheterocystous (isolate LBK), unbranched heterocystous (isolate NOC and NOK) and true branched heterocystous (isolate WEC and WEK) based on LM and FESEM (Table 2). Based on morphotaxonomy, LBK isolate resembled *Phormidium* exhibiting comparatively thinner (1–2 μm broad) and loosely entangled filaments in mat-like formation (Fig. 1a, b). The NOC and NOK isolates showed morphological properties with overlapping characteristics and resembled *Nostoc ellipsosporium*. In case of NOC isolate, the filament width ranges from 2 to 4 μm whereas for NOK the filament ranges from 2.5 to 3.5 μm (Fig. 1c, d). The remaining two isolates, WEK and WEC, showed typical cellular dimension and uniform morphology resembling *Westiellopsis* (Fig. 1e, f). Based on FESEM, finer morphological details including surface texture variation, cell dimension and constriction at cross wall were also noted for all the five isolates (Fig. 2a–f) (Table 2).

Molecular phylogeny of isolates Partial 16S rRNA sequences (approx 298 bp) representing the five isolates showed 97–100 % identity with published 16S rRNA sequences of cultured filamentous cyanobacteria (Table S1). Five isolates identified by morphological attributes were subjected to molecular phylogenetic study to get a clear understanding of their taxonomic affiliation. The top Blastn result for each isolate has been provided in Table S1. Based on ML approach, five isolates showed strong taxonomic affiliation with 16S rRNA sequences of cultured cyanobacteria and represented across four clades with strong bootstrap support for some of them (Fig. 3). In clade A, the isolate LBK clustered with sequences belonging to the genus *Leptolyngbya*. The 16S rRNA sequence of isolate NOK was found to be clustered with cultured

Table 1 Physico-chemical parameters of arsenic contaminated rice field soils collected from Karimpur (KMP) and Chakdaha (CHK) block; ($n = 3$)

| Sampling site | Temperature ($^{\circ}\text{C}$) | pH | Conductivity (dS m^{-1}) | Available nitrogen (mg kg^{-1}) | Available phosphate (mg kg^{-1}) | Organic carbon (%) | Total As (mg kg^{-1}) |
|---------------|------------------------------------|------|-------------------------------------|--|---|--------------------|----------------------------------|
| KMP | 29 | 7.74 | 1.98 | 92.3 | 24.7 | 0.34 | 24.05 |
| CHK | 28 | 7.6 | 2.3 | 88.5 | 22.02 | 0.36 | 16.4 |

Table 2 Morphological variation of five cyanobacterial isolates as observed using light microscopy; ($n = 5$)

| Cyanobacterial isolate | Cell dimension (μm) | | Constriction at cross wall | Sheath | Heterocyst | Akinete |
|------------------------|----------------------------------|-------------|----------------------------|-----------------------|------------------------|--------------|
| | Length | Breadth | | | | |
| LBK | 3–3.5 | 1–2 | Distinct | Present, very thin | Absent | Absent |
| NOC | 6–8 | 2.5–4 | Distinct | Present, mucilaginous | Intercalary | Distinct |
| NOK | 6–8 | 2.5–3.5 (4) | Distinct | Present, mucilaginous | Intercalary | Present, few |
| WEC | 8–10 | 4–6 (7) | Distinct | Present, thin | Intercalary or lateral | Distinct |
| WEK | 8–12 | 4–7 | Distinct | Present, thin | Intercalary or lateral | Distinct |

sequences of *Nostoc* and represented in clade B. In clade C, the isolate NOC was found to be clustered with 16S rRNA sequences of cultured cyanobacteria belonging to *Nostoc commune*, *Nostoc punctiforme* and *Nostoc verrucosum*. The remaining two isolates, WEK and WEC, were part of the clade D along with cultured 16S rRNA sequences of belonging to *Westiellopsis*, *Fischerella* and *Hapalosiphon*; however, both the isolate sequences diverged from the rest indicating that these two isolates are novel.

Growth rate, generation time and EC_{50} of cyanobacterial isolates exposed to As(III) Effect of As(III) on five cyanobacterial isolates was tested using 10, 50, 100, 200, 400, 500 and 1000 μM (1.3, 6.6, 13, 26, 52, 65 and 130 mg L^{-1} , respectively) sodium arsenite. After treatment with 10–100 μM As (NOEC), growth was found to be accelerated in four isolates as evident from generation time (67.2 to 79 h) except for LBK (95 h) (Fig. 4). However, when As concentration reached 200–500 μM , growth in all the tested isolates were found to be inhibited by 50–60 % (generation

time, 124.2–178.5 h) (Fig. 4). From this growth experiments, we calculated EC_{50} and SEC_{50} of each cyanobacterial isolate. The EC_{50} of NOC, NOK, WEC, WEK and LBK isolates were found to be 62.7, 60.3, 47.5, 50.2 and 28.60 mg L^{-1} , respectively. The SEC_{50} was found to be 52 mg L^{-1} (400 μM) for NOC and NOK, 39 mg L^{-1} (300 μM) for WEC and WEK, and 26 mg L^{-1} (200 μM) for LBK, respectively. In all cases, NOEC was found to be 13 mg L^{-1} (100 μM). Among the five isolates, NOC was most tolerant to As(III) toxicity followed by NOK, WEC, WEK and LBK.

Photosynthetic pigments profile under As(III) exposure

The fluorescence emission spectra acquired by CLSM were retrieved from vegetative cells present in As filaments treated (NOEC and SEC_{50}) and untreated biomass representing each isolate. In vivo maximum wavelength (λ_{max}) typical of four distinct pigments was detected in this study (Table 3). The PBP's are attached to the thylakoid membrane and transfer energy to Chl-*a* in the photosynthetic reaction centres. The changes in the PBP's that occur during As treatment consist of changes in

Fig. 1 LM micrographs of five cyanobacterial isolates: **a** LBK filaments loosely arranged; **b** LBK trichome; **c** NOC with heterocyst; **d** NOK with heterocyst; **e** WEC branch with lateral heterocyst; **f** WEK branch with intercalary heterocyst (scale bars = 10 μm)

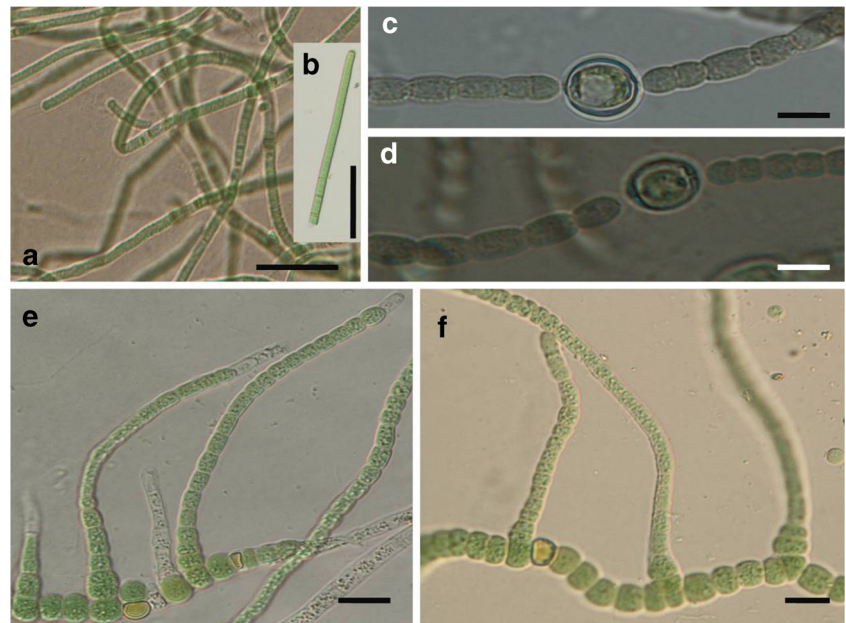
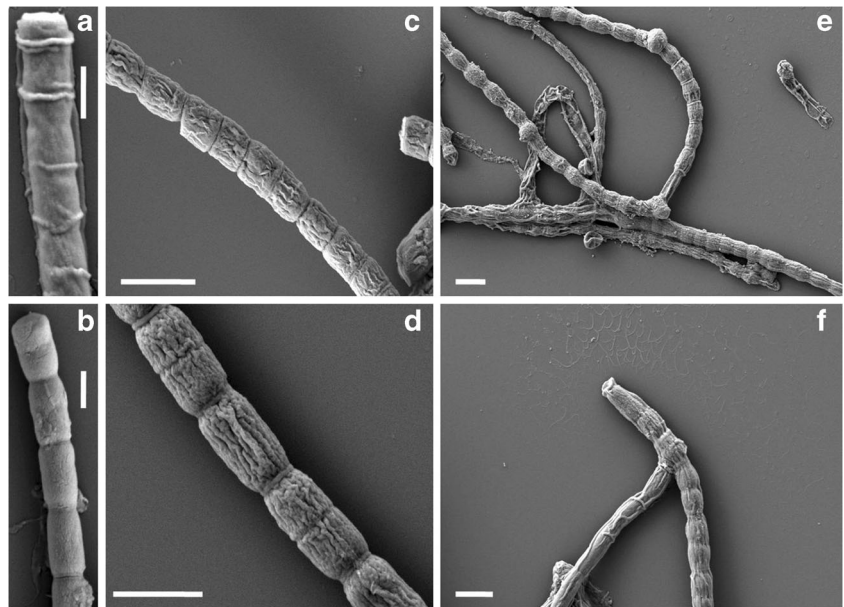


Fig. 2 FESEM micrographs of five cyanobacterial isolates: **a** LBK filament; **b** LBK trichome; **c** NOC with intact and dividing cells; **d** NOK with intact and dividing cells; **e** WEC branch with short cell; **f** WEK branch with elongated cells (scale bars: **a, b** = 2 μm, **c–f** = 5 μm)

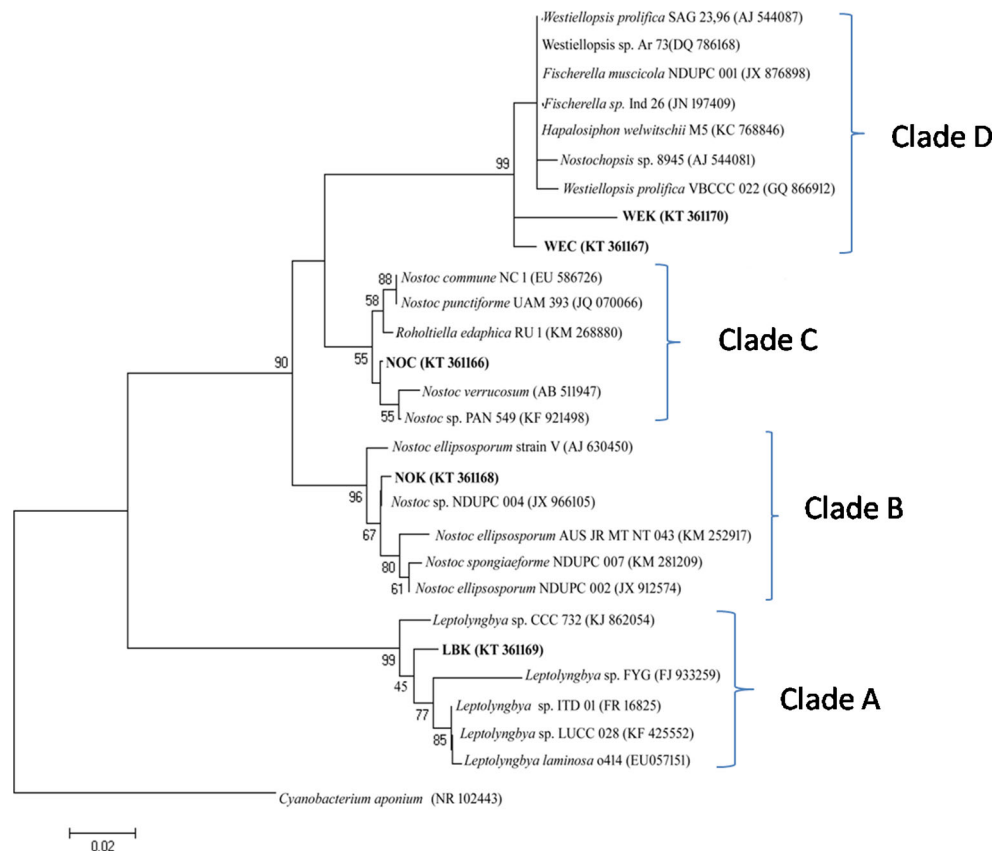


the antenna molecule PBPs content and reaction centre (PSII) Chl-*a*. By nature, PBPs are also highly autofluorescent proteins. Three PBPs were found in the studied cyanobacterial isolates. PBPs exhibited distinct wavelengths of fluorescence emission maximum (λ_{emax}): PE, λ_{emax} ~575 nm; PC, λ_{emax} ~635–645 nm; and APC, λ_{emax} ~656 nm. The Chl-*a* pigment also is

autofluorescent and has λ_{emax} ~675–685 nm, which sometimes may overlap with PC and APC (λ_{emax} ~630–685) (Fig. 5).

Under NOEC, fluorescence intensity of PE and Chl-*a* decreased whereas PC and APC increased compared to control in case of all the cyanobacterial isolates (Fig. 5). A clear decrease in fluorescence intensity was detected for each isolate

Fig. 3 ML tree represents relationship among 16S rRNA sequences of five cyanobacterial isolates of this study (NOC, NOK, WEC, WEK and LBK) and related cultured cyanobacteria based on 16S rRNA sequences. The 16S rRNA sequence of *Cyanobacterium aponium* used as outgroup. The ML tree was bootstrapped with 1000 replicates to produce consensus tree retaining significant branching of >45 %



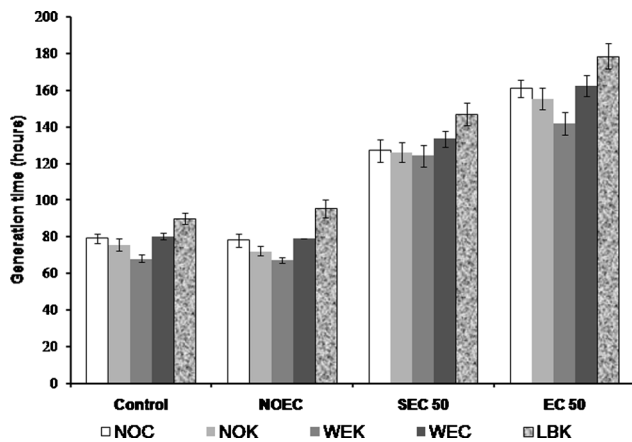


Fig. 4 Generation time (hour) in terms of chlorophyll-*a* of five cyanobacterial isolates exposed to different concentration of sodium arsenite in vitro; (\pm SD; $n=3$)

when exposed to SEC₅₀. For WEK and WEC isolates, very weak fluorescent intensity of PE was detected compared to other three isolates. Maximum PE intensity was recorded in NOK isolate followed by NOC and LBK isolates. More of less stable fluorescence intensity of APC was found in every set of experiments followed by PC in every tested cyanobacterial isolate (Fig. 5).

Nitrogenase activity and EPS production In the five cyanobacterial isolates, nitrogenase activity in terms of ARA activity was measured. ARA activity in four heterocystous isolates increased about twofold at NOEC (100 μ M) compared to control (0.0 μ M). Maximum activity was noted in WEK isolate and lowest activity was found in LBK isolate (Table 4). At SEC₅₀, almost 50 % decrease in enzyme activity was noted in all the five isolates (Table 4).

Except in case of LBK isolate, EPS was detected for the remaining isolates under control condition which increased (40–60 %) in dose-dependent manner when exposed to NOEC and SEC₅₀ (Table 4).

Table 3 Physiological characterization of five cyanobacterial isolates based on growth and pigment profile (Chl-*a*, PE, PC and APC)

| Cyanobacterial isolate | Pigments profile | | | |
|------------------------|------------------|-----|-----|-----|
| | Chl- <i>a</i> | APC | PC | PE |
| LBK | +++ | ++ | +++ | ++ |
| NOC | ++ | ++ | ++ | +++ |
| NOK | ++ | ++ | ++ | +++ |
| WEC | +++ | +++ | + | ++ |
| WEK | +++ | +++ | + | ++ |

+ low, ++ moderate, +++ high

Arsenic absorption by cyanobacterial isolates Intracellular accumulation of total As in the studied cyanobacterial isolates under NOEC (100 μ M) and SEC₅₀ (200–400 μ M) were analysed after 7 days of exposure to sodium arsenite. Absorption experiment was not performed at EC₅₀ to avoid selective low absorption value below 50 % or less viable biomass. After 7 days incubation, all isolates showed ability to absorb As based on BCF values (Fig. 6). Intracellular concentrations of total inorganic As increased with the increase of As(III) dose (Table 5) but BCF value decreased in all isolates. In NOC isolate, highest BCF (0.150) and As absorption (1.96 mg kg⁻¹ at NOEC) was recorded and lowest was found in LBK isolate while intermediate values were found in NOK, WEK and WEC isolates for both treatments (Fig. 6).

After 7 days of incubation, amount of total As in cyanobacterial growth medium also decreased compared to initial concentrations (NOEC and SEC₅₀ of each isolate). At NOEC, maximum total As was present in the medium which was used to grow WEC isolate (~85 %) followed by growth media representing WEK, LBK, NOK and least in case of medium used to grow NOC isolate (72.30 %) (Table 5). At SEC₅₀ (200–400 μ M), higher residual As was detected compared to NOEC (100 μ M) which ranged from 0.03 to 4.1 % (Table 5).

Arsenic transformation in cyanobacterial growth medium and biomass Arsenic transformation in cyanobacterial growth medium and within cyanobacterial biomass was performed from the same set of samples used for total As determination under NOEC and SEC₅₀. A trend for isolate-specific As transformation was observed when treated with sodium arsenite.

In growth medium, under both the concentration (NOEC and SEC₅₀), As(III) and As(V) forms were detected. In growth medium, As(III) fraction was dominant in case of media used to grow NOK and NOC isolates, whereas As(V) was dominant in case of WEK and WEC growth media (Table 6). In the growth medium and biomass of LBK, As(V) was more (~60 %) compared to As(III) (~40 %) at NOEC but almost reverse at SEC₅₀ (Table 6). In all representative As(III)-treated samples except biomass of NOK isolate (at NOEC, 100 μ M), both As(III) and As(V) were found in varying amounts (Fig. S1). In biomass representing all the isolates, As(V) was most predominant (Table 6). After 7 days of incubation, intracellular concentration of As(III) increased with the increase in As(III) dose. Highest transformation to As(V) form was recorded in NOC isolate and lowest in LBK isolate and intermediate transformation was found for NOK, WEK and WEC isolates under both treatments (Table 6).

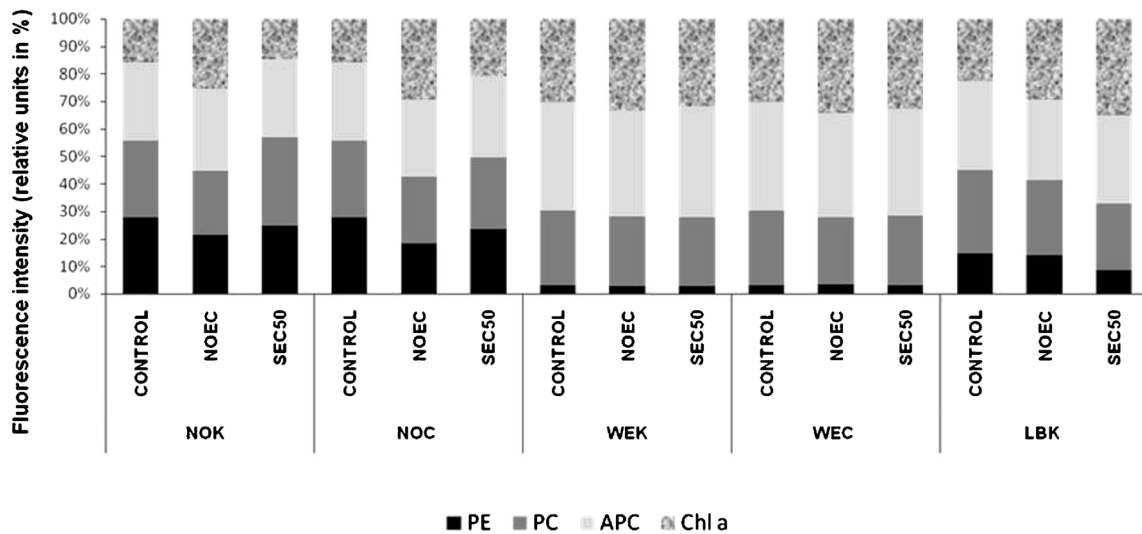


Fig. 5 Percent representation of relative autofluorescence intensity of PBPs and Chl-a emission spectra (after 488 nm laser excitation) of five cyanobacterial isolates exposed to different concentration of arsenite ($n=3$)

Discussion

Reclamation of contaminated environment and thereby human health safety are two major issues that may be linked to sustainable development (Harnandez et al. 1998; ATSDR-2001) Microorganisms are key players in mediating these processes due to their high ability to thrive in degraded environments such as As-contaminated rice field soils (Costa et al. 2014). It is also believed that native or indigenous population of microorganisms such as cyanobacteria are the best candidates for long-term remediation of contaminated environments (Satoh et al. 2005).

In the present study, soil samples analysed from the two study sites, which are known to have higher As level as reported in previous studies (Nath et al. 2011; Ghosh et al. 2015), were found to be slightly alkaline. As evident, available phosphate and organic carbon concentrations were found to be comparatively lower, and these attributes are known to control the mobility and bioavailability of As(III) (Chatterjee et al. 2010).

Five indigenous diazotrophic cyanobacterial isolates (LBK, NOC, NOK, WEC and WEK) were obtained from

the study sites and subsequently characterized to determine potential for arsenite biotransformation and sorption. Polyphasic taxonomic approach was adopted in this study to assign taxonomic rank (genus and species) for cyanobacterial isolates. The LBK isolate showed morphological similarity with *Phormidium* sp. based on light microscopy. However, using FESEM, deeply constricted trichome with thin and firmly attached sheath was clearly visible in LBK isolate and therefore the taxonomic placement of the same was confirmed within the genus *Leptolyngbya* (Komarek and Anagnostidis 2005). Additionally, molecular phylogenetic approach reconfirmed the taxonomic affiliation of this isolate with the genus *Leptolyngbya*. Two other isolates, namely NOC and NOK, showed morphological features which were mainly overlapping and therefore clear taxonomic discrimination was difficult based on available keys (Desikachary 1959; Anagnostidis and Komárek 1990). Morphologically, NOC and NOK isolates closely resembled *N. ellipsosporium*. The cell length for both isolates showed overlapping with maximum and minimum values as stated earlier in Table 1. Given their quite similar colony and filament morphology and the occurrence of these taxa in the same habitat, it was nearly

Table 4 Effect of arsenite on nitrogenase activity (ARA) and EPS production in studied cyanobacterial isolates; (\pm SD; $n=3$)

| Cyanobacterial isolate | ARA activity (nmol C ₂ H ₄ μ g Chl- <i>a</i> ⁻¹ h ⁻¹) | | | Carbohydrate in EPS (μ g mL ⁻¹) | | |
|------------------------|--|-----------------|-------------------|--|----------------|-------------------|
| | Control | NOEC | SEC ₅₀ | Control | NOEC | SEC ₅₀ |
| LBK | 0.70 \pm 0.04 | 0.68 \pm 0.03 | 0.32 \pm 0.03 | ND | ND | ND |
| NOC | 2.98 \pm 0.12 | 5.6 \pm 0.22 | 1.43 \pm 0.05 | 225 \pm 3.05 | 390 \pm 5.02 | 310 \pm 4.0 |
| NOK | 2.9 \pm 0.14 | 5.76 \pm 0.26 | 1.39 \pm 0.06 | 229 \pm 3.2 | 410 \pm 6.02 | 359 \pm 5.07 |
| WEC | 6.70 \pm 0.3 | 13.2 \pm 0.59 | 3.2 \pm 0.22 | 29 \pm 0.72 | 70 \pm 0.02 | 63 \pm 1.75 |
| WEK | 7.2 \pm 0.29 | 13.9 \pm 0.72 | 3.4 \pm 0.25 | 34 \pm 1.02 | 82 \pm 0.02 | 78 \pm 2.3 |

ND not detected

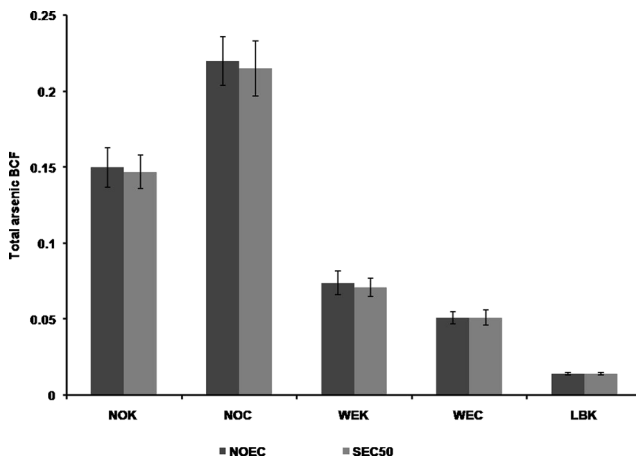


Fig. 6 Inorganic arsenic absorption ability expressed as bioconcentration factor (BCF) of five cyanobacterial isolates exposed at NOEC and SEC₅₀ in vitro evaluated for respective biomass (\pm SD; $n=3$)

impossible to distinguish in culture plate using light microscopy. Therefore, the observed morphological characteristics could not be used as diacritical features for assigning these two isolates. In FESEM, surface texture was found to be almost similar in NOC and NOK isolates except the presence of constriction at the cross wall and pattern in line of division in dividing cells in case of NOC isolate. In molecular phylogenetic tree, the 16S rRNA sequences representing NOC and NOK isolates clustered with two different clades with strong bootstrap support; however, both the isolates showed strong taxonomic affiliation with several 16S rRNA sequences representing cultured species belonging to the genus *Nostoc*. Additionally, the partial 16S rRNA sequence of NOK differed by only 16 bp from the NOC 16S rRNA sequence. These findings also indicate that thorough study involving application of multiple molecular markers may be necessary for species delineation. For the remaining two isolates WEK and WEC, the observed morphological characters including

cellular dimensions resembled those of the genus *Westiellopsis* based on taxonomic keys (Desikachary 1959; Komárek and Anagnostidis 1990). In FESEM, no distinct differences were found between the two isolates except that the cell dimension of branching vary which could be due to phase of development as also reported for the genus *Westiellopsis* (Komárek and Anagnostidis 1990). The molecular phylogeny representing these two isolates revealed that they belong to the *Westiellopsis* clade; however, these isolates may represent possibly new species from As-contaminated rice field soil environments as evident from divergent branch length.

Many As-resistant cyanobacterial genera such as *Synechocystis*, *Synechococcus*, *Phormidium*, *Oscillatoria*, *Nostoc*, *Anabaena* and *Hapalosiphon* have been reported as dominant taxa in As-contaminated aquatic environments globally (Maeda et al. 1998; Shaheen et al. 2007; Banerjee 2008; Yin et al. 2012; Sánchez-Riego et al. 2014; Zutshi et al. 2014). Cyanobacteria isolated from the study sites and belonging to the genus *Westiellopsis* and *Leptolyngbya* are the first report in terms of their presence in As-contaminated rice field ecosystems.

Arsenic metabolism has been widely reported in microbes and plants (e.g., Rosen 2002). In the present investigation, As(III) was used for toxicity assessment on cyanobacterial isolates in vitro. Under NOEC (100 μ M) and SEC₅₀ (200–400 μ M), cyanobacterial isolates were able to accumulate As and grow; however, growth rate slowed with increasing As concentration. In particular, the isolates NOC and NOK (*Nostoc*) were most tolerant followed by WEC and WEK isolates (*Westiellopsis* clade) and LBK isolate (*Leptolyngbya*). Similar observations were also reported for *Synechocystis* sp. PCC6803 (Yin et al. 2011, 2012), *Microcystis* sp. PCC7806 (Yin et al. 2011), *Microcystis aeruginosa* (Wang et al. 2013), *Oscillatoria tenuis*, *Nostoc*

Table 5 Intracellular arsenic absorption ability (C_{BM}) and residual arsenic content (medium) of five cyanobacterial isolates exposed under initial NOEC and SEC₅₀ (C_{IN}) in vitro (mean value, $n=3$) after 7 days of exposure

| Cyanobacterial isolate | Initial total As (C_{IN}) in growth medium (ppb) | Total As in cyanobacterial biomass (C_{BM}) | | Total As in growth medium (ppb) |
|------------------------|--|---|---------------------|---------------------------------|
| | | ppb | mg kg ⁻¹ | |
| NOK | 13,000 (NOEC) | 1960.5 | 1.961 | 9495 |
| | 52,000 (SEC ₅₀) | 7641.6 | 7.642 | 37,992 |
| NOC | 13,000 (NOEC) | 2870.5 | 2.871 | 9400 |
| | 52,000 (SEC ₅₀) | 10,482 | 10.482 | 37,648 |
| WEK | 13,000 (NOEC) | 958.5 | 0.959 | 10,365 |
| | 39,000 (SEC ₅₀) | 2777.5 | 2.778 | 32,750 |
| WEC | 13,000 (NOEC) | 660.5 | 0.661 | 11,045 |
| | 39,000 (SEC ₅₀) | 1992.5 | 1.993 | 34,135 |
| LBK | 13,000 (NOEC) | 185 | 0.185 | 9875 |
| | 26,000 (SEC ₅₀) | 370 | 0.370 | 19,973 |

Table 6 Transformation of inorganic arsenic [sodium arsenite As(III)] to arsenate [As(V)] by five cyanobacterial isolates exposed at NOEC and SEC₅₀ in vitro evaluated in biomass and respective growth medium (mean value, n = 3) after 7 days of exposure

| Cyanobacterial isolate | Initial As in growth medium (ppb) | As in cyanobacterial biomass (ppb) | | As in growth medium (ppb) | |
|------------------------|-----------------------------------|------------------------------------|---------|---------------------------|-----------|
| | | As(III) | As(V) | As(III) | As(V) |
| NOK | 13,000 (NOEC) | ND | 1857.70 | 8144.13 | 862.87 |
| | 52,000 (SEC ₅₀) | 370.22 | 6888.98 | 33,317.592 | 2740.41 |
| NOC | 13,000 (NOEC) | 144.43 | 2585.77 | 9738.98 | 971.21 |
| | 52,000 (SEC ₅₀) | 621.37 | 9241.63 | 33,108.82 | 3597.18 |
| WEK | 13,000 (NOEC) | 98.17 | 809.94 | 2896.06 | 6933.93 |
| | 39,000 (SEC ₅₀) | 337.43 | 2340.57 | 8099.46 | 23,700.54 |
| WEC | 13,000 (NOEC) | 108.64 | 519.35 | 2263.03 | 8213.96 |
| | 39,000 (SEC ₅₀) | 414.60 | 1461.40 | 9091.67 | 24,053.33 |
| LBK | 13,000 (NOEC) | 53.63 | 123.36 | 3803.0 | 5572.0 |
| | 26,000 (SEC ₅₀) | 233.16 | 114.84 | 1097.46 | 789.84 |

ND not detected

sp. PCC7120 (Yin et al. 2011), *Nostoc muscorum* and *Nostoc minutum* (Ferrari et al. 2013), *Anabaena affinis*, *Tolypothrix tenuis* (Ferrari et al. 2013) and *Hapalosiphon fontinalis*-339 (Zutshi et al. 2014) isolated from diverse aquatic environments and when exposed to As(V) or As(III). As(III) exerted strongest toxic effect causing cell death in the range of 200–500 μM concentration among the isolates and more so at the intragenetic level. Ferrari et al. (2013) showed that sensitivity to As(III) varied among different species of the genus *Nostoc* and the same has been observed in our study between NOC and NOK isolates.

In this study, CLSM approach was adopted to detect physiological change in cyanobacterial isolates in terms of in vivo fluorescence properties of photosynthetic pigments as an indicator of cell adaptability and survivability (Billi et al. 2011; Schulze et al. 2011). Cyanobacteria possess highly abundant autofluorescent PBPs and Chl-*a*, which can be used as intrinsic fluorescent markers for probing the regulation of their synthesis, accumulation and photosynthetic activity under natural and stress conditions (Bordowitz and Montgomery 2010). In the present study, PBPs (10–25 %) and Chl-*a* (20–30 %) autofluorescence decreased with the increase in As(III) concentration as evident from SEC₅₀ values (Fig. 6). Loss of pigment fluorescence (Geider et al. 1993) has been correlated with decreased enzyme activity and increased membrane permeability which could be a useful indicator of senescence for any species of algae (Franklin et al. 2012). We also found loss of pigment fluorescence in our study. Once this process begins, degradation of the photosynthetic pigments, in particular chlorophyll (Berges et al. 1998), leads to the final stage of autolysis and this was observed in our study based on quantified fluorescence loss at SEC₅₀. However, the persistence of PBPs autofluorescence serves as a survival marker and has been related to genome stability, undamaged plasma

membranes and dehydrogenase activity (Billi et al. 2011). Thus, in our study, consistent or even higher APC fluorescence was found in all the tested cyanobacterial isolates thereby indicating adaptability under As(III) exposure for survival and performance of essential cellular functions such as efficient energy transfer from PC to Chl-*a* via APC.

In four out of five isolates, namely NOC, NOK, WEC and WEK, nitrogenase activity increased by almost twofold when exposed to NOEC (100 μM) but reduced to half under SEC₅₀ (200–400 μM) compared to control conditions. Nitrogen fixation and its incorporation in cyanobacterial cell (ammonium assimilation) depend on cellular energy and represent a complex interdependent metabolic cycle. This energy and reducing power normally come from cell’s metabolic energy through TCA cycle and electron transport chain (Wallsgrove et al. 1983). In heterocystous cyanobacteria, photosynthetically fixed carbon which is essential for nitrogen fixation is supplied to the heterocyst by the vegetative cells (Stewart 1980) and plays a key role in the linking of nitrogen and carbon metabolism (Chen et al. 1988). Thus, in our study under NOEC, almost twofold increase in ARA activity might be coupled with the increased APC fluorescence in terms of photosynthetic energy transfer from antenna molecule to reaction centre PS II. These phenomena might enhance photosynthetic activity and energy production processes for cellular metabolism. The reduced nitrogenase activity under increased concentration (SEC₅₀) of As(III) in LBK isolate may be related to reduction in photosynthetic activity due to lack of reducing agent pool (Leganés and Fernández-Valiente 1992) under As stress as indicated from our CLSM results. Thus ARA activity might be hampered in two ways, either by deficiency in energy supply by inhibition of photosynthesis or by inhibition of energy metabolism and reduction of supply of reducing molecules such as NADH or NADPH (Debnath et al. 2012). On

the other hand, at SEC₅₀, when nitrogen fixation was seriously inhibited, cyanobacterial cells may suffer from nitrogen deficiency. Nitrogen stress affects energy transfer from PC and Chl-*a*, and therefore, their spectral properties under both in vivo and in vitro conditions may change (Peter et al. 2010) and these may lead to low levels of photosynthesis during nitrogen limitation (Duke et al. 1989; Sauer et al. 2001). Therefore in our study, decrease in ARA activity corresponded to decrease in pigment fluorescence intensity.

In NOC, NOK, WEC and WEK isolates, EPS production increased under NOEC and SEC₅₀ treatments. In the natural environment, bacteria usually grow within structured microbial communities embedded in EPS composed of sugars, proteins and DNA (Hall-Stoodley et al. 2004) which help them to thrive in high As environment (Muller et al. 2007) as also reported for *Thiomonas arsenitoxydans* when exposed to As(III) (Marchal et al. 2011). This is an adaptive response to changing ecological niche. The hyper-production of exopolysaccharide in cyanobacteria in response to metals also reflects one of the strategies for survival under stress, as these polymers have potential to interact strongly with cations as metal chelators (Kiran and Kaushik 2008; Huertas et al. 2014). This property has also been demonstrated in the green alga *Chlorella* sp. (Kaplan et al. 1987). The stimulation of exopolymer biosynthesis by Cu, Ni or Hg cations was first reported in the cyanobacterium *Nostoc spongiforme* (Singh et al. 1999) contrary to previous suggestions about the role C/N ratio in regulation of EPS (Bergmann 1986). In our study, increased EPS production may possibly indicate the role of As-mediated regulation which warrants further investigation.

Based on the As bioabsorption and transformation experiments, it was found that the As-tolerant cyanobacterial isolates not only grow at high As(III) concentration, but also have the ability to absorb substantial amounts of As. It has been reported that prokaryotes adapt to high concentration of As by activation or deactivation of As transporter genes or efflux pump genes (e.g., Yin et al. 2011; Sounders and Rocap 2015). In this study, absorption of As increased with increase in As(III) concentration in the growth media but not based on BCF values. Previous studies have documented similar finding in *Synechococcus leopoliensis*, *Chlorella vulgaris*, *Synechocystis* sp. PCC6803, *Microcystis* sp. PCC7806, *Nostoc* sp. PCC7120, and *M. aeruginosa* (Budd and Craig 1981; Maeda et al. 1992a, b; Yin et al. 2011; Wang et al. 2013). Three out of five isolates, namely NOC, NOK and WEK, exhibited higher capability to bioabsorb As (~0.958–2.870 mg kg⁻¹ and BCF ~0.074–0.220 at NOEC) based on analysis of dry biomass compared to available reports involving freshwater algae (Levy et al. 2005; Yin et al. 2011; Wang et al. 2013). Therefore these isolates can be potentially used for bioremediation of As-contaminated rice fields located in BDP.

During exposure studies, the major As species detected in cyanobacterial isolates was the less toxic inorganic As(V).

The transformation ability was found to be isolate-specific and highest in the experimental biomass of NOK isolate. Similar findings have been also reported for different species of algae (Yin et al. 2011; Wang et al. 2013). In our study, oxidation of As(III) to As(V) was detected in the growth medium as well as in cyanobacterial biomass when exposed to As(III). The process of As(III) to As(V) conversion in the culture was directly mediated by the cyanobacterial cells. The toxicity of As is dependent on speciation, with inorganic arsenicals thought to be more toxic than organic forms (Tamaki and Frankenberger 1992). It must therefore be assumed that As-resistant organisms either compartmentalize and/or transform As to other less phytotoxic arsenic species in order to withstand high cellular arsenic burden (Meharg 1994; Qin et al. 2009). Our results indicate that such pathways may exist in the five tested cyanobacterial isolates. It has been reported that As(III) within the cyanobacterial cell may be complexed with phytochelatins (PCs) (Sneller et al. 1999). When these PCs are extracted in media, PC-complexes may dissociate to form free PCs and As(V) [reduction of the PCs and oxidation of the As(III) to As(V)]. Thus, when As(V) was detected in biomass extracts as part of our study, it may have originated from PC complexed As(III). Yin et al. (2011) investigated As biotransformation in three cyanobacterial species (*Microcystis* sp. PCC7806, *Nostoc* sp. PCC7120 and *Synechocystis* sp. PCC6803). They encountered inorganic As(V) and As(III) as the predominant species, with As(V) making up more than 80 % of total As.

Bacterial adaptive response to As has been demonstrated in two ways (Cleiss-Arnold et al. 2010). First, bacterial cells express various genes involved in defense mechanisms and next several metabolic activities including for As(III) oxidation. Oxidation of As(III) to As(V) is an energy-producing reaction that is also believed to be a detoxification process since As(V) is less toxic than As(III) (Páez-Espino et al. 2009) and play a crucial role in microbial physiology (Stolz 2011). Generally, bio-oxidation is the first and primary transformation mechanism for As(III) biotransformation, while bioreduction together with subsequent methylation may play an important role in As(V) biotransformation (Qin et al. 2009; Bhattacharjee and Rosen 2007) and biological production of volatile As compounds is considered to be an important part in global As biogeochemical cycling (Michalke and Hensel 2004). Rapid changes of As speciation that occurred in the growth media containing inorganic As species indicated that the cyanobacterial isolates were able to detoxify inorganic As. Decrease of As(III) and increase of As(V) occurred synchronously in the growth medium after 7 days As(III) exposure. Moreover, total As in the medium decreased to 72–84 % within 7 days depending on the cyanobacterial isolate tested. The decrease in total As from culture medium showed the biological potential of these isolates to be able to remove As from contaminated flooded rice fields located in BDP. In particular,

the dominant As species in cyanobacterial cells was As(V) regardless of exposure concentration, thereby illustrating the detoxification potential of these cyanobacterial isolates.

Previous studies have shown that the abundance of As(III)-oxidizing microbes could enhance As(III) oxidation to As(V) in the rice rhizosphere, resulting in decreased As mobility and bioavailability since As(V) can be sequestered on Fe/Mn hydroxide/oxyhydroxide in rhizosphere soil as well as in rice roots (Liu et al. 2006; Seyfferth et al. 2010). In several heterotrophic and chemoautotrophic microorganisms, As(III) oxidation is catalysed by As(III) oxidases, which are encoded by *aioA* and *aioB* genes of the *aio* enzyme (Hamamura et al. 2009; Slyemi and Bonnefoy 2012). In autotrophic cyanobacteria such as *Synechocystis* sp. PCC 6830, arsenic oxidation, reduction and methylation are mediated by the *arsBHC* operon (Sanchez-Reigo et al. 2014 and references therein for details). In the cyanobacteria *Synechocystis* sp. PCC 6803 and *Nostoc* sp. PCC7120, *ArsM* pathway exist with three *ArsM* homologs for As(III) resistance and to catalyse methylation of As(III) and the *ArsM* from *Nostoc* sp. PCC7120 exhibits highest methylation capacity (Yin et al. 2011 and references therein for detail). In *Prochlorococcus* populations of global marine ecosystem, the importance of the *ArsM* pathway (Qin et al. 2006; Paes-Espino et al. 2009) as well as As(III)-specific efflux detoxification pathway has been also reported (Saunders and Rocap 2015). In another study, Zhang et al. (2015) undertook a study to show microbe-mediated As biotransformation in paddy soils across South China. They showed wide distribution of arsenite oxidase genes (*aioA*), respiratory arsenate reductase genes (*arrA*), arsenate reductase genes (*arsC*) and arsenite adenosylmethionine methyltransferase genes (*arsM*) in paddy soils. The abundance of *aioA* genes was almost 10 times higher than *arsM* gene abundance, but the diversity and the OTU richness of *arsM* genes were much higher than *aioA*, suggesting highly diverse microbial communities are involved in As methylation in paddy fields. It would be therefore important to investigate genetic mechanisms of As bioaccumulation and biotransformation for our cyanobacterial isolates targeted from the As-contaminated rice fields of BDP as part of future studies.

Overall, as part of this study, five most abundant cyanobacterial isolates with varying metabolic potential have been screened for the first time from As-contaminated rice fields of Bengal Delta Plains. Our findings indicate that all the indigenous isolates showed ability to absorb and transform As(III), an inorganic form of As, and could be potentially used for bioremediation of As-contaminated rice field environments through extensive trials. Additionally, these fast-growing cyanobacterial isolates with moderate nitrogen fixation ability could additionally used for as a soil biofertilizer. To conclude, application of cyanobacterial biomass representing these isolates, which can significantly bioabsorb

and biotransform As in rice fields, may ultimately result in less bio-available As for uptake by rice plants via plant transport system, thereby rendering food grains safe for human consumption in BDP as well as in South Asia.

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