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Enhancement of biosorption capacity of cyanobacterial strain to remediate heavy metals

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ABSTRACT

The present study comprises a new method for enhancing biosorption capacity of isolated cyanobacterial strain to remediate metals (Cr, Pb, Ni, Zn and Cu) from different industrial effluent including dairy, sugar mill, pharma and steel plant by the means of controlled minimum electric current. Random Amplified Polymorphic DNA analysis was performed for determination of genomic variation produced within the cyanobacterial cells due to electricity. Initial metal concentrations were found maximum in steel plant effluent followed by pharma, sugar and dairy effluent. Maximum concentration of metals was recorded in an order of Zn > Cu > Cr > Pb > Ni in dairy and steel plant effluents. While the sugar mill effluent contains metals in Cr > Cu > Zn > Pb > Ni order and in pharma effluent it was found as Cu > Cr > Zn > Pb > Ni. For determination of biotechnological importance and significant role of wild and electricity treated improved strain of *A. variabilis GITAM RGP*, biosorption experiments were carried out in batch conditions. The electric current treated improved strain was found to be more efficient over wild strain as it was shown maximum removal of copper (87.50%), chromium (82.96%), lead (86.44%), zinc (86.59%) from the sugar mill effluent and nickel (82.76%) which was removed maximum from pharma city effluent.

Keywords: Biosorption; Cyanobacteria; Minimum electric current; Improved strain; Industrial effluent

1. Introduction

One of the vital aspects of life is "Water". Various natural and anthropogenic activities lead surface water pollution which causes human health hazards [1–4]. Metals are one of the major and hazardous contaminants of water pollution. Even low concentrations of certain heavy metals like lead, nickel, chromium, etc. as pollutants have the potential to cause effects such as inhibition or modification of vital biological entities, including enzymes, DNA and protein [5–8]. Mobilized or dissolved metals can also exhibit the bio-magnification phenomenon, increasing in concentration as they accumulated through food webs [9]. After entering to the body, these toxic metals hastily spread to the whole system via the bloodstream [10]. Initially, clinical symptoms such as nausea, vomiting, stomach cramps appear followed by diarrhea. Reduction of liver and plasma iron levels, and fall in hemoglobin level can also be observed during hematological investigations [11]. Chronic conditions such as severe damage of proximal convoluted tubules of kidney,

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hepatocellular necrosis, myocarditis and aseptic meningitis lead to failure of heart, kidney, liver, central nervous system (CNS) *etc.* and finally may cause death [12]. Therefore removal of these toxic pollutants from effluents is important in view of negative health effects.

Bioremediation is one of the superlative ecofriendly and precisely sensible techniques for resolving this quandary because of its special properties including minimization of chemical and biological waste, elevated efficiency, low cost, renaissance capability and possible metal renewal ability, etc. [13]. Earlier research revealed the affirmative impact of *cyanobacteria* as important bioremediator and with the ability to remove various metal ions and metal complexes from soil and solutions [9,14]. Cyanobacteria are ubiquitous in nature occupying a wide range of ecological habitats, distributed from the Arctic and Antarctic valleys to hot sulphur spring [15]. They easily colonize terrestrial and aquatic ecosystems as free living or symbiotic forms, this makes them largest photosynthetic group of prokaryotic on the planet [16–20]. Paddy fields are well known ecological habitats which support the growth and survival of these diazotrophs [21]. In addition, Different species of *Anabaena*, *Lyngbya*, *Phormidiumbohneri*, *Microcoleus* and *Scytonema* were shown to be able to grow in water containing contaminants from industry and sewage [22].

These Cyanobacteria can uptake and accumulate metals from the environment by physicochemical and biological mechanisms [14]. A role for extracellular polysaccharides, intracellular polyphosphates, mucilaginous outer sheath and specific proteins has been suggested in resistance to or absorption of toxic metals [23]. The processes resulting in the uptake of heavy metals by biomass are: cell surface binding, intracellular accumulation and extracellular accumulation. The binding of heavy metals on the cell surface may be exhibited by either living or inactivated microorganisms [9]. This process does not depend on the metabolism of the Cyanobacteria. The other two processes can occur only within living cells as they require energy [10]. The indigenous microorganisms of a toxic metal environment are potential candidates for metal tolerance, and thus bioremediation as resistance mechanisms are often enriched with inbuilt abilities which may be restricted via plasmid encoded. Such features include bio-surfactant, extrusion, catabolic potential, biotransformation, production of exopolysaccharide (EPS) and synthesis of metallothioneins abilities [9,24].

Physical factors (including temperature, pH, salt and sugar concentrations, vibrations, radiations, electric field, etc.) have ability to modify survival and growth characteristics of an organism. They may induce alterations in cell permeability, metabolic activity, structural and chemical composition of various biomolecules and even genomic information. For decades, theory and principle of stress are used for controlling growth of various microbes present in solids, liquids and gaseous mediums [25]. Recently application of electric current for the same purpose was used as pulsed electric current technique in juice and beverage industries, where, a single pulse of 30 kV/cm for a very short time (240 or 480 μs) is applied for sterilization of product [25]. Usually these factors when in extremes are inhibitory, however at lower levels they may also induce modifications or mutations thereby improving their beneficial functions. Earlier studies illustrate the positive impact of these factors when used in a controlled manner [26]. In medical field the electric field exposures have variously been explored in aiding therapy such as for healing corneal epithelial cell injury and for treatment of open ulcers in diabetic patients which demonstrated the role of electric current as novel therapeutic technique [27–30]. Role of electric stimulation in an agricultural discipline has also been reported. Black et al. were reported that the significant enhancement in growth of tomato plants in terms of linear growth and metal uptake capacity when subjected to direct electrical stimulation [31]. They reported activation of growth regulation compounds and ion pumps due to electric shock.

Keeping in view the above, we started present work with cyanobacteria as the test organisms. Though the natural isolates exhibit lot of applications, but their efficiency is not sufficient for industrial or large-scale usage. Thus, there is a need to develop improved strains for their extensive exploitation. The aim of present study was to determine the possibility of inducing improvement in the isolated cyanobacterial strain "*Anabaena variabilis GITAM RGP*" via controlled electric current for enhancement of bioremediation ability towards toxic metal pollutants from industrial effluents. Present work was started with the major objectives which includes isolation, screening and for strains showing metal absorption ability, improvement and determination of post electric treatment effects in improved strains and finally, assessment of enhanced bioremediation potential. We planned to assess the obtained varieties (wild strain and improved strain), as potential bioremediator for biosorption of chromium, nickel, lead, copper and zinc in different industrial effluents including dairy, sugar mill, pharma city and steel plant. In addition FT-IR, GCMS and RAPD analysis were performed for determination of chemical and genomic variation produced within the cyanobacterial cells due to electricity.

2. Material and methods

2.1. Collection of cyanobacterial strain

The filamentous cyanobacterial strain was obtained from the effluent of Visakhapatnam Steel Plant, Dist-Visakhapatnam, AP, India (17°38′04.97″N–83°10′25.47″E). The samples were collected in sterilized plastic bags and transferred to the ice box, immediately. The samples were brought to the Laboratory for further processing.

2.2. Isolation and identification of cyanobacterial sample

The isolation and purification techniques used were similar to those described earlier by Newton and Herman 1979 [32]. Identification of the isolated cyanobacteria was based on morphological characters, microscopic studies, pigment production and 16S rRNA sequencing [33]. Identification on the basis of genomic study was performed by isolation of DNA from exponentially growing cyanobacterial pure culture using an ultraclean plant DNA isolation kit (MoBioInc, USA). Amplification of 16S rRNA gene was

carried out using universal primers fD1 (5′-AGAGTTTGATCCTGGCTCAG-3′) and rD1 (5′-AAGGAGGTGATCCAGCC-3′) using Quanta-Bio (Biotron, USA) thermal cycler. Montage PCR Clean up kit (Millipore) was used for the removal of unincorporated PCR primers and dNTPs. The PCR amplified 16S rRNA genes were sequenced by Big Dye terminator cycle sequencing kit (Applied Biosystems, USA) by using M13 forward and reverse primers. Resolvesion of sequenced products was carried out on an Applied Biosystems model 3730XL automated DNA sequencing system (Applied Biosystems, USA). Isolated sequence of cyanobacterial strain was rearranged and compared with those available in the NCBI databases. After identification, the sequence was submitted to NCBI GenBank and finally a phylogenetic tree was constructed with Clustal W and Tree view 2 software.

2.3. Electric shock treatment method

An electrophoresis unit with perforated diaphragm was used as an electric chamber for this study (Fig. 1). This diaphragm separates stage within the chamber and allows electric current and broth medium to circulate. Both anode and cathode terminals were joined to an electrophoresis power supply unit. A cyanobacterial cell suspension of 5% (OD : 0.15 at 560 nm) was transferred in centric position of the stage after filling the chamber with 142.5 ml of autoclaved BG 11⁻ broth medium. Desired amperes of current and time were set by using the setting knobs for current and time on electrophoresis power supply unit. Mass production of algal culture was carried out by inoculating the culture in sterile BG 11⁻ medium. The culture was incubated under photon flux density (300 µmol m^{-2} s^{-1}) at a temperature of 25°C ± 1 for 15 days and then used for further investigation [34].

2.4. Random amplified polymorphic DNA (RAPD) of cyanobacterial isolates

Random Amplified Polymorphic DNA was performed as per Williams et al. methods for which 5 ml of *cyanobacterial* sample was ground in a mortar gently and centrifuged at 3000 rpm for 5 min [35]. The pellet was re-suspend in 467 µl TE buffer by repeated pipetting in which 30 µl of 10% SDS and 3 µl of 20 mg/ml proteinase K were added, mixed and incubated at 37°C for one hour. After incubation equal volume of pre warmed phenol was added and gently mixed by inverting the tube and again centrifuged at 3000 rpm for 2–3 min. The upper aqueous phase was collected in a fresh tube in which 2 volumes of chloroform: isoamyl alcohol (24: 1) was added, mixed and centrifuged at 10,000 rpm for 5 min. 500 µl of upper aqueous phase was collected into a new tube in which 2 volumes of 100% ethanol and 0.1 volume 3 M Sodium acetate were added and the pH was adjusted at 5.2. The mixture was mixed gently and kept at –20°C for one hour. After incubation the mixture was centrifuged at 10,000 rpm for 3 min. The pellet containing DNA was washed with 70% ice cold ethanol (500 µl), dried and dissolved in 50–100 µl of TE buffer solution. The concentration of DNA was measured by diluting 2 µl of DNA in 198 µl of TE (1: 100 dilution) and measured absorbance at 260 nm and 280 nm. Concentration of original DNA solution in µg/ml = OD at 260 nm × 50 × dilution factor.

2.5. Polymerase Chain Reaction (PCR) analysis

Genomic DNA from both the strains of *A. variabilis GITAM RGP* was obtained and amplification of the product was carried out by PCR with 36 cycles in three-step PCR (thermal cycler). Appropriate annealing temperatures and additives were optimized for the system. The product was

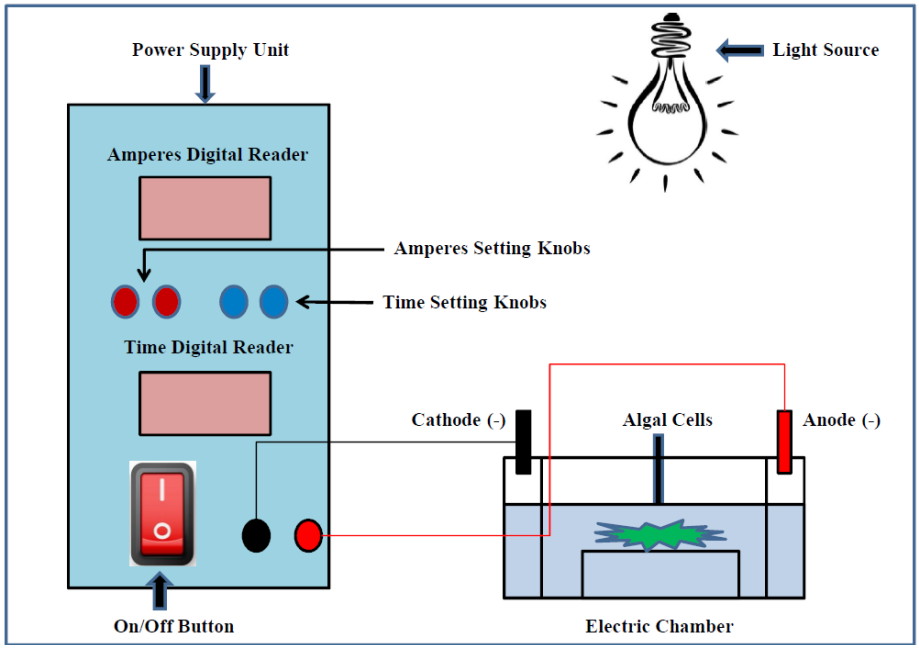

Fig. 1. Electric shock treatment setup.

amplified by polymerase chain reaction by RAPD in a 20 µl reaction volume containing 10 mM TrisHCl (pH 8.0), 50 mM KCl, 15 mM $MgCl_2$; 10 mM each dNTPs; 10 pmol primer, and 1U *Taq* DNA polymerase and 100 ng of genomic DNA.

PCR protocol			
Template	100 ng of genomic DNA/ reaction volume of 10 µl.		
RAPD primer	5′- CCG CAG CCA A - 3′		
dNTPs	Final concentration 200 µM each		
Buffer	Final concentration 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM TrisHCl (pH 7.6 - 8.0)		
Taq DNA Polymerase	0.50 U/reaction volume of 10 µl.		
20 µl volume PCR reaction mixture			
Double distilled water	11.40 µl		
1.5 mM $MgCl_2$ PCR mix	2.00 µl		
dNTPs:	1.60 µl		
Primer:	1.00 µl		
Taq polymerase:	1.00 µl		
0.02% glycine	1.00 µl		
DNA sample	2.00 µl		
PCR conditions			
Initial temperature	94^0C	5 min.	
Denaturation	94^0C	1 min.	45 cycles
Annealing	37^0C	1 min.	
Extension	72^0C	2 min.	
Final extension	72^0C	7 min.	
Hold	4^0C	α	
Gel	1.5% agarose in 40 ml 1x TEB buffer		

2.6 .Agarose gel electrophoresis

Amplified products from each sample were separated electrophoretically on 1.5% agarose gel (Fisher Biotech, USA) containing ethidium bromide in 1 X TAE buffer at 110 V for 30 min. To determine molecular weight a DNA marker (Ø X 174 DNA/Hae III digest and /or 100 bp DNA ladder) was electrophoresed alongside RAPD products. DNA bands were observed on Bio Rad Molecular Imager Gel Doc XR + Gel Documentation system and labeled.

2.7. Sampling of industrial effluents

In this study, the effluent samples after secondary treatment were collected from various industries includes Visakha Dairy, Ankapalle Sugar Mill, Pharma City and Steel Plant, Visakhapatnam, Andhra Pradesh, India. Effluent samples were collected in sterile plastic bags of 1 L volume each and in sterile sample collection screw cap bottles of 1 L volume. At least ten samples each were collected from the sampling sites. The samples were labeled, kept in dark ice box and transported to the laboratory andthe collected samples were stored in the refrigerator at about 4^0C prior to analysis.

2.8. Bioremediation assay

To evaluate the bioremediation potential of cyanobacterial strains on industrial effluent samples from the four industries viz., Dairy, Sugar Mill, Pharma and Steel Plant, experiments were designed. For each industrial effluent, three sets of flasks (each set contains 3 flasks) were taken. The first and second sets were dispensed with 1 L of effluent, in which 100 g dehydrated log phase wild and improved cultures (15 d old) were inoculated. While third set containing 1 L effluent was maintained as an experimental control. All the three sets were kept for 10 d at 16 : 8 h, light : dark conditions under photon flux density of 300 µmol m^{-2} s^{-1} at 25/20 ± 2°C, day/night temperature. After the period of incubation, 3 ml of 1 N HNO_3 solution was added to all of the experimental flasks as to destroy the matrix, which interfere during atomization and to converts all form of metal into a single oxidation state. After gentle mixing, filter sample with Whatmann filter paper-42. For enhancing selective and efficient metal extraction, 100 ml of filtrated was transferred to different conical flasks and mixed with 15 ml diacid mixture (HNO_3 : $HClO_4$ – 9 : 4 ratio) and kept on hot plate for evaporation. After complete evaporation, 100 ml of distilled water was added, filtered and collected in different labeled bottles and finally used for atomic absorption studies. For detection of metals such as chromium, lead, nickel, copper and zinc in the samples atomic absorption spectrophotometer instrument (AAS4141) was used for analysis.

2.9. Statistical analysis

All tests were conducted in three replicates and data is reported as means ± standard deviation (n = 3). Results were analyzed statistically by two way analysis of variance (ANOVA) followed by Post Hoc analysis (Tukey HSD) through SPSS [36]. In addition, null (H_0) and alternate (H_1) hypothesis were also reported for the significant difference between the groups. P-Value < 0.05 was considered statistically significant.

3. Results and discussion

3.1. Primary identification

Cyanobacteria are oxygenic prokaryotes of morphologically diverse group which adopted both PS II and PS I photosynthetic pathways. They are well known for their potential applications in nutritional, agricultural and medicinal areas, due to their facility to produce variety of chemical substances and enzymes [9,10,16]. In addition, biosorption ability for toxic metals from effluents is another interesting feature of these prokaryotes which increase research interest among them [34]. In this study, cyanobacterial sample was collected aseptically from Visakhapatnam steel plant effluent. The sample when plated on solid BG 11^- medium, showed the appearance of morphologically three types of colonies. These colonies were clearly distinguishable after 20 d of growth based on distinct color and appearance.

Microscopic examination of type one colonies revealed presence of slightly curved, coiling or sinuous appearance of the individual filament. Each filament showed beaded appearance with two types of cells – vegetative cells and

heterocysts. Vegetative cells exhibited a round to oval shape, whereas heterocysts were oval to elongated. Type two cyanobacterial colonies were made up of straight or bent narrow filaments, no heterocysts were present in the filaments. These colonies were initially small pin pointed but later on rapidly spread irregularly, producing flat colonies. Third category was small pale yellowish green colonies which appeared circular under microscope. They exhibited only a single type of cell forming a rosette like structure with granular appearance of the cell. Cell dimensions of these colonies were largest when compared to the first two categories (Fig. 2).

The first type of algal cells from steel plant effluent was used for further experiments, based on its higher growth. Colony morphology, cell types and structure, including the cell dimensions were used to identify the cyanobacterial colony, as per Desikachary 1959 [37]. It was tentatively identified as *Anabaena species.* Axenic colonies of *Anabaena sp*. were obtained by repeating plate methods.

3.2. Molecular identification

Identification upto the species level was determined as a 16S rRNA gene sequence similarity of ≥96% with that of a prototype strain sequence in the GenBank [38] and submitted at NCBI GenBank and named as "*Anabaena variabilis GITAM RGP*" (Fig. 3) [Accession Number details – (JX134587) 1 sequence (20th Aug 2012) Authors: Pant, G &Prasuna, R. G.].

3.3. The development of electricity adapted improved strain of A. variabilis GITAM RGP

The exposure time of 80 min at 10 A electric field strength was the maximum initial exposure of electric current, survived by the cyanobacterium. The particular cyanobacterial sample which survived the exposure to the electric stress as above was used as the starting material to develop a current adapted cyanobacterial strain (improved strain). These suspensions, after incubation for 15 d under optimum growth conditions, were again given a series of electric treatment in different concentrations for one or two hours (Table 1). The electric exposure was repeated with incubation periods of 15 d in the standard growth conditions between successive current exposures. The ability of the adapted cultures was seen to increase, with survival and growth observed, even after electric shock treatment of 10 A for 5–6 h as compared to the control cells (wild strain). This electric current adapted culture was used for biosorption studies as an improved strain and unexposed isolate as the wild strain.

3.4. Random Amplified Polymorphic DNA (RAPD) of wild and improved strains

Random amplified polymorphic DNA (RAPD) profile using randomly selected 10 bp length primer (5′-CCGCAG-CCAA-3′) was done to reveal any modification of genomic information caused by the means of electric shock treatment in improved *A. variabilis* strain. The use of smaller

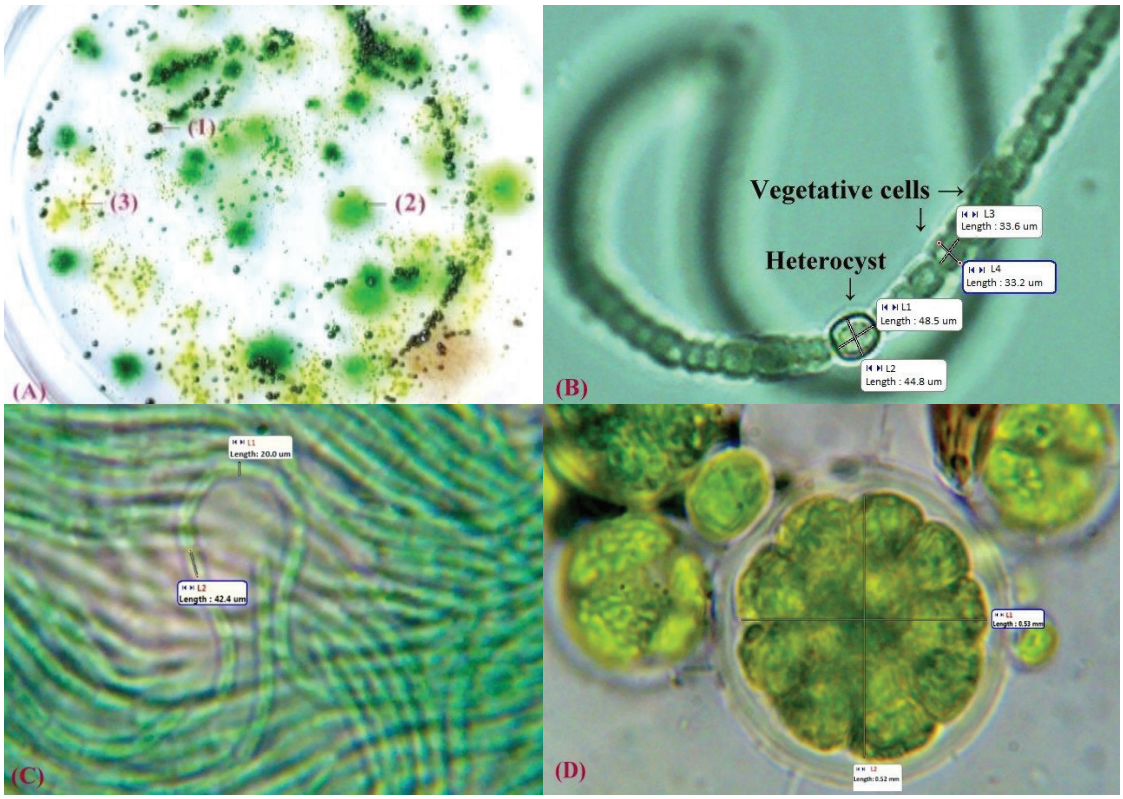

Fig. 2. Growth of algal sample on solid BG 11 agar plate: (A) after 20 d of incubation, (B) micrographs of type one, (C) type two and (D) type three colonies.

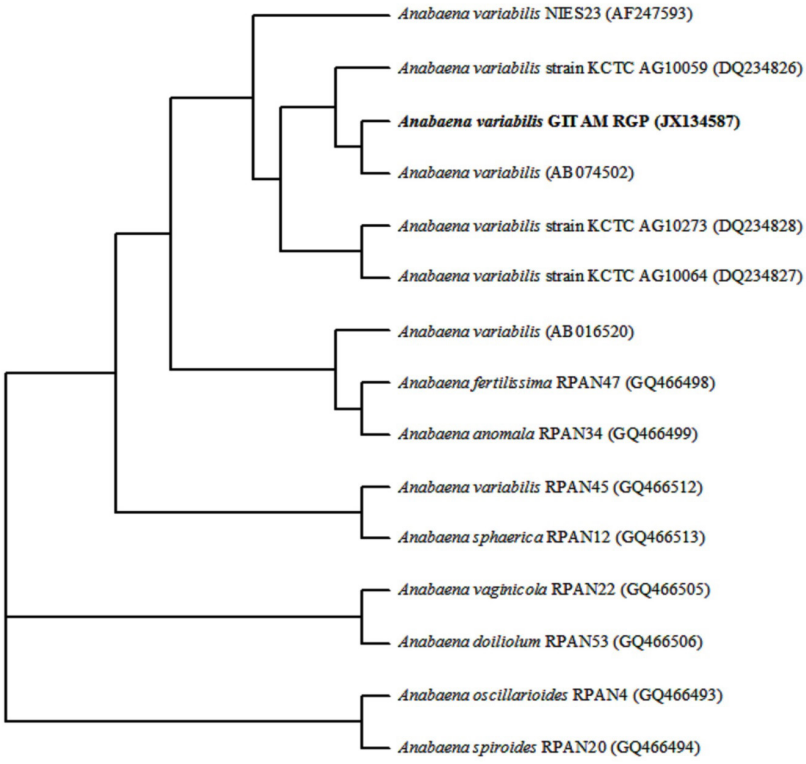

Fig. 3. Phylogenetic tree of *Anabaena variabilis GITAM RGP.*

Table 1
Effect of different current levels and time on the survival of cyanobacterial strain

S. no	Current (A)	Time (h)	Survival of cyanobacterial cells
1.	1	1	+
2.	3	1	+
3.	5	1	+
4.	7	1	+
5.	9	1	+
6.	2	2	+
7.	4	2	+
8.	6	2	+
9.	8	2	+
10.	10	2	+

(+) Survival

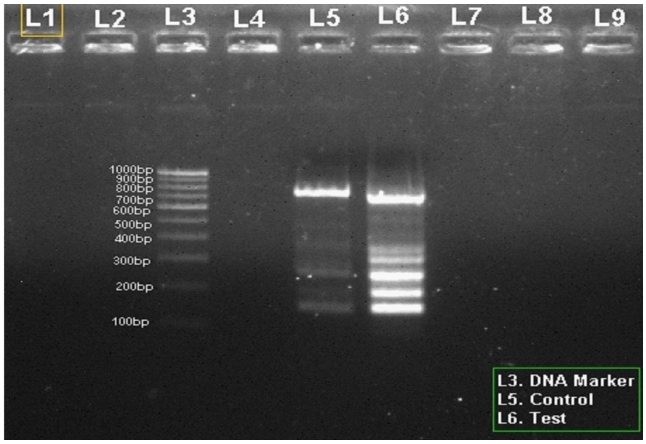

Fig. 4. RAPD profile of *A. variabilis* strains: Lane 3 (L3) - DNA Marker, Lane 5 (L5) – Wild strain and Lane 6 (L6) - Improved strain.

length of primer leads to much higher numbers of bands, and a larger primer may mean decreased pairing with the template. It is evident from the gel photograph that wild strain of *A. variabilis* and electricity adapted variant strain produced different size of the bands. One prominent band of 800 bp appeared in the wild *A. variabilis* strain while a prominent band of 750 bp appeared in the improved strain, the difference in the size of the bands could mean a shift due to a decrease by loss of DNA within the particular sequence in the improved strain. In addition, five bands of much smaller size ranging from 150 bp to 350 bp were observed. These clearly indicate changes at the DNA level in the improved strain (Fig. 4).

3.5. Initial studies of effluents

On the basis of triplicate results readings were taken for metal concentration in different effluent samples (Visakha Dairy, Ankapalle Sugar Mill, Pharma City and Steel Plant) as shown in Table 2 and all values are expressed in ppm (mg/l).

Table 2
Initial concentration of metals in different industrial effluents (mg/L)

Sites / Accepted values	Cu	Cr	Pb	Ni	Zn
DAIRY	0.33 ± 0.04	0.29 ± 0.02	0.16 ± 0.04	0.07 ± 0.02	0.36 ± 0.03
SUGAR	1.28 ± 0.03	1.35 ± 0.05	0.59 ± 0.04	0.11 ± 0.02	0.82 ± 0.03
PHARMA	1.74 ± 0.03	1.42 ± 0.03	0.48 ± 0.05	0.29 ± 0.04	0.52 ± 0.05
STEEL PLANT	1.93 ± 0.03	1.85 ± 0.05	1.44 ± 0.02	0.38 ± 0.02	2.26 ± 0.02
WHO guideline	2.00 ± 0.05	0.05 ± 0.01	0.01 ± 0.005	0.02 ± 0.001	2.00 ± 0.05

Here, Data is given as Mean ± Standard Deviation of three replicates (n = 3).

For determination of different metals selective wavelengths were used as Cr (λ) = 357.9 nm, Cu (λ) = 324.8 nm, Pb (λ) = 217.00 nm, Ni (λ) = 232.00 nm and Zn (λ) = 213.86 nm in atomic absorption spectrophotometer (AAS4141).

Metal concentrations were found maximum in steel plant effluent followed by pharma, sugar and dairy effluent. Comparative studies of heavy metals demonstrated maximum concentration of Zn in steel plant and dairy effluent while Cr and Cu were found maximum in sugar and pharma effluent respectively. For both dairy and steel plant effluents maximum concentration of heavy metals were in an order of Zn > Cu > Cr > Pb > Ni. Sugar mill effluent contains heavy metals in Cr > Cu > Zn > Pb > Ni, while in pharma effluent order of heavy metals were changed and found as Cu > Cr > Zn > Pb > Ni.

The biosorption experiments were carried out in batches to evaluate potential of wild and electricity treated improved strain of *A. variabilis GITAM RGP* towards Cu, Cr, Ni, Pb and Zn in various industrial effluents. Cyanobacterial treatment significantly reduced the concentration of above mention metals, however the electricity treated improved strain of *A. variabilis GITAM RGP* was found to be more efficient than that of the wild strain. Results are represented as Mean ± Standard Deviation of three replicates.

In all studied industrial effluents, only copper was found 0.33–1.93 ppm which is within the WHO recommended permissible limit (2 ppm) [39]. While the range of other studied metals was found above the permissible limits of 0.05 ppm, 0.01 ppm, 0.02 ppm and 2 ppm for chromium, lead, nickel and zinc respectively. In studied industrial effluents chromium was found in the range of 0.29–1.85 ppm, lead 0.16–1.44 ppm, nickel 0.07–0.38 ppm and zinc 0.36–2.26 ppm.

In present study, improved strain shows higher removal percentage of metal ions in industrial effluents when compared with wild strain. Among all effluents, maximum removal percentage of copper, chromium and lead were observed in sugar mill effluent as 87.50%, 82.96% and 86.44% respectively, followed by the wild strain of *A. variabilis GITAM RGP* i.e. 66.41%, 72.96% and 62.71% respectively after 10 d (Figs. 5A, B, C). Nickel was found below detection limits after cyanobacterial treatment (for both wild and improved strains) in dairy and sugar mill effluent (Fig. 5D). While comparative studies of pharma and steel plant effluents demonstrate maximum biosorption ability of improved strain 82.76% and 78.95%, followed by wild strain 55.17% and 60.53% respectively. Similarly, lowest concentration of zinc was detected in sugar mill effluent when compared with other studied effluent samples after treatment with cyanobacterial strains (Fig. 5E). Comparative studies demonstrate 68.29% removal of zinc by wild strain in sugar mill effluent which was 18.3% more with improved strain of *Anabaena variabilis GITAM RGP*.

In order to check the concentration of Cu, Cr, Pb, Ni and Zn metal in the industrial effluent, we applied t-test (right tailed) between the two different parameters 3 times i.e. UE to WSTE, WSTE to ISTE and UE to ISTE. The null and alternate hypotheses were defined as:-

$H_0: \mu_x = \mu_y$, i.e. There is no significant difference between the mean heavy metal concentration in UE (μ_x) to WSTE (μ_y)

$H_1: \mu_x > \mu_y$, i.e. The mean heavy metal concentration has decreased after treating effluent with wild strain. (Right-tailed test)

Clearly, after applying t-test through SPSS, we found the t-calculated value at 5% level of significance and 11 (n –1) degree of freedom for Cu, Cr, Pb, Ni and Zn as 4.052287, 3.6444002, 2.602736, 3.044839 and 2.711072624 respectively. On comparing with the critical t-value = 1.786 ($\alpha = 0.05$, $\nu = 11$), Cu, Cr, Pb, Ni and Zn concentration between WSTE to ISTE is 3.249545, 2.8628185, 2.017701, 2.404411 and 1.830659 respectively and that for UE to WSTE is 5.653328, 5.1624268, 3.737952, 4.625487 and 3.56738656 respectively. On the basis of these calculated t-values, null hypothesis is rejected and confirmed the ability of cyanobacterial potential for all the heavy metal remediation in each case.

4. Conclusions

The Random Amplified Polymorphic DNA (RAPD) analysis showed presence of additional bands in the improved strain, giving an indication of a minor but permanent change at the genetic level due to the electricity exposure. After ascertaining the development of a new strain based on various analytical techniques the two strains were assessed with respect to their application for removal of metals. Results proved the biotechnological importance and significant role of wild and improved strain for promising removal of investigated metals contaminants (Cr, Pb, Ni, Cu and Zn) from various industrial effluent samples such as dairy, sugar mill, pharma and steel plant. The present study revealed the positive impact of wild *Anabaena variabilis GITAM RGP* strains in the environmental area as they exhibited good metal biosorption ability. In addition present study also demonstrates a new method for enhancing this faculty by the means of controlled "minimum electric current" (MEC). Effluents obtained from secondary treatment plants from various industries can use an additional step i.e. application of such biotechnologically improved strains, for removal of toxic pollutants from waste water. These

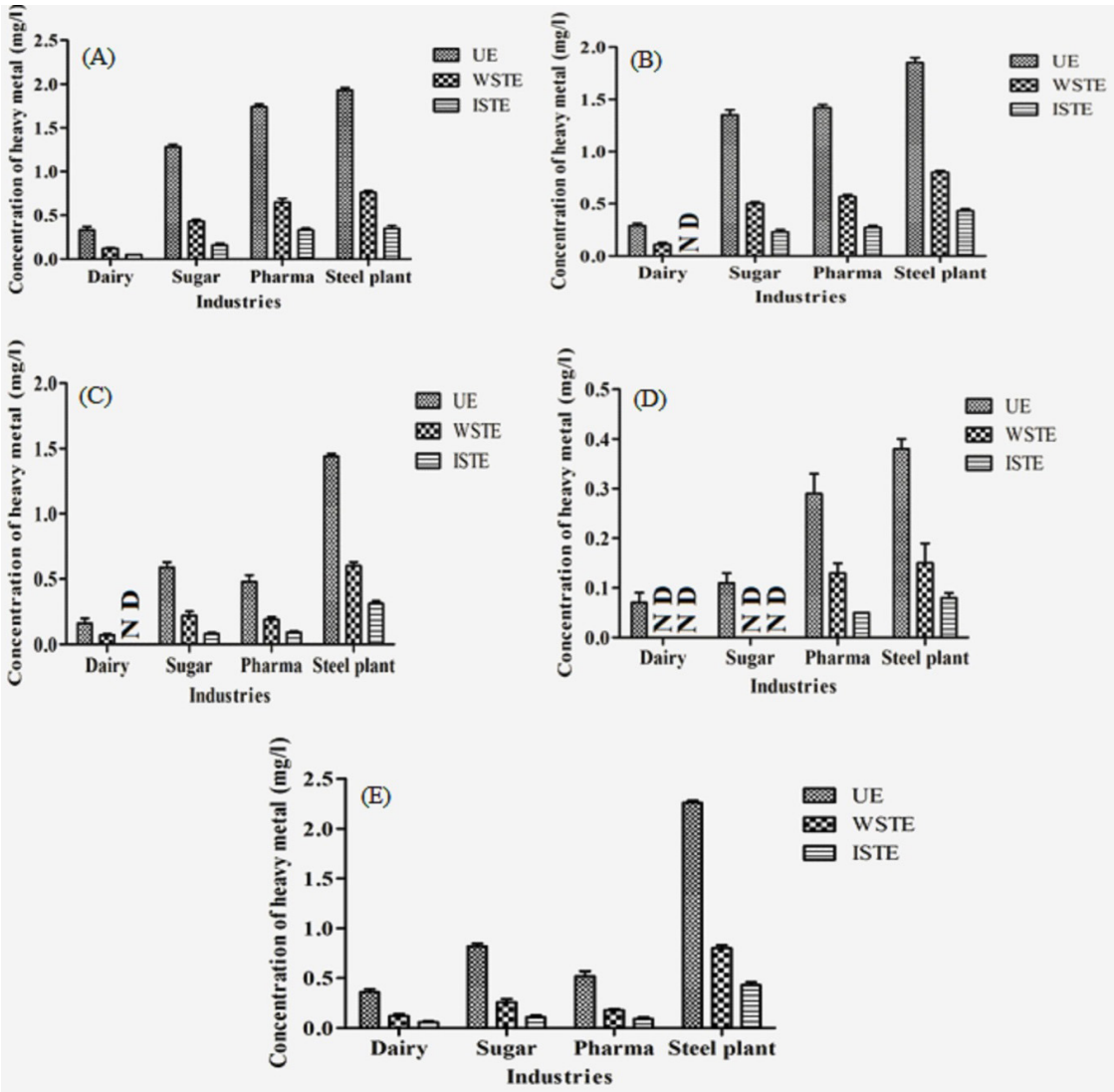

Fig. 5. Biosorption potential of different metals on wild and electricity treated improved *A. variabilis GITAM RGP* strain: (A) copper, (B) chromium, (C) lead, (D) nickel and (E) zinc. (UE – untreated effluent, WSTE – wild strain treated effluent and ISTE – improved strain treated effluent. Data are given as mean ± standard deviation of three replicates).

modified algal strains could be used in large scale treatment plants for their extensive exploration. In future, such studies may give a better idea on the principle behind the improvement of technologies and performance of adapted strain for solving environment problems. However, further research needs to focus on the mechanism behind the shock based improvement on genetic and molecular levels to finally facilitate their exploitation.

Conflict of interests

The authors have declared no conflict of interests

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