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Screening, characterization and molecular identification of metal tolerant microalgae from the natural habitats

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Abstract
The growing industrialization and modern agricultural practices are spread worldwide and leads to adverse impact on the ecosystem. In particular, contamination of heavy metals in the environment becomes a major global concern due to their toxicity and threat to human life and environment. However, in nature, Microalgae have developed an extensive spectrum of mechanisms to cope with heavy metal toxicity. Their wide-spread occurrence along with their ability to grow and concentrate heavy metals, ascertains their suitability in practical applications of waste water bioremediation. Therefore, present study was focused on screening of metal tolerant microalgal strains isolated from natural ecosystem. Four microalgal strains were isolated from fresh water and cultivated in BG 11 medium under controlled condition. All the four strains were identified *viz*., *Lyngbya* sp. (Blue green algae), *Oscillatoria* sp (Blue Green algae), *Chlorella* sp1 (Green algae) and *Chlorella* sp2 (Green algae). Followed by all the four microalgal strains were screened for its metal tolerance activity against Zinc, Nickel and Chromium under laboratory conditions. Out of four isolates only two microalgal isolates such as *Lyngbya* sp and *Chlorella* sp2 were showed better metal tolerant activity. These two metal tolerant microalgae were authenticated through SEM imaging analysis and molecular sequencing. 16S rRNA molecular sequence data revealed that *Lyngbya* sp. Showed 95% query similarity with *Lyngbyahieronymusii* and based on 18S rRNA sequence data *Chlorella* sp2 showed 97% query similarity with *Chlorella sorokiniana*.

Keywords: microalgae, heavy metals, ecosystem, metal toxicity and bioremediation

Introduction

In recent years, heavy metal pollution has become one of the most serious environmental hitch, most of the heavy metal ions are toxic to living organisms. These metals are non-degradable and persistent in the environment. Hence the elimination of heavy metal ions from environment is important to protect public health.

Algae as a photosynthetic organism which is a large and diverse group found in natural habitats like freshwater, brackish water or on the surface of moist soil or rocks and terrestrial environment. Algae are one of the first organisms to come into existence in the ocean more than 3 billion years ago, when the earth's environment formed. Algae are mostly unicellular in nature and some are multi-cellular. It is an autotrophs which has ability to trap the sunlight and the light energy converted into food molecules by photosynthesis. These are also called phytoplanktons. These organisms have chlorophyll and produce oxygen by sequestering CO2 in the atmosphere through photosynthesis. There are about 100,000 different types of microalgae living not only in oceans but also in fresh water (Isao, 1997).

Algae are the primary producers in the ocean that covers 71% of the earth's surface, is the original source of fossil carbon which is found in crude oil and natural gas. Some algae have secondary heterotrophic, uptaking the complex organic molecules by organography or heterotrophy (Tuchman, 1996) [2], but the fundamental genetic affinities with their photosynthetic relatives are still retained (Pfandl *et al*., 2009) [29]. The phytoplankton production and the biomass in most lentic systems is controlled by grazing, light availability, temperature are involved in regulating phytoplankton and only nutrients are responsible to regulation. (Schindler, 1978; Wetzel, 1983; Hecky and Kilham, 1988) [31, 38, 11].

In particularly, microalgae are the most ecologically diverse groups of photosynthetic and nonphotosynthetic organisms inhabiting marine, freshwater and terrestrial habitats (Thajuddin and Subramanian, 2005; Pondey, 2013; Peter *et al*., 2015) [36]. The microalgae are capable of producing negatively charged exopolysaccharides and having the good sorbent capacity (De philippic *et al*., 2007; Paperi *et al*., 2006; Micheletti *et al*., 2007; Balakiran *et al*., 2016) [27].

The ability of algae can sorbs high concentrations of heavy metals and are removed from the waste waters. This is possible by building an efficient and commercialisms viable algal technology based upon the knowledge of metal sorption by algae. The heavy metal pollution in aqueous systems is a big problem in today's world, technologies for remediation have developed. These technologies target the bioremediation of heavy metal contaminated water by living algal cells, or non-living algal cells that on important concern. Microalgae can uses in bioremediation of metal contaminated area. Because it has the ability to tolerate those metals and it has high yields of recovery per unit mass, and it has high specific outer area coupled with a cell wall loaded with ionisible groups (Gakuistei *et al*., 2012).

Bioremediation refers to the use of microorganisms to degrade contaminants that pose environmental and human risks. Bioremediation processes typically involve the actions of many different microbes acting in parallel or sequence to complete the degradation process. Both in situ (in place) and ex situ (removal and treatment in another place) remediation

approaches are used. The versatility of microbes to degrade a vast array of pollutants makes bioremediation a technology that can be applied in different soil conditions (Sylvia *et al*, 2005).
Microorganisms have the capability to grow in condition with high concentration of heavy metals and maintain a homeostasis within the cell that keeps the heavy metals an optimal sub-toxic level. To survive under metal-stressed conditions, microbes have evolved several types of adaptation mechanisms to tolerate the uptake of heavy metal ions. The chemical form of a metal in the environment is constantly changing due to a wide spectrum of dynamic biochemical processes. This changes are influenced by biotic (interactions with living organisms like microorganisms, plants, and animals) and abiotic factors like temperature, pH, organic matter, and ionic strength (Hafeburg and Kohe, 2007).
Microalgae possess high metal absorbing capacities due to the presence of the polysaccharides, proteins or lipids on the surface of their cell walls containing some functional groups such as amino, carboxyl, hydroxyl and sulphate, which has an ability to bind with metals. The microalgal biomass has highly effective, as well as reliable and predictable in the removal of heavy metals from the aqueous solutions (Priyadharshini *et al.*, 2011).
Hence, the present study was focused on Screening of metal tolerant Microalgae from the natural habitats and to authenticate metal tolerant microalgal strains through SEM imaging and molecular sequencing analysis.

Methodology

Sample collection from natural sources

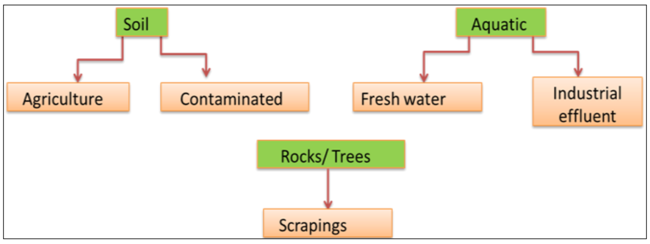

Fig 1

Samples from the different sources *viz*., tannery effluent lagoon, fresh water streams, tree scrapings were collected and transported to the laboratory, Department of Biology, The Gandhigram Rural Institute (Deemed to be University), Gandhigram, Tamil Nadu, India for further analysis.

Isolation and identification

Isolation of microalgal strains from different sources was done using BG 11 medium by standard procedures (Anderson, 1944). Identification of selected microalgal strains was done using standard Algal taxonomic publications Desikachary (1959) [7]; Komarek and Anagnostidis (1998, 2005).

Screening of microalgal isolates for metal tolerance

The four selected microalgal strains (*Lyngbya* sp., *Oscillatoria* sp., *Chlorella* sp 1 and *Chlorella* sp 2.) were subjected to screening for their metal tolerance efficiency by standard procedure (Hellio *et al*., 2000) [14]. Based on its better metal tolerant activity two microalgal strains such as *Lyngbya* sp. and *Chlorella* sp 2, were selected for characterization study.

Characterization of selected microalgal strains with various environmental conditions

The growth performance of two selected metal tolerant microalgal strains in various environmental conditions *viz*., different pH (5 and 9) and various concentrations of metal ions (6ppm and 8ppm) were characterized following standard procedures (Shang, Yu, and Romero- Gonzalez, 2015) [32].

Determination of cell count and the growth rate of two potential metal tolerant microalgal strains

The growth performances of two potential metal tolerant microalgal isolates *viz*., *Lyngbya* sp and *Chlorella* sp2 grown in BG 11 medium was determined through direct cell count and by following the estimation chlorophyll, carotenoid, protein and total carbohydrates contents using standard procedures(Lund *et al*., 1958) [32].

Direct cell count

The direct cell count determination was done through two main methods, Lackey drop transect and Petroff Hauser chamber.

Lackey drop-transect method

The direct cell count in Lackey drop-transect was done by the standard procedure (Sunita *et al*., 2007). In this method, a drop of algal culture was placed in the center of clean cover slip placed on the slide by inverted position and examined by parallel overlapping strips to count the total cells present in the drop. Depending on the concentration of algal cells in the sample one or more horizontal strips were counted. The number of algal units counted were then multiplied by a factor (Total area of cover slip/Area of strips were counted) and thus determine the number of algal units ml^{-1} of sample.

Haemocytometer method

The direct cell count was done using Haemocytometer (Hallegraeff *et al*, 2004) [10]. This method requires a haemocytometer, a cover slip and a medicine dropper or fin pipette. The haemocytometer used has a Neubauer ruling with two counting grids onto which the sample should be placed. Each counting grid consists of 25 large squares (each square have 16 cells). The haemocytometer chamber is 1mm in size and 0.1mm deep. The volume of sample in one square is therefore, $0.1mm^3$. The samples were thoroughly mixed and one drop, withdrawn from each sample, was applied to each haemocytometer grid at the position of application. On each counting grid a total number of 4 squares were counted resulting in a total volume count $0.4mm^3$ (0.0004 ml). The unit counts were then used to calculate the algal biomass in unit ml^{-1}.

Determination of total carbohydrate

The total carbohydrate of two metal tolerant microalgal cultures (*Lyngbya* sp. and *Chlorella* sp2) was estimated following the procedure of Hedge and Hofreiter (1962) [12]. 0.1gram of algal dry biomass was taken and hydrolyzed using dilute hydrochloric acid to release simple sugars. The hydrolyzed product is allowed to react with Anthrone reagent and subjected for spectrophotometric analysis at 630 nm using spectrophotometer.

Determination of protein

The total protein found in two selected metal tolerant microalgal cultures (*Lyngbya* sp. and *Chlorella* sp2) was determined through standard procedure as described by Lowry *et al* (1951) [21]. 0.5gram of algal dry biomass was taken react with reagent Folin-Ciocalteau and subjected for spectrophotometric analysis at (660) nm.

Estimation of chlorophyll

The total chlorophyll content of two potential metal tolerant microalgal cultures (*Lyngbya* sp. and *Chlorella* sp2) was determined by standard procedure (Arnon, 1949).The Microalgal pellet was taken and 5 ml of acetone was added in a Stoppard test tube and incubated for an hour. After incubation, it was centrifuged at 5000 rpm for 5 mins. The absorbency of the supernatant was measured at 663nm and 645nm using spectrophotometer. The chlorophyll content was calculated using following formula.

$$\text{Chlorophyll a } (\mu g/ml) = 12.7\,(A_{663}) - 2.69\,(A_{645})$$

$$\text{Chlorophyll b } (\mu g/ml) = 22.9\,(A_{645}) - 4.68\,(A_{663})$$

$$\text{Total Chlorophyll } (\mu g/ml) = \text{Chlorophyll a} + \text{Chlorophyllb}$$

$$\text{Total Chlorophyll } (\mu g/ml) = 20.2\,(A_{645}) + 8.02\,(A_{663})$$

Estimation of Carotenoid

A 0.5gram of algal wet biomass from two metal tolerant microalgal cultures (*Lyngbya* sp. and *Chlorella* sp2) was homogenized and extracted repeatedly with acetone. The pooled extracts absorbance was read at 470nm and total carotenoid contents were quantified using following formula, (Lichtenthaler, 1983) [20].

$$\text{Carotenoid } (\mu g/ml) = (1000\,A_{470} - 1.82C_a - 85.02\,C_b) / 198$$

SEM imaging studies of two potential metal tolerant algal strains.

In order to study the detailed morphological characteristics of algal cultures, SEM imaging was done for two selected algal strains using Scanning Electron Microscopy (Tescan-Vega 3) as per standard method (Baldi *et al.*, 1990) [28]. The selected two metal tolerant microalgae *viz. Lyngbya* sp (Blue Green Algae) and *Chlorella* sp2 (Green algae) were authenticated through nanoscale microscopic imaging using SEM.

Microalgal cell sediments collected and thus determined the morphology of two microalgal strains.

Molecular sequencing analysistwo potential metal tolerant algal strains.

In order to authenticate the microalgal strains, molecular sequencing of 16 S rRNA for *Lyngbya*sp and 18 S rRNA for *Chlorella* sp2 was done by standard protocols (Talavera and Castresana, 2007). The program Tree Dyn 1983 (Dereeper *et al.*, 2008) [6] was used for tree rendering of two potential metal tolerant microalgal strains.

Results

Isolation and identification of microalgae

Based on the colony morphology and microscopic images, the four microalgal isolates were identified as *Lyngbya hieronymusii*, *Oscillatoria* sp, *Chlorella* sp 1, *Chlorella* sp 2, (Plate 1).

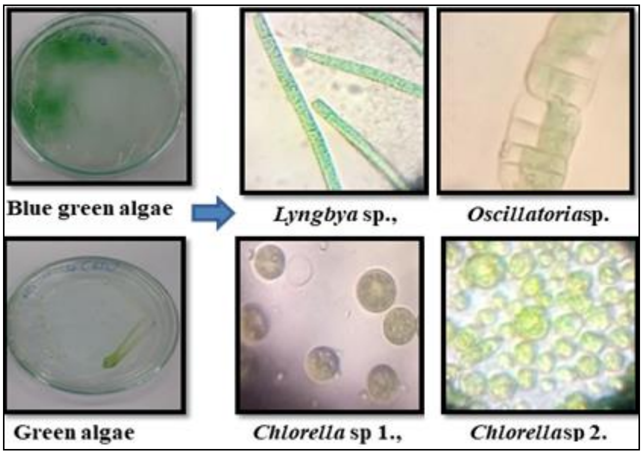

Plate 1: Colony morphology and microscopic views of four selected metal tolerant microalgal isolates

Screening of microalgal isolates for metal tolerance

The screening results of four selected metal tolerant microalgal isolates in various metal ions *viz*., Zinc, Nickel, and Chromium were presented in Tables 1 and 2.

Table 1: The growth performance of four microalgal isolates in three different metal ions (4ppm each) at 7 days (values are mean of three replicates ± standard error).

Microalgal species	Growth of microalgae (OD at 645 and 663 nm)					
	Zinc (4 ppm)		Nickel (4ppm)		Chromium (4 ppm)	
	645 nm	663 nm	645 nm	663 nm	645 nm	663 nm
Lyngbya sp	0.152±0.006	0.162±0.005	0.125±0.009	0.153±0.004	0.087±0.0004	0.081±0.002
Oscilatoria sp	0.147±0.073	0.28±0.003	0.139±0.0017	0.137±0.00	0.13±0.0041	0.126±0.003
*Chlorella sp*1	0.241±0.0841	0.229±0.006	0.067±0.004	0.064±0.001	0.106±0.001	0.099±0.002
*Chlorella sp*2	0.245±0.039	0.236±0.005	0.123±0.0017	0.126±0.001	0.106±0.0024	0.096±0.002

Table 2: The growth performance of four microalgal isolates in three different metal ions (4ppm each) at 14 days (values are mean of three replicates ± standard error)

Microalgal species	Growth of microalgae (OD at 645 and 663 nm)					
	Zinc (4 ppm)		Nickel (4 ppm)		Chromium (4 ppm)	
	645 nm	663 nm	645 nm	663 nm	645 nm	663 nm
Lyngbya sp	0.047±0.003	0.085±0.004	0.042±0.002	0.041±0.002	0.1±0.002	0.103±0.002
Oscilatoria sp	0.191±0.011	0.183±0.003	0.068±0.004	0.076±0.001	0.166±0.001	0.159±0.003

*Chlorella sp*1	0.149±0.007	0.158±0.006	0.089±0.002	0.091±0.003	0.127±0.001	0.128±0.00
*Chlorella sp*2	0.142±0.004	0.172±0.001	0.156±0.002	0.164±0.001	0.112±0.008	0.13±0.003

Characterization of two selected metal tolerant algal strains with different environmental conditions

Based on the metal tolerance screening study, selected two microalgal strains having better metal tolerant activity and were characterized with different growth conditions *viz*., different pH (5 and 9) and various concentrations of metal ions (6 ppm and 8 ppm) of Zn, Ni and Cr. The results of characterization study are presented in Tables 3 to 6. Both the strains were able to tolerate basic pH condition than acetic condition.

Table 3: Growth performance of two metal tolerant microalgal isolates grown in medium supplemented with different metal ions at (4ppm each) pH 5 and pH 9 on 7th day (values are mean of three replicates± standard error)

Microalgal species	Growth measured nm	Growth at different pH					
		Zinc (4ppm)		Nickel (4ppm)		Chromium (4ppm)	
		pH5	pH9	pH5	pH9	pH5	pH9
Lyngbya sp	645	0.104±0.002	0.072±0.002	0.055±0.00	0.045±0.002	0.034±0.003	0.055±0.002
	663	0.125±0.00	0.085±0.003	0.045±0.002	0.054±0.002	0.046±0.001	0.074±0.003
Chlorella sp2	645	0.036±0.001	0.054±0.001	0.035±0.002	0.055±0.002	0.034±0.003	0.061±00.003
	663	0.039±0.003	0.069±0.003	0.042±0.002	0.078±0.004	0.042±0.002	0.046±0.002

Table 4: Growth performance of two metal tolerant microalgal isolates grown in medium supplemented with different metal ions(4ppm each) at pH 5 and pH 9 on 14th day (values are mean of three replicates± standard error)

Microalgal species	Growth measured nm	Growth at different pH					
		Zinc (4ppm)		Nickel (4ppm)		Chromium (4ppm)	
		pH5	pH9	pH5	pH9	pH5	pH9
Lyngbya sp	645	0.0106±0.004	0.105±0.00	0.101±0.007	0.113±0.004	0.085±0.004	0.086±0
	663	0.098±0.002	0.112±0.003	0.1±0.003	0.108±0.003	0.089±0.002	0.097±0.005
Chlorella sp2	645	0.069±0.002	0.127±0.007	0.064±0.002	0.097±0.002	0.08±0.005	0.111±0.004
	663	0.066±0.005	0.0134±0.009	0.07±0.001	0.089±0.008	0.091±0.002	0.109±0.006

Table 5: Growth performance of two metal tolerant microalgal isolates in medium supplemented with differentmetal ions with 6ppm and 8ppm on 7th day (values are mean of three replicates± standard error)

Microalgal species	Growth measured nm	Growth at different metal concentrations					
		Zinc		Nickel		Chromium	
		6ppm	8ppm	6ppm	8ppm	6ppm	8ppm
Lyngbya sp	645	0.206±0.004	0.257±0.002	0.424±0.003	0.08±0.003	0.22±0.004	0.036±0.002
	663	0.227±0.002	0.266±0.003	0.457±0.01	0.082±0.002	0.214±0.001	0.036±0.002
Chlorella sp2	645	0.269±0.009	0.268±0.002	0.183±0.01	0.147±0.002	0.042±0.002	0.107±0.00
	663	0.319±0.002	0.264±0.003	0.217±0.007	0.158±0.002	0.4±0.002	0.126±0.002

Table 6: Growth performance of two metal tolerant microalgal isolates in medium supplemented with different metal ions with 6ppm and 8ppm on 14th day (values are mean of three replicates± standard error)

Microalgal species	Growth measured nm	Growth at different metal concentration					
		Zinc		Nickel		Chromium	
		6ppm	8ppm	6ppm	8ppm	6ppm	8ppm
Lyngbya sp	645	0.17±0.008	0.15±0.004	0.104±0.004	0.083±0.004	0.165±0.002	0.13±0.003
	663	0.174±0.007	0.141±0.002	0.123±0.006	0.15±0.002	0.212±0.004	0.151±0.00
Chlorella sp2	645	0.153±0.004	0.182±0.008	0.205±0.003	0.146±0.004	0.254±0.004	0.216±0.012
	663	0.171±0.012	0.152±0.004	0.141±0.005	0.209±0.005	0.245±0.008	0.252±0.007

Determination of cell count the growth rate of two metal tolerant microalgal strain

The direct cell count of two selected metal tolerant microalgal strains were determined and the results are present in (Table 7)

Table 7: Total cell count of two selected metal tolerant microalgal strains growth in BG11 medium (values are mean of three replicates ± standard error)

S. no	Cell count method	Total cell count×10^2	
		***Lyngbya* sp**	***Chlorella* sp2**
1	Lackey drop transect	17±0.8	57±0.8
2	Haemocytometer	3±0.6	404± 0.70

The growth rate of two selected metal tolerant microalgal strains was assessed based on direct cell count and biochemical constituents like total Chlorophyll & Carotenoid pigments, total Carbohydrates and protein contents and the results of biochemical contents of two selected microalgal isolates were presented in Table 8.

Table 8: Biochemical constituents of two potential metal tolerant microalgal strains grown on BG 11 medium (values are mean of three replicates ± standard error)

S. No	Biochemical parameters	*Lyngbya* sp	*Chlorella* sp2
1.	Carbohydrates (mg/g)	0.95±0.00	0.40±0.00
2.	Protein (mg/g)	17.73±0.02	19.0±0.00
3.	Chlorophyll (mg/g)	4.23±0.01	3.28±0.00
4.	Carotenoid (mg/g)	1.98±0.01	0.90±0.01

SEM imaging studies of two potential metal tolerant algal strains.

In order to study the detailed morphological characteristics of algal cultures, SEM imaging was done for two selected algal strains (*Lyngbya* sp and *Chlorella* sp2) using Scanning Electron Microscopy (Tescan – Vega 3) and results are shown in Plate 2.

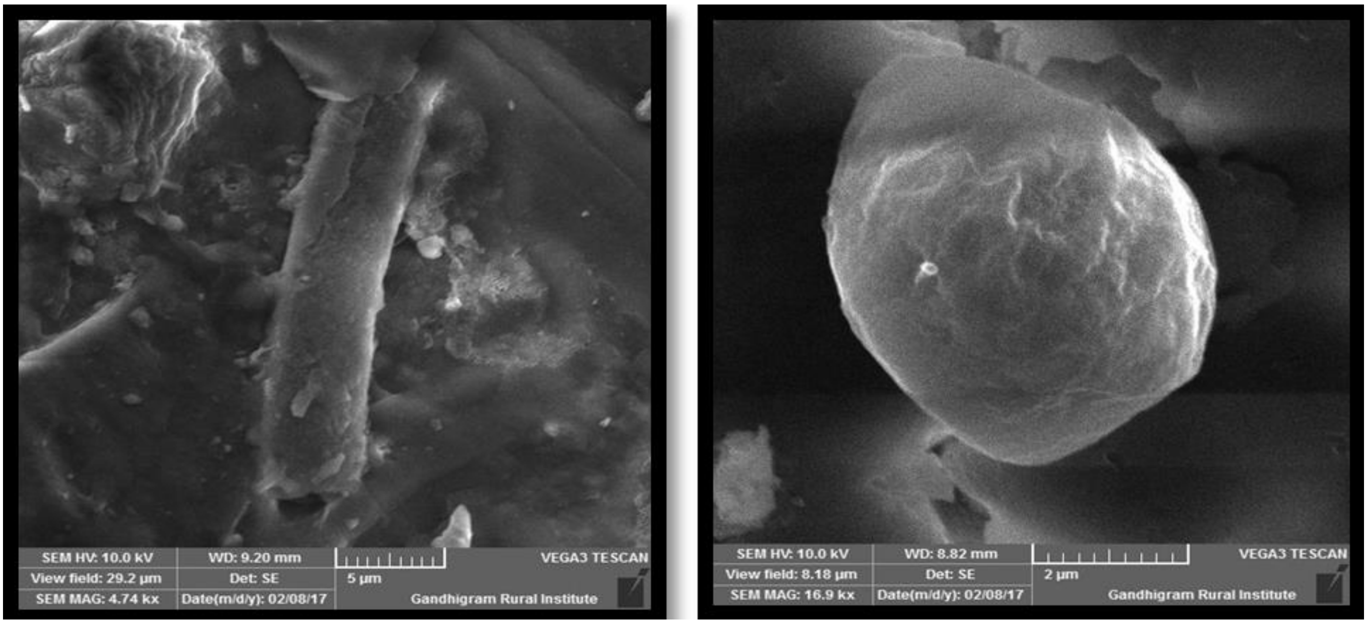

Plate 2: SEM Images of two metal tolerant microalgal isolates

Molecular sequencing analysis

In order to authenticate the two selected metal tolerant microalgal strains, molecular sequencing of 16 S rRNA for *Lyngbya* sp., and 18 S rRNA for *Chlorella* sp2 was done by standard procedure. The genomic DNA of the two selectedmicroalgal strains such as *Chlorella* sp., and *Lyngbya* sp was extracted by using INSTA GENE^{TM} MATRIX GENOMIC DNA ISOLATION KIT. 18S r RNA and 16S r RNA genes of respectable microalgal strains were amplified and the sequences was provide base pair product for Strain1 and strain 2 are 1352 base pair product. The sequence was processed for trimming both 3' and 5' ends. The software "Biosystems ABI 3730xl sequencer" was used to process sequencing data. The National Center for Bioinformatics (NCBI) USA, online tool Blast N were used to compared Strain sequence with archived NCBI nucleotide database. The Blast sequence with data base of *Chlorella* sp., strain-1 showed 97% query similarity with *Chlorella sorokiniana*, while *Lyngbya* sp. showed 95% query similarity with *Lyngbyahieronymusii.*

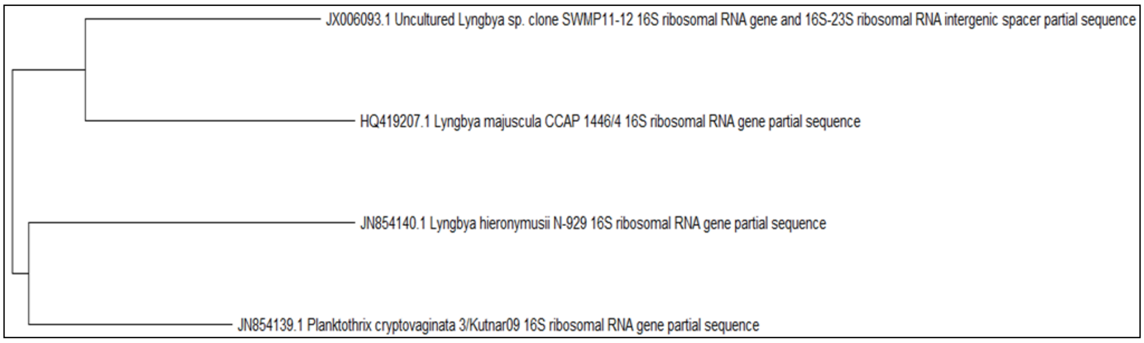

Fig 2: 16SrRNA gene sequence-based phylogenetic trees of *Lyngbya* sp isolated from natural habitat

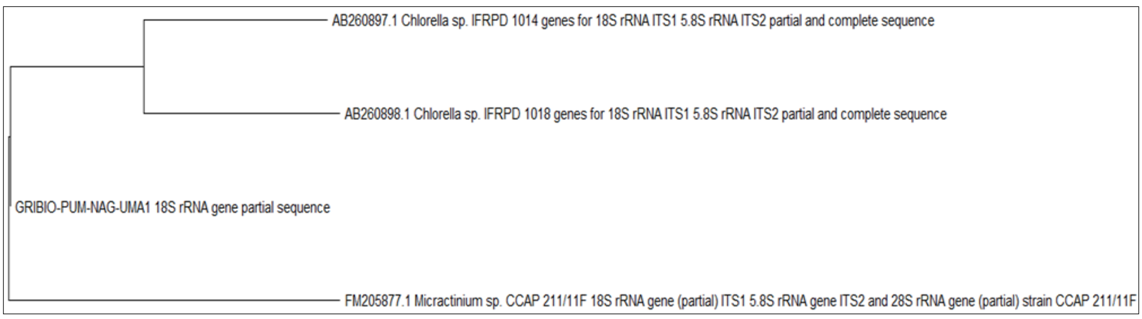

Fig 3: 18SrRNA gene sequence-based phylogenetic trees of *Chlorella* sp isolated from natural habitat

Phylogenetic analysis of microalgal isolates *Lyngbya hieronymusii* 16 S rRNA and *Chlorella sorokiniana* 18S r RNA were performed using Neighbor-Joining method. The optimal tree with the sum of branch length = 2.33023926 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. This analysis involved 8 nucleotide sequences. Evolutionary analyses were conducted in MEGA X.

Discussion

Microalgal strains were isolated from natural sources and cultured in BG 11 medium under continuous illumination [3000 *lux*] and with proper temperature (Plate 1). The individual cells of the colonies were appears green colour, unicellular and spherical in shape. Laura and Paolo (2006) demonstrated the BG 11 medium was used to isolate the microalgal cultures in very short period. Lots of microalgal isolates were grown by using this medium. Light intensity and culture medium are the main and important factors for algae culturing. A photo inhibition effect can be caused by too high light intensities, i.e. direct sunlight during cultivation.

Zinc is a trace element, important for the physiological functions of living tissues and regulates many biochemical processes (Oyaro *et al*., 2007) [26] as well as Nickel (Borba *et al*., 2006) [3] and Chromium (Khezami and Capart, 2005) [15]. Heavy metal concentration is the major constituent in the biosorption by microalgae. Initial concentration values are directly proportional to the biosorption values (Singh *et al*., 2010) [33].

In this study four microalgal isolates *viz*., *Lyngbya* sp. (Blue green algae), *Oscillatoria* sp (Blue Green algae), *Chlorella* sp1 (Green algae) and *Chlorella* sp2 (Green algae) were screened for their potential to remove heavy metals. I ml of exponential culture was inoculated in 50 ml of BG 11 broth with known quantity 4 ppm concentration at pH 7 (Tables 1 and 2). For further optimization studies carried out by varying pH and varying concentration of metals. The pH is one of the major growth promoting parameter when compared to other conditions. In case of varying pH but better growth observed in neutral pH followed by alkaline (pH9) and acidic condition (pH5). Acidic conditions of pH5 contribute no growth in both culture *C. sorokiniana* and *L. hieronymusii* with the presence of three metals Zinc, Nickel and Chromium (Tables 3 and 4). Alkaline pH (pH9) showed lower growth in both culture medium.

The growth properties of algae can be determined by measuring the cell number per unit volume of cell suspension. Lackey drop-transect method was used to enumerate the algal count in a short period of time. The main disadvantage of the Lackey drop- transect method is that a small sample volume is permitted (Sunita *et al*., 2007). In this study the total cell count counts were determined and the results are presented in (Table 7).

Chlorophyll is a green pigment found in several varieties in plants and algae referred by Amnot and Rey (2000). Chlorophyll a possess green - blue colour and chlorophyll b possess a green yellow colour (Biochemii, 1999et; Mlodzinska, 2009) and the bioremediation capacity is vary from strain to strain (Monteiro *et al*., 2010) [24]. In this study the growth characterization of two metal tolerant microalgal isolates in different environmental conditions were determined by estimating by biochemical constituents like total chlorophyll, carotenoid, total carbohydrate and protein contents. The Chlorophyll and carotenoid contents were higher in *Lyngbya hieronymusii* than the *Chlorella sorokiniana* (Table 8). Regarding protein determination, *C. sorokiniana* exclaimed rich protein content when compared to *L. hieronymusii*in while *L. hieronymusii* exhibited high carbohydrate content than *C. sorokiniana* (Table 8). SEM imaging studies of two potential metal tolerant microalgal strains *viz*., *L. hieronymusii* and *C. sorokiniana* clearly reveals the morphological features (Plate 2). Based on 18S rRNA sequence data *Chlorella* sp., showed 97% query similarity with *Chlorella sorokiniana* and 16S rRNA sequence data of *Lyngbya* sp., showed 95% query similarity with *Lyngbya hieronymusii* (Figures 1 and 2).

Conclusion

Microalgal based biosorption of heavy metals has become a sustainable alternative for wastewater treatment. The findings of the present study stated that *Lyngbya hieronymusii* is the efficient metal tolerant microalgae when compared to *Chlorella sorokiniana*. With the fast and robust growth in varying industrial waste and resistance to contaminants, microalgae serve a promising feedstock for heavy metal biosorption.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgment

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Reference

1. Arnon DI. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. Plant physiology,1949:24(1), 1. (DOI: 10.1104/pp.24.1.1)
2. Ballaban-Gil K, Rapin I, Tuchman R, Shinnar S. Longitudinal examination of the behavioral, language, and social changes in a population of adolescents and young adults with autistic disorder. Pediatric neurology,1996:15(3):217-223. (DOI: https://doi.org/10.1016/S0887-8994 (96) 00219-6)
3. Borba CE, Guirardello R, Silva EA, Veit MT, Tavares CRG. Removal of nickel (II) ions from aqueous solution by biosorption in a fixed bed column: experimental and theoretical breakthrough curves. Biochemical Engineering Journal,2006:30(2):184-191. (DOI: https://doi.org/10.1016/j.bej.2006.04.001)
4. Dach R, Lutz S, Walser P, Fridez P. Bernese GNSS software version 5.2, 2015. (DOI: 10.7892/boris.72297)
5. De Philippis R, Paperi R, Sili C. Heavy metal sorption by released polysaccharides and whole cultures of two exopolysaccharide-producing yano bacteria. Bio degradation,2007:18(2):181-187. (DOI: https://doi.org/10.1007/s10532-006-99053-y)
6. Dereeper A, Guignon V, Blanc G, Audic S, Buffet S, Chevenet F *et al*. Phylogeny. fr: robust phylogenetic analysis for the non-specialist. Nucleic acids research,2008:36(2):W465-W469. (DOI: https://doi.org/10.1093/nar/gkn180)

7. Desikachary TV. Cyanophyta. New Delhi: Indian Council of Agricultural Research, 1959, 2.
8. Falini B, Bolli N, Shan J, Martelli MP, Liso A, Pucciarini A *et al*. Both carboxy-terminus NES motif and mutated tryptophan (s) are crucial for aberrant nuclear export of nucleophosmin leukemic mutants in NPMc+ AML. Blood,2006:107(11):4514-4523. (DOI: https://doi.org/10.1182/blood-2005-11-4745)
9. Haferburg G, Kothe E. Microbes and metals: interactions in the environment. Journal of basic microbiology,2007:47(6):453-467. (DOI: https://doi.org/10.1002/jobm.200700275)
10. Hallegraeff GM, Anderson DM, Cembella AD, Enevoldsen HO. Manual on harmful marine microalgae. UNESCO, 2004.
11. Hecky RE, Kilham P. Nutrient limitation of phytoplankton in freshwater and marine environments: a review of recent evidence on the effects of enrichment 1. Limnology and oceanography,1988:33(4, 2):796-822. (DOI: https://doi.org/10.4319/lo.1988.33.4part2.0796)
12. Hedge JE, Hofreiter BT, Whistler RL. Carbohydrate chemistry. Academic Press, New York, 1962, 17.
13. Hefeeda M, Habib A, Botev B, Xu D, Bhargava B. PROMISE: peer-to-peer media streaming using CollectCast. In Proceedings of the eleventh ACM international conference on Multimedia, 2003, 45-54. (DOI: https://doi.org/10.1145/957013.957022)
14. Hellio Le, Graverand MP, Ou Y, Schield-Yee TERESA, Barclay L, Hart D *et al*. The cells of the rabbit meniscus: their arrangement, interrelationship, morphological variations and cytoarchitecture. Journal of Anatomy,2000:198(5):525-535. (DOI: https://doi.org/10.1046/j.1469-7580.200019850525.x)
15. Khezami L, Capart R. Removal of chromium (VI) from aqueous solution by activated carbons: kinetic and equilibrium studies. Journal of hazardous materials,2005:123(1-3):223-231. (DOI: https://doi.org/10.1016/j.jhazmat.2005.04.012)
16. Kiran B, Pathak K, Kumar R, Deshmukh D. Statistical optimization using central composite design for biomass and lipid productivity of microalga: A step towards enhanced biodiesel production. Ecological Engineering,2016:92:73-81. (DOI: https://doi.org/10.1016/j.ecoleng.2016.03.026)
17. Kumari S, Ghosh C, Jayaraman SG. Phytoplankton composition, community structure and regional climatic variations in two tropical model ponds in India. Asian Journal of water, environment and pollution,2007:4(2):123-128.
18. Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: an overview. The scientific world journal, 2013. (DOI: https://doi.org/10.1155/2013/162750)
19. Kumaraswamy P, Lakshmanan R, Sethuraman S, Krishnan UM. Self-assembly of peptides: influence of substrate, pH and medium on the formation of supra molecular assemblies. Soft Matter,2011:7(6):2744-2754. (DOI: https://doi.org/10.1039/COSM00897D)
20. Lichtenthaler HK, Wellburn AR. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents, 1983. (DOI: https://doi.org/10.1042/bst0110591)
21. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. Journal of biological chemistry,1951:193:265-275.
22. Lund JWG, Kipling C, Le Cren ED. The inverted microscope method of estimating algal numbers and the statistical basis of estimations by counting. *Hydrobiologia*,1958:11(2):143-170. (DOI: https://doi.org/10.1007/BF00007865)
23. Młodzińska E. Survey of plant pigments: molecular and environmental determinants of plant colors. Acta Biologica Cracovienca Series Botanica,2009:51(1):7-16.
24. Monteiro CM, Castro PM, Malcata FX. Cadmium removal by two strains of Desmodesmus pleiomorphus cells. Water, Air, and Soil Pollution,2010:208(1):17-27. (DOI: https://doi.org/10.1007/s11270-009-0146-1)
25. Odaka M, Fujii K, Hoshino M, Noguchi T, Tsujimura M, Nagashima S *et al*. Activity regulation of photoreactive nitrile hydratase by nitric oxide. Journal of the American Chemical Society,1997:119(16):3785-3791. (DOI: https://doi.org/10.1021/ja962179c)
26. Oyaro N, Ogendi J, Murago EN, Gitonga E. The contents of Pb, Cu, Zn and Cd in meat in nairobi, Kenya, 2007.
27. Paperi R, Micheletti E, De Philippis R. Optimization of copper sorbing-desorbing cycles with confined cultures of the exopolysaccharide-producing cyanobacterium Cyanospiracapsulata. Journal of applied microbiology,2006:101(6):1351-1356. (DOI: https://doi.org/10.1111/j.1365-2672.2006.03021.x)
28. Parks EJ, Olson GJ, Brinckman FE, Baldi F. Characterization by high performance liquid chromatography (HPLC) of the solubilization of phosphorus in iron ore by a fungus. Journal of industrial microbiology,1990:5(2-3):183-189. (DOI: https://doi.org/10.1007/BF01573868)
29. Pfandl K, Chatzinotas A, Dyal P, Boenigk J. SSU rRNA gene variation resolves population heterogeneity and eco physiological differentiation within a morphospecies (Stramenopiles, Chrysophyceae). Limnology and Oceanography,2009:54(1):171-181. (DOI:https://doi.org/10.4319/l0.2009.54.1.0171)
30. Satyapal S, Petrovic J, Read C, Thomas G, Ordaz G. The US Department of Energy's National Hydrogen Storage Project: Progress towards meeting hydrogen-powered vehicle requirements. Catalysis today,2007:120(3-4):246-256. (DOI:https://doi.org/10.1016/j.caltod.2006.09.022)
31. Schindler DW. Factors regulating phytoplankton production and standing crop in the world's freshwaters. Limnology and oceanography,1978:23(3):478-486. (DOI: https://doi.org/10.4319/l0.1978.23.3.0478)
32. Shang Y, Yu X, Romero-González ME. Screening of algae material as a filter for heavy metals in drinking water. Algal research,2015:12:258-261. (DOI:https://doi.org/10.1016/j.algal.2015.09.003)
33. Singh R, Chadetrik R, Kumar R, Bishnoi K, Bhatia D, Kumar A *et al*. Biosorption optimization of lead (II), cadmium (II) and copper (II) using response surface methodology and applicability in isotherms and thermodynamics modeling. Journal of hazardous materials,2010:174(1-3):623-634. (DOI: https://doi.org/10.1016/j.jhazmat.2009.09.097)

34. Southall R, Daniel J, Habib A. State of the Nation: South Africa 2003-2004, 2003
35. Talavera G, Castresana J. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Systematic biology,2007:56(4):564-577. (DOI:https://doi.org/10.1080/10635150701472164)
36. Thajuddin N, Subramanian G. Cyanobacterial biodiversity and potential applications in biotechnology. Current science, 2005, 47-57.
37. Velasco R, Zharkikh A, Troggio M, Cartwright DA, Cestaro A, Pruss D *et al*. A high quality draft consensus sequence of the genome of a heterozygous grapevine variety. PloS one,2007:2(12):e1326. (DOI: https://doi.org/10.1371/journal.pone.0001326)
38. Wetzel DL. Near-infrared reflectance analysis. Analytical chemistry,1983:55(12):1165A-1176A. (DOI: https://doi.org/10.1021/ac00262a001)