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Isolation, screening and identification of cyanobacteria and its uses in bioremediation of industrial effluents and chromium sorption

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Abstract

Blue Green Algae are Prokaryotic, Oxygenic photosynthetic, Autotrophic and some of them are able to fix atmospheric nitrogen. Cyanobacteria used in aquaculture, wastewater treatment, food, fertilizers. Its play an important role in maintainance and build-up of soil fertility, consequently increasing cereals growth and yield as a natural biofertilizer. In the present study, total 25 water sample were taken from of various ponds, lakes and rivers in valsad, vapi and surat region. A total of 8 cyanobacteria among 25 sample were isolated. Cyanobacteria grows well on algal culture broth and urea broth. The incubation period taken was 15-20 days for the growth of the cyanobacteria by using light energy. In microscopic examination cyanobacterial strain like *oscillatoriasps*, *Nostocsps*, *Anabaena sps*, ,*Gloeocapsasps*, *Plectonemasps*and*Gloeothecesps*etc were identified. The isolated cyanobacteria were used for further study of bioremediation of waste water and chromium sorption.

Keywords: Cyanobacteria, Photosynthetic, microscopic, Prokaryotic.

Introduction

The name "Cyanobacteria" comes from the color of the bacteria. In 1955- Edondson discovered Cyanobacteria in the lake and Stanier first classified Cyanobacteria in 1977, Oldest organisms on the earth (Fossil record-3.5 billons years). Cyanobacteria is the only bacteria that can produce photosynthesis using light energy. They are oxygenic photosynthetic prokaryotes found in soil and some of them are able to fix in atmospheric nitrogen (Malik et al. 2001).They grow at any place and in any environment where moisture and sunlight are available. However, specifc algae grow in specific environment. It is found in marine sediments freshwater, lakes, ponds, terrestrial, soil etc (Ganpati,1940; Philipose, 1960; Vijayakumar et al., 2005; Muthukumar et al., 2007).Cyanobacteria are larger than bacteria, and they contain chlorophyll-A. Some of the forms have terminally differentiated specialized structure called heterocyst. All heterocyst bearing cyanobacteria are aerobic photodiazotrophs Cohen et al. 1986). Blue green algae are very susceptible to sudden physical and chemical alterations of light, salinity, temperature and nutrient composition. Also have many more biotechological applications that await possible uses in mariculture, food, fuel, fertilizers and production of various secondary metabolities including toxins, vitamins, enzymes, bioremediation and pharmaceuticals. Cyanobacteria play an important role in maintenance and build-up of soil fertility, consequently increasing cereal growth and yield as a natural biofertilizer. (Song et al. 2005).

Heavy metal contamination in industrial effluents is one of the serious environmental issues. The presence of toxic heavy metals in the environment cause human health risks. Chromium is one of the toxic heavy metals which are discharged into the environment from various industries such as leather tanning, electroplating, metal finishing, chromate preparation, wood preservation and manufacture of dyes and pigments (Krishna *et al.,* 2004; Shali and Indu 2005).The maximum allowable limit for total chromium in drinking water is 0.05 mg/l (World Health Organization (WHO), 2004). Chromium occurs in aqueous systems in trivalent and hexavalent forms. Chromium (III) is used in tanneries as chromium sulphate which may be converted to Chromium (VI) in the effluent. Cr (VI) may be converted to Cr (III) under reduced environment, which is much less toxic and less soluble by several microorganisms which possess chromate reductase and thus reduction by these enzymes affords a means of chromate bioremediation (Shaili and Indu, 2005).

In huge quantities chromium can be toxic and carcinogenic. The hexavalent form of chromium is considered to be a group "A" human carcinogen because of its mutagenic and carcinogenic properties (Cieslak, 1996). Conventional methods used for the treatment of heavy metal contaminated effluents include precipitation, adsorption, ion exchange oxidation and reduction. This leads to some difficulties such as large quantities of sludge generation and operational cost. Also they are less specific to the heavy metals. So the attention has been changed to biological methods of remediation. Biosorption is one of the efficient methods used for the removal of chromium VI from industrial effluents. Biosorption can be defined as the passive uptake of toxicants by dead/inactive biological materials (Gadd and White, 1993). Bacteria, fungi, algae, industrial wastes, etc. are most commonly used biosorbents (Dongee*et al*., 2010) which decrease the concentration of heavy metal ions in solution from ppm to ppb level (Wang and Chen, 2006).

The functional groups such as acetamido, amines, sulphydral, carboxylates, imidazoles and hydroxyls present in the biosorbent takes up the metals by biosorption. The pretreatment of the biosorbent enhances the surface groups for metal adsorption (Vieira and Volesky, 2000). There are various factors which influence the biosorption such as pH, temperature, biosorbent dosage and size, initial solute concentration etc. (Vijayaragavan, 2008). Bioremediation is a pollution control technology that uses biological systems to catalyze the degradation or transformation of various toxic chemicals to less harmful forms. Bioremediation is a cost effective and efficient method of decontamination that has become increasingly popular now-a- days to reduce environmental pollution. In urban and semi-urban colonies, sewage disposal has become an ecological problem (Moore, 1998). The effluents from residential and industrial discharge constitute a major source of water pollution. The industrial effluents were discharged into open drains which finally joins the rivers (Kumari *et al*, 2006).

Wastewater discharge of industries are major issues of water pollution, contributing to oxygen demand and nutrient loading of the water bodies promoting toxic destabilized aquatic ecosystem (Morrison *et al,* 2001; DWAF and WRC, 1995). High or low pH values in a river have been reported to affect aquatic life which alters the toxicity of other pollutant in one form or the other (DWAF, 1996c). A low pH value in a river impairs recreational uses of water and affects aquatic life. A decrease in pH values reduces the solubility of certain essential element such as selenium and increases the solubility of many other elements such as Al, B, Cu, Cd, Hg, Mn and Fe (DWAF, 1996c). High level of pollutants in river water causes an increase in Biological oxygen demand (BOD), Chemical oxygen demand (COD), Total suspended solids (TSS), Total dissolved solids (TDS).

Materials and Methods

Media and reagents

Sodium nitrate, dipotassium phosphate, magnesium sulphate, ammonium chloride, calcium chloride, ferric chloride, potassium dichromate, 1, 5 diphenyl carbazide, methanol, hydrogen sulphate, glucose, ferrous ammonium sulphate and all other chemical used were of analytical grade. All this reagents and media were prepared in distilled water.

Collection of Samples

For the present study, water samples were collected from different site i.e. various ponds, lakes and rivers in different localities from Vapi ,Valsad and Surat , Gujarat, India.

Isolation and Screening of Cyanobacteria

Water sample was inoculated in Erlenmeyer flask having algal culture medium (Composition in gm/lit; sodium nitrate 0.1gm, dipotassium phosphate 0.25 gm, magnesium sulphate 0.051 gm, ammonium chloride 0.0051gm, calcium chloride 0.005gm and ferric chloride 0.0005 gm) and incubated at room temperature under continuous dark and sunlight period for 15-20 days, growth from the incubated flask were spreaded on algal culture plate and incubated at room temperature under continuous dark and sunlight period for 15-20 days as described by patil.et.al.(2015). Isolated colonies were observed in microscope for morphological characterization.

Identification of Blue green algae

Microscopic observation was done by spreading isolated culture on glass slide using forceps. culture were covered with glass cover slips and observed under low (10X) and high power (45X) objective lens of compound light microscope.

Study on biosorption of chromium by algal species

Batch studies on biosorption of chromium

Batch experiments were carried out in 100 ml Erlenmeyer flasks containing 40 ml algal culture medium (Composition in gm/lit; sodium nitrate 0.1gm, dipotassium phosphate 0.25 gm, magnesium sulphate 0.051 gm, ammonium chloride 0.0051 gm, calcium chloride 0.005gm and ferric chloride 0.0005 gm). The flask was sterilized at 121^0C for 15 min at 15 lbs. The sterilized flasks were cooled and inoculated with 10% v/v algal culture (7.352 mg algal biomass) and 0.4 ml of stock chromium solution (3000 ppm) to make final concentration of 30 ppm chromium in experimental flask. The inoculated flasks were gently agitated on a shaker with a constant shaking rate at 120 rpm for 4 days until sorption equilibrium was obtained. Samples were taken from the solution at regular time intervals for the determination of residual metal ion concentration in the solution. The harvested sample were centrifuged at 5000 rpm for 20 minutes and the residual concentration of chromium ions in the supernatant was determined spectrophotometrically at 540 nm using diphenylcarbazide as the complexing agent (Gilcreas *et al.*,1995).

The biosorption capacities (q_{eq}) at equilibrium were calculated as follows:

$$q_{eq} = \frac{(C_0 - C_{eq})V}{X}$$

Where,

C_0 was the initial Cr (VI) concentration ($mg1-^1$)
C_{eq} the Cr (VI) concentration at equilibrium($mg\ 1-^1$)
V the volume of solution used
X was biosorbent mass ($g1-^1$).

The adsorption yield or efficiency (*Ad*%) was defined as ratio of metal ion concentration at equilibrium to the initial metal ion concentration and was calculated as follows:

$$Ad\% = \frac{C_0 - Ce_q}{C^0} \times 100$$

Where,

C_0 was the initial metal ion concentration ($mg\ l^{-1}$)
Ceq is the residual metal ion concentration in solution at equilibrium ($mg\ l-^1$).

Study on industrial waste water treatment by isolated algal sp.

Batch studies on COD reduction

Batch experiments were carried out in 100 ml erlenmayer flasks containing 40 ml algal culture medium (Composition of medium in gm/lit; Sodium nitrate 0.1gm, dipotassium phosphate 0.25 gm, magnesium sulphate 0.051 gm, ammonium chloride 0.0051 gm calcium chloride 0.005 gm and ferric chloride 0.0005 gm).The flask were sterilized at 121^0C for 15 min at 15 lbs. The sterilized flasks were cooled and inoculate with 10% v/v algal culture and 10% v/v waste water in experimental flask. The inoculated flasks were gently agitated on a shaker with a constant shaking rate at 120 rpm for 21 days. Samples were taken from the flask at regular time intervals for the determination of residual COD in the experimental flask.

Calculation:

$$COD\ (mg/l) = \frac{(A-B)*N*1000*8}{\text{Volume of sample (ml)}}$$

Where,
A = Volume of ferrous ammonium sulphate used in "Blank"
B = Volume of ferrous ammonium sulphate used in "Test"
N = Normality of ferrous ammonium sulphate 0.1 N
8 = Gram equivalent weight of O_2

Results and Discussion

Isolation and screening of cyanobacteria

In present study various water sample from different regions of Vapi, Valsad, Surat, Gujarat, India were screened on algal culture plate and a total of 8 algal isolate were isolated in pure form. The pure form of the isolate were maintained in 250 ml Erlenmeyer flask containing 100 ml algal culture medium at room temperature under continuous dark and sunlight period as described by Patiel et al., (2015). Fig1 shows growth of all cyanobacterial isolates in algal culture medium. All the isolates were subjected for microscopic observation (Fig 2) and identified on the basis of their cellular morphology as described by Khare et al. (2014). The morphological characteristics and its identification is shown in Table 1.

Table 1. Morphological and cultural characteristics of isolates

Sr. no.	Cyanobacterial Isolate	Microscopic and Macroscopic observation	Morphotype
1	PL-1	Soft, green gelatinous, membranous, Short, straight, parallel, filamentous and heterocystous	*Anabaena sps*
2	PL-2	Bluish green, gelatinous,tightly packed, cells oblong, irregular, filamentous and heterocystous	*Nostoc sps*
3	PL-3	Single, yellowish brown, filamentous, irregularly curved, bulbous at the base and heterocystous	*Calothrix sps*
4	PL-4	Expanded, filamentous, entanlaged and non heterocystous	*Plectonema sps*
5	PL-5	Cells spherical, unicellular, non-filamentous & non-heterocystous	*Gloeothece sps*
6	PL-6	Unicellular cells, mucilaginous, loosely arranged in a group, blue green, nanocytes present and non heterocystous	*Aphanocapsa sps*
7	PL-7	Cushion like, filamentous, repeatedly branched, rounded cylindrical, solitary or in a row and heterocystous	*Tolypothrix tenuis*
8	PL-8	Vescicular, lobed, gelatinous, long, basal cells broad, filamentous and heterocystous	*Rivularia sps*

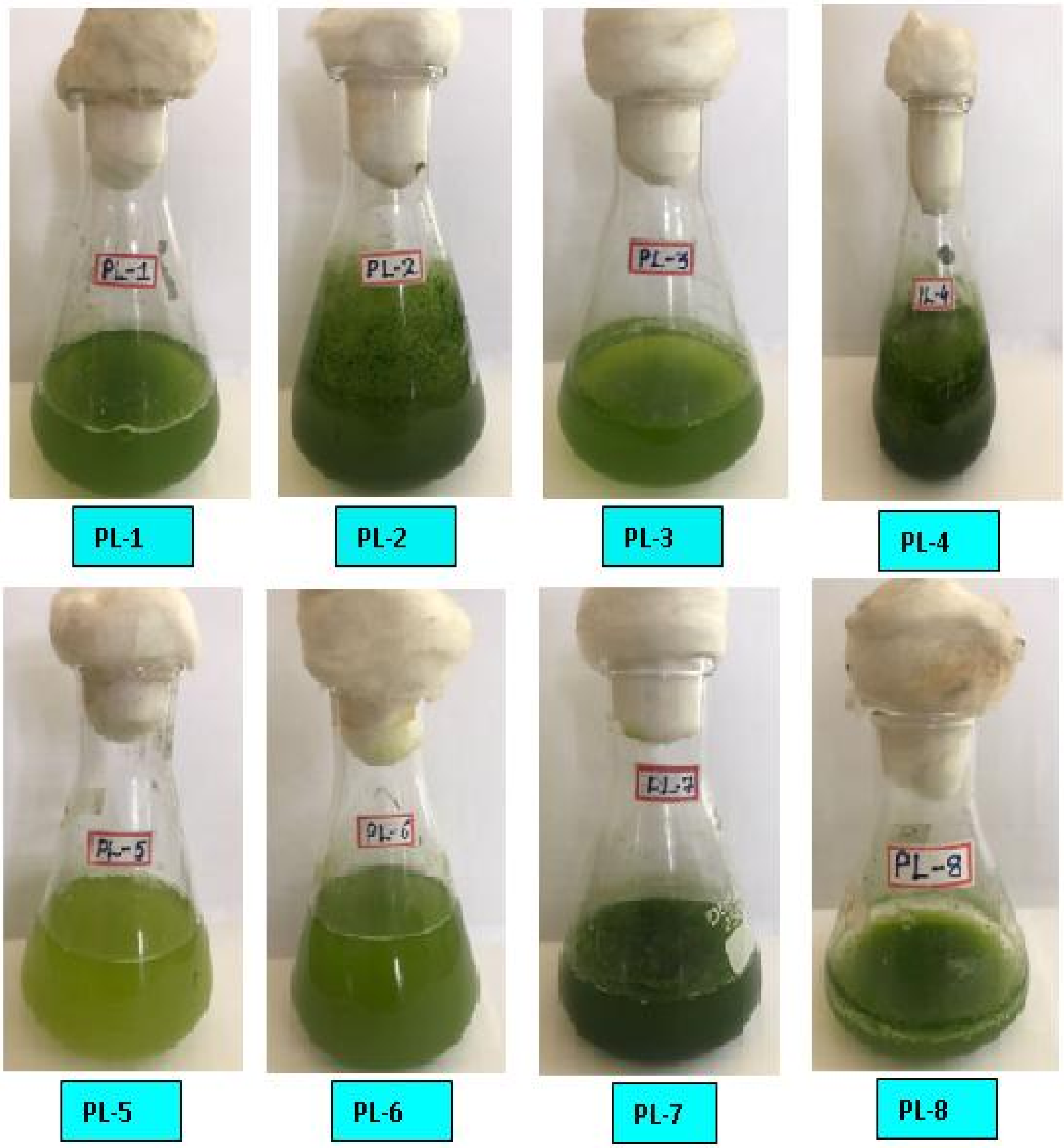

Figure-1. Cyanobacteria growth in algal culture medium

The morphological characteristics of the eight cyanobacterial species applied in this study. The isolate cyanobacteria was recorded from water sample of Vapi. Eight morphotypes were observed with heterocystous and non heterocystous cyanobacterial morphology.

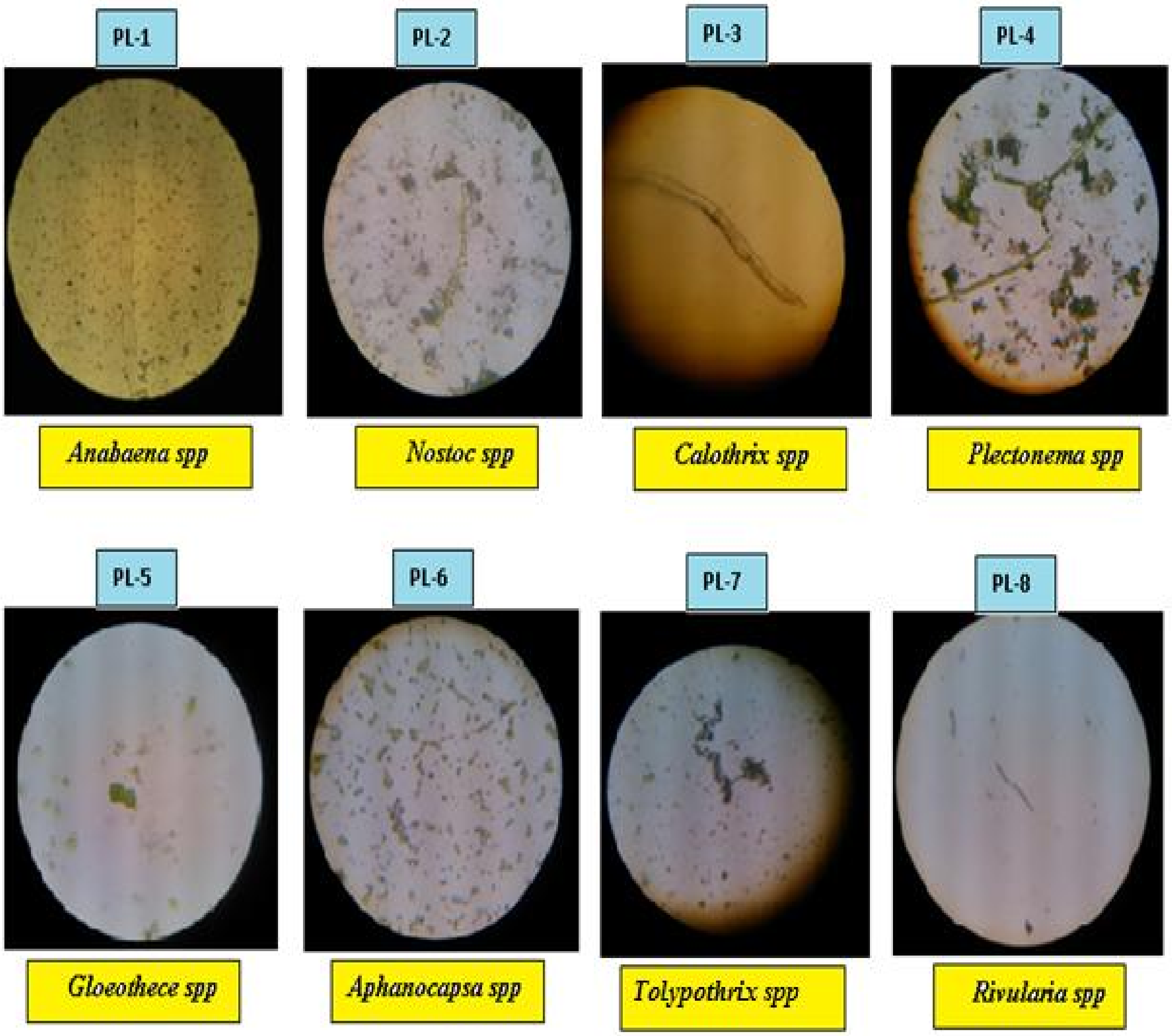

Figure-2. Microscopic observation of cyanobacterial isolate

Study on chromium biosorption

In present study the live algal biomass of isolated cultures were used for chromium biosorption study.

Screening of cyanobacterial isolate for Cr (VI) biosorption

A total of 8 algal culture were selected for the biosorption of chromium expirement using algal species. The results observed shows that all the algal isolate shows a good amount of chromium biosorption efficiency. All the algal isolate shows the biosorption efficiency in the range of 50 – 80%. Amongst various isolate algal strain PL-5 shows maximum biosorption efficiency of chromium i.e. 81.7 % at 30°C after 72 hrs of incubation under shaking condition. Thus isolate PL-5 was selected for further study.

Table 2. Screening of cyanobacterial isolate for Cr (VI) biosorption

Serial no	Culture no	Chromium adsorption efficiency (%)			
		“24” hrs	“48” hrs	“72” hrs	“96” hrs
1	PL-1	57.3	70.8	73.2	74.1
2	PL-2	61.0	73.2	74.4	74.8
3	PL-3	53.7	76.9	80.7	81.1
4	PL-4	74.4	78.1	79.3	80.2
5	PL-5	54.9	76.9	81.7	82.1
6	PL-6	64.7	76.9	79.3	79.9
7	PL-7	62.2	75.6	78.1	79.2
8	PL-8	62.2	74.4	79.3	80.1

Screening of cyanobacterial isolate for COD reduction

A total of 8 algal culture were selected for the COD reduction of industrial waste water. The results observed that, all the tested algal isolate showed COD reduction in the range of 15-35%. Isolate PL 5 shows maximum COD reduction of 34.7% at 30°C after 9 days of incubation under shaking condition. Thus isolate PL-5 was selected for further study. Valderramna et al., (2002) obtained 61% removal efficiencies for COD by *C. vulgaris* in the treatment of ethanol & citric acid production industry wastewater.

Table 3. Screening of Cyanobacterial isolate for Bioremediation of industrial effluents

		% COD reduction				
Serial no	**Culture**	**3 days**	**6 days**	**9 days**	**12 days**	**15 days**
1	PL-1	20.2	25.3	30.0	31.0	31.0
2	PL-2	25.1	26.6	24.2	26.2	25.3
3	PL-3	25.6	28.4	30.8	30.4	30.1
4	PL-4	26.0	27.1	32.4	32.9	33.2
5	PL-5	24.4	28.7	34.7	35.7	36.0
6	PL-6	15.1	18.3	22.5	25.1	26.7
7	PL-7	24.6	26.0	31.8	32.5	33.0
8	PL-8	13.4	15.2	18.2	19.1	21.1

Conclusion

In present study 8 different caynobacterial culture were isolated in pure form and were tested for biosorption of heavy metals and waste water treatment. Amongst eight tested isolate PL-5 isolate show maximum removal of chromium and COD.

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