CyanoBacteria: A Potential Natural Source for Drug Discovery and Bioremediation

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INTRODUCTION

Cyanophyta or cyanobacteria are a phylum of bacteria which obtain their energy by performing photosynthesis. Cyanobacteria are the oldest photosynthetic organisms that evolved on Earth approximately 2.6 to 3.5 billion years ago (Hedges, et al., 2001). They are generally called as blue-green algae, which is a misnomer, as cyanobacteria are prokaryotic and algae are eukaryotic (Allaby, 1992). Cyanobacteria are morphologically diverse, existing as free-living or colonial or forms symbiotic relationships with plants or with lichen-forming fungi or with other organisms (Al-Thani, 2015).

They are diverse (Klymiuk, et al., 2014) and Omni present on earth and their habitats range from geothermal hot springs, volcanic vents etc., to frozen ponds of Antarctica and from marine to terrestrial ecosystems (Panosyan, 2015). Cyanobacteria have evolved many adaptations to survive, compete and achieve dominance in different environments (Rastogi and Madamwar, 2015; Menamo and Wolde, 2015). It is hypothesized that photosynthetic organelle evolution in eukaryotes is by the process of endosymbiosis between a phagotrophic host and a cyanobacterium (Cohen, et al., 1986). Each individual cell of a cyanobacterium has a thick gelatinous cell wall but lacks flagella, but hormogonia of some species helps in gliding movement along surfaces.

ABSTRACT

Cyanobacteria are one of the earliest inhabitants of Earth and they contribute towards biogeochemical cycles of carbon and nitrogen. Cyanobacteria have the capability of producing wide variety of bioactive compounds and these compounds have wide applications in various fields of agriculture, biotechnology, pharmacology, etc. They also release exopolysaccharides and other compounds which possess antimicrobial and anticancerous properties. Cyanobacteria needs minimal and unsophisticated growth conditions and the manipulations in their genome can be done easily, thus making it a best choice for biotechnological applications. Many strains of cyanobacteria produce commercially high-valued compounds like pigments, ‘vitamins, and enzymes.

Cyanobacteria are potential bioremediation agents due to their capability of thriving in extreme conditions and metabolizing wide variety of compounds. Cyanobacteria are promising in degrading some peculiar pollutants and also removing heavy metals from the environment. Use of cyanobacteria for the treatment of wastewater or sludge has been increased recently. Moreover, the developments in biophysical and genetic engineering techniques have made it possible to amplify the production of desired molecules in cyanobacteria which can be used for industrial scale production. In this review, the applications of cyanobacteria as sources of bioactive compounds for drug development and bioremediation applications were discussed.

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Though cyanobacteria have no internal membrane bound organelles, they perform photosynthesis in distinctive folds unlike green plants which use specialized organelles for photosynthesis called chloroplasts. Cyanobacteria are considered as the causative agents for the "rusting of the earth" leading to 'Great Oxygenation Event', that changed the composition of life forms and increased biodiversity on earth. Their metabolism alters in the absence of light and they show different properties in different conditions (Sekhar, et al., 2012). Cyanobacteria fix atmospheric nitrogen into ammonia (NH$_3$), nitrates (NO$_3^-$) or nitrates (NO$_2^-$) in anaerobic conditions through specialized cells called heterocysts with the help of nitrogenase enzyme. Cyanobacteria accumulate metal ions such as Cr, Co, Cu and Zn very effectively and can utilize ammonia, nitrogenous compounds, and phosphates for their metabolism. They play a major role in biogeochemical cycles by making necessary transformations to the compounds. Cyanobacteria can perform different modes of metabolism and can rapidly switch from one mode to another (Stal, 1985). Cyanobacteria are the only prokaryotes that synthesize vitamin C (Ascorbic acid) (Mazid, et al., 2011).

Many bioprocesses in industries rely on heterotrophic bacteria to produce various desired compounds. The carbon source and complicated culture media of normally used bacteria cost high when compared with the culture media of cyanobacteria. For sustainable production of bio-chemicals and biofuels cyanobacteria require sunlight, carbon dioxide, water as major nutrient sources which are inexpensive and minimal cost nutrients. Moreover cyanobacteria are equipped with superior photosynthesis capabilities and biomass production rates compared to plants (Dismukes, et al., 2008). Due to simple genetic makeup, ease in culture and genetic manipulation even they are capable of replication viroid RNA genomes in their cell (Latifi, et al., 2016).

Their easiness in handling for bio manipulations had gained attention in research community and currently more than 300 cyanobacterial genomes are sequenced. So, cyanobacteria are most preferred organisms in effluent treatment, industrial production of enzymes, toxins, bioactive compounds etc. Among the commercially available marine origin biomedical research compounds, almost 24% are of cyanobacterial origin (Tan, 2013).

**Cyanobacteria in drug discovery**

Cyanobacteria are a rich source of primary and secondary metabolites like nonribosomal proteins, polyketides, terpenes and alkaloids, which have anticancer, antiviral, UV protective activities but some compounds are known to have hepatotoxicity and neurotoxicity (Herrero and Flores, 2008). Secondary metabolites are natural organic compounds which are diverse, having low molecular weight. These are not essential for normal growth and development of an organism but, they perform very potent biological activities like helping in defensive mechanisms against stress, and facilitate effective reproductive processes. Many secondary metabolites act as antibacterial or antifungal or anticancer, cholesterol-lowering, and antiparasitic agents. Some secondary metabolites acts as diagnostics, herbicides, immunosuppressants and some help in prevention of biological disorders. The word 'Parvome' has been coined for these small organic molecules group (Davies and Ryan, 2012).

The cyanobacteria of tropical marine and freshwater habitats are widely studied for their metabolites. Notably, cyanobacteria compounds isolated from different environments show different bioactivities and structural properties. *Lyngbya majuscula* lives in tropical marine habitats and produce cytotoxic compounds, which are having high potential for drug development. These metabolites, possess a polyketide backbone, and often contain amino acid constituents (eg., alysiatoxin, barbamide, curacin A, hectochlorin, jamaiamide, lyngbyatoxin) (Jones, et al., 2009). Freshwater cyanobacteria produce a variety of peptide backbones having polyketide side chains which are effective against various proteases or protein phosphataes (eg., aeruginosin, anabaenopeptin, cyanopeptolin, microcystin, microginin, and microviridin) (Welker and Dohren, 2006). Cyanobacteria of terrestrial habitat produce different types of compounds, such as cryptophycin, mycosporic like amino acids, nostocyclopeptide, scytomenin, and nostopeptolide (Kehr, et al., 2011).

**Production of bioactive compounds**

Most of the cyanobacterial secondary metabolites are produced through nonribosomal peptide synthetases (NRPS) or polyketide synthases (PKS) route (Hoffmann, et al., 2003). Cyanobacteria are having multiple biosynthetic metabolic pathways for synthesizing bioactive secondary metabolites suggesting that, their richness in bioactive compounds is an evolutionary adaptation (Engene, et al., 2013; Pagliara, 2015). Gerwick, et al. found that secondary metabolites are mostly found in *Oscillatoriales* (49%), nostocales (26%), chroococcales (16%), pleurocapsales (6%) and stigonematales (4%) of cyanobacteria phylum (Gerwick, et al., 2008). These metabolites include different compounds like...
cytotoxic (41%), antitumor (13%), antiviral (4%), antimicrobial (12%) and other compounds (18%) (antifeedant, antimalarial, antimycotics, herbicides, immune suppressive agents and multidrug resistance reversers). These cyanobacterial origin bioactive compounds will be of amides (9%), amino acids (5.6%), fatty acids (4.2%), lipopeptides (40%) and macrolides (4.2%).

Cyanobacteria release some compounds into surrounding environment which is an allelopathic strategy to overcome the competition with other microorganisms in their ecosystem (Gross, 2003). Extensive research has been undergoing to search new compounds from cyanobacterial extracts, which can be used as new drugs or antibiotics.

Production of anti-microbial compounds

*Nostoc commune* produces Noscomin, a diterpenoid compound which shows antibacterial activity against *Bacillus cereus*, *E. coli* and *Staphylococcus epidermidis* at minimum inhibitory concentration (MIC) which is similar to standard drugs (Jaki, et al., 1999) and nostofungicide which is effective against *Aspergillus candidus*, *Nostoc ellipsosporum*. It has been found that that extracts of, *Fischerella* sp. ARM 354, *Hapalosiphon hibernicus* ARM 178, *muscorum* ARM 221, *Scytonema* sp. and *Westiellopsis prolife* ARM 365 exhibited antibacterial activity against *Bradyrhizobium* sp. *Bacillus subtilis*, *E. coli*, and *Pseudomonas striata* (Tyagi, et al., 2014). In another investigation, extracts of *Anabaena* sp. exhibited antibacterial activity against *E. coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococcus aureus* (Chauhan, et al., 2010).

Production of antioxidant compounds

Phycobiliproteins are soluble protein complexes which are abundantly present in cyanobacteria. These protein molecules are ecologically and economically important biomolecules which show high level of anti-oxidant activity and are now highly explored for synthesizing anti-aging drugs (Sonani, et al., 2014). Some studies concluded that the antioxidant properties of some edible cyanobacteria have significantly reduced oxidative stress in athletes (Sakhthivel and Kathiresan, 2015).

Production of antiviral compound

Many species of marine cyanobacteria produce anti-viral compounds such as cryptophycin which interferes with tubulin formation in viral lifecycle; and bicyclic peptide which inhibit histone deacetylation (Sivanesan, et al., 2014). It is hypothesized that consumption of spirulina potentially slows the growth and spread of ovarian cancer by interfering in the lysophosphatidic activity which is a growth factor (McCarty, 2015). Spirulina consumption also showed potent anti-proliferative activity on human pancreatic adenocarcinoma due to the presence of bilirubin-like tetrapyrrolic compounds (Koníčková, et al., 2014). *Oscillatoria terebriformis* extracts has potential anti-cancerous property which induced apoptosis in human lung cancer A549 cells (Mukund and Sivasubramanian, 2014). *Scytonema* sp., produces cyanoxinf-N, scytoxin, and sulfoglycolipid which exhibited potent antiviral activity against human immunodeficiency virus (HIV) which will helps in AIDS treatment (Boyd, et al., 1997; Loya, et al., 1998; Bokesch, et al., 2003).

Cyanobacterial compounds in the environment

Some isophenolic compounds produced by cyanobacteria like *Anabaena wisconsinense*, *Nostoc muscorum* and *Oscillatoria curvisp* have protective effect on fish by exhibiting antimicrobial activity against bacteria, fungi and other pathogens causing diseases in fishes (El-Sheekh, 2013; Mostafa, et al., 2008). Some cyanobacteria show allelopathic effect on submerged macrophytes, living in their vicinity by producing bio active compounds (Chang, et al., 2013). By further exploring these compounds we can prepare fertilizers with bio-origin.

Cyanobacteria and genetic engineering

Cyanobacteria are potential source for genes which can express proteins that give high level of stress resistance. Through genetic engineering it is possible to transfer the stress resistance genes to the target plant, by which abiotic stress resistance crops can be produced (Jyotsna, et al., 2014 ). Cyanobacterial extracted pigments, such as astaxanthin, β-carotene, and phycocyanin are used in feeds and foods, these are proved to be having many health applications for example phycocyanin a phycobilin protein, plays a major role in cholesterol degradation (Gnagne, et al., 2015; Eriksen, 2016). Research is going on to produce mosquitoicidal and mosquito larvicidal toxins by using cyanobacteria with specific toxicity to mosquitoes (DiMasi, et al., 2003). Many cyanobacteria are known to produce two types of sunscreen compounds scytonemin and mycosporine when stressed under UV radiation and these compounds are used in preparation of sunscreen lotions.

Cyanobacteria in drug delivery

Glycotherapy using cyanobacterial glycoconjugates is in progress, for example sulfated homopolysaccharides and heteropolysaccharides extracted from cyanobacteria have potent activity against retroviruses, including human
immunodeficiency virus (HIV) and herpes simplex virus (Schaeffer and Krylov, 2000). Cyanobacterial glycans have potential drug delivering capabilities to the targeted site. These glycans are hydrogels which imbibe large amounts of biofluids (Peppas, et al., 2000) and this property has been exploited for delivery of low-molecular weight drugs and macromolecular payloads (hormones, peptide and protein drugs) to specific targeted site (He, et al., 1999; Agnihotri, et al., 2004; Oh, et al., 2009).

**Cyanobacteria and bioinformatics**

However, optimizing new drugs with novel therapeutic capacity is a lengthy and tedious process (DiMasi, et al., 2003). Both the chemical and genetic screening of drugs is very laborious, time consuming, making the whole process very costly. To bypass this hurdle, nowadays variety of high speed automated technologies like DNA sequencing (Kramer and Cohen, 2004), microarray or two-dimensional (2D) gel electrophoresis (Onyango, 2004), spectrometry assays, high-speed robotized library screens (Comess and Schurdak, 2004) have been developed. Moreover, most advanced in silico approaches which uses genetic/genomic information to assess the genetic potential of microorganisms for their ability to produce required bioactive compounds (Walsh and Fischbach, 2010) can minimize the cost and time for screening cyanobacteria. Sophisticated software tools like SMURF, anti SMASH, and NP are also being used to predict the gene expression and screening of the expressed products (Weber, 2014).

**Cyanobacteria in bioremediation**

Cyanobacteria have capacity to oxidize a variety of complex organic compounds in the environment (Mansy and El-Bestway, 2002). They are potent accumulators and degraders of different kinds of pollutants such as catechols (Cerniglia, et al., 1980; Megharaj, et al., 1987), crude oil (Al-Hasan, et al., 2001; Cerniglia, et al., 1980b), naphthalene (Cerniglia, et al., 1980a; Narro, et al., 1992), phenanthrene (Ellis, 1997), pesticides (Sokhoh, et al., 1992), phenols (Shashirekha, et al., 1997), Heavy metals (Anas, et al., 2015) and xenobiotics (Varsha, et al., 2011). Cyanobacteria are efficient biosorbents for eliminating heavy metals from environment (Narro, et al., 1992; Ellis, 1997). Studies conducted by various researchers had shown that bio-degradation and biosorption capacity of cyanobacterial species like *Cyanothece* sp. *Nodularia* sp., *Nostoc* sp. *Oscillatoria* sp., and *Synechococcus* sp., dominated the industrial effluents. However, their capacity and spectrum of activity increases when combined with other bacteria for example *Oscillatoria* a cyanobacteria in presence of *gammaproteobacteria* degrade dibenzothiophene, *n*-octadecane, phenanthrene, and pristine (Abed and Köster, 2005). Immobilized bacteria coated with cyanobacteria were capable of degrading wide variety of pollutants (Radwan, et al., 2002).

**Industrial effluent treatment**

In the present global scenario industrial effluents on a large-scale are released into surroundings causing significant irreversible damage to environment. When they are released into the nearby water source, they change colour and give odour to the water which diminishes the quality of water making it non-potable and toxic to the creatures living in it.

Cyanobacterial treatment system with widely available species like *Anabaena variabilis, Lyngbya majuscula, Nostoc muscorum*, and *Oscillatoria salinas* are being used for bioremediation of textile industry effluents. These cyanobacteria are efficient in reduction of metal levels like zinc, nickel and other pollutant like sulphates, calcium ions and they also help in raising the pH value along with reduction of biological oxygen demand (BOD), and chemical oxygen demand (COD) (Noel and Rajan, 2014). *Oscillatoria* growth helps in removing BOD, colour and COD by 30.4%, 39.82% and 57.6% after 4 weeks (Abraham and Nanda, 2015). *Tolypothrix tenuis* (Kutz.) has the capability to reduce the toxic effects of fertilizer industry effluents by 65% (Kumar, 2015). However immobilized cyanobacteria are more efficient in reducing pollutant levels than free cyanobacteria (Vijayakumar and Manoharan, 2012).

Some cyanobacteria like *Oscillatoria willei* BDU130791, *Phormidium valderianum* BDU20041, has potential mechanism in biocalcification of calcium pollutants like the ossein effluents which were released during gelatin production from cattle bones (Vaithiyalingam, et al., 2014; Uma, et al., 2014).

Cyanobacterial treatment is an efficient and a low-cost method for converting dairy waste water and dissolved nutrients into biomass. This treatment is also used for removal of dissolved inorganic pollutants from fish farms which can enhance the productivity of fish. Some filamentous cyanobacteria like *Anabaena* sp. and *N. ellipsosporum* has natural ability to degrade chlorinated aliphatic pesticides such as lindane (ghexachlorocyclohexane) and the efficacy of the degradation can be enhanced by genetic engineering techniques.

**Heavy metal removal**

Heavy metals are naturally present in the
environment at very low levels, but due to anthropogenic activities like discharge of industrial wastes, lead-acid batteries, use of fertilizers, mining, vehicle emissions, etc., have drastically increased their concentration to toxic levels in the environment. These heavy metals like cadmium, lead, mercury and arsenic will bind to some cellular components, like enzymes, proteins, nucleic acids etc., and disrupts their normal functioning (Landis, et al., 2000). Long-term exposure to these toxic heavy metals can lead to defects in nervous system, blood circulation and cancer.

Several cyanobacterial strains produce extracellular polymeric substances (EPS), which have different roles like protection from UV radiation, desiccation, etc (Singh and Mishra, 2015). EPS-producing cyanobacteria and EPS polymers can potentially absorb heavy metals from its surroundings. Cyanobacteria like Oscillatoria trichoides Szafer and Oscillatoria laetevires Gomont are common inhabitants of polluted environments and are potential remediators of hexavalent chromium (Cr⁶⁺). Spirulina platensis potentially absorbs cadmium, trivalent chromium, zinc and their efficiency can be increased by immobilizing them even the dried mass of it, which is a good biosorbent of chromium (Murugesan, et al., 2008; Shashirekha, 2008; Meng, 2011). Anabena sp., are very potent absorbers of toxic metals like lead, cadmium, zinc etc (El-Bestawy, et al., 2014).

Now a days disposal of waste water like sewage into rivers or other water bodies has become a major concern due to the presence of very high level of pollutants. These pollutants alter the natural environment of the water body by changing BOD, COD, etc. Cyanobacteria are now being used for treating wastewater especially to remove toxic metals and other pollutants before discharging them into the environment (Frontasyeva, et al., 2015; Fawzy, 2015).

Clearing oil spills

Seas, oceans and coastal zones are greatly threatened by crude oil pollution, which is a major threat to the biodiversity and sustainability of planet earth (Halpern, et al., 2009). The environmental impact of oil spill or crude oil leakage is multiplied when the oil reaches shoreline of lagoons, salt marshes, etc., which are low energy habitats. The entire food web gets destroyed making the ecosystem uninhabitable for certain period. These hydrocarbons are stable molecules, but their presence on the earth for millions of years has led to the evolution of organisms that use them as a major or sole source of carbon and energy (Prince, et al., 2010). Hydrocarbon contaminated environments are remediated majorly through biodegradation by cyanobacteria and bacteria.

Cyanobacterial mats of the Arabian Gulf coast of Saudi Arabia experience extreme salinity and temperature as they are exposed to continuous oil pollution. They are ideal models for biodegradation of oil under extreme environmental conditions (Raeid, et al., 2006). Oil and ammonia concentrations play a key role in determining distribution of these cyanobacterial mats’ microbial communities in oil field wastewaters (Abed, et al., 2014). Marine cyanobacteria Phormidium sp. removes almost 40% of spilled diesel oil and hexadecane within 10 days and has capability to remove C10-C28 hydrocarbons under aerobic autotrophic conditions (Morales and Michel, 2013). These bacteria form mats which are generally seen in petroleum-polluted environments of tropical regions. These mats are of with high biodiversity microbial consortium with efficient hydrocarbons degraders. However, in axenic conditions, Phormidium sp. will not degrade C13-C35 hydrocarbons either in autotrophic or heterotrophic conditions (Chaillan, 2006). Cyanobacteria like Aphanocapsa sp., Oscillatoria salina Biswas, forms excellent cyanobacterial mats when grown together, these mats are highly potent in mitigating oil pollution on seashores (Kumar et al., 2001).

Negative aspects of cyanobacteria

However, many cyanobacteria are toxic and show negative effect on humans and other animals in the surroundings. Some cyanobacteria produce taste-and-odour producing compounds (TOC) such as geosmin and 2-methylisoborneol (MIB) especially in freshwater aquaculture which affects fish growth in both open fishponds and tank production systems (http://www.omicsgroup.com/conferences/ACS/conference/pdfs/1325-Keynote-Pdf.pdf).

β-N-methylamino-l-alanine (BMAA) is a neurotoxin produced by nostoc, which gets into human food chain via sea food and water. It potentially affects the functioning of brain, which may lead to early neurosis (Govindaraju, et al., 2012). Nodularia spumigena grows in shrimp growth tanks and produces toxic pentapeptide, nodularin; this compound can easily enter into the human’s body through consumption of these prawns. Nodularin-R is a potent hepatotoxin which damages liver when consumed (Yunes, et al., 2016).

Many cyanobacterial blooms produce significant quantities of microcystins (MCs) which are toxic and they decrease the potable quality of water (Mbukwa, et al., 2015; Fenta and Kidanemariam, 2016). MCs are
hepatoxic they effect mainly aquatic animals and increase their mortality (Sonia and Ramanibai, 2015). *Cylindrospermopsis raciborskii* a cyanobacterium produces neurotoxins which effect mammalian nervous system; and other toxins which effects the growth of phytoplanktons and zooplanktons disturbing the food chain in the ecosystem (Zagatto and Ferrão-FilhoAdS, 2013).

For detecting the presence of cyanobacterial toxins in the samples, several diagnostic methods and kits are available (Qiu, *et al.*, 2013; Pierce and Ouellette, 2014; Nyoni, *et al.*, 2015; Bagchi, 2015) which helps us to prevent from consuming.

**CONCLUSION**

Cyanobacteria are primitive photosynthetic ubiquitous microorganisms playing a major role in biogeochemical cycles of nitrogen, carbon etc. Due to the presence of huge number of diversified bioactive compounds, special interest has been shown to evaluate its potential in novel drug discovery. In the recent decade cyanobacteria have gained importance in fields of bioremediation, industrial microbiology and drug discovery especially for the treatment of incurable diseases such as HIV, cancer, etc. Cyanobacteria are being extensively used in bioremediation because of its capability to degrade pollutants in industrial effluents, waste water, and they can also degrade hydrocarbons such as crude oil and its components from oil spills in ocean and soil. Some cyanobacteria act as biosorbents and absorb heavy metals, from the environment. Because of their simple growth requirements such as sunlight, carbon dioxide; easiness in their genome manipulations and well documented applications, there is a need and a good chance for optimal utilization of cyanobacteria.

Even though they have many advantages, cyanobacteria still need to be explored for using their potential at maximum levels. The industrial application of cyanobacteria for large-scale synthesis of desired compound is technologically challenging for which high efficiency photosynthetic bioreactors are to be designed with minimum operation costs. There is a need to develop cultivation systems which harness the photosynthetic capability of cyanobacteria for discovering green paths to produce industrial products.

Still extremophilic cyanobacteria living in various extreme habitats are not yet explored. Many potential drug metabolites could be developed from them. In addition to exploring new compounds, genome mining can help us in better understanding of production and manipulation of cyanobacterial products. For successful drug discovery using cyanobacterial compounds new and innovative approaches are required in a systematic manner to exploit their true bio-potential. Several genome mining strategies, informatics analyses of their metagenomes have become successful in identifying new metabolites that were unnoticed under normal experimental conditions. Cyanobacterial PKS-NRPS (polyketide synthases-nonribosomal peptide synthetases) metabolic process makes it possible for heterologous expression of natural products which helps in biosynthesis of new hybrid compounds. However, more research is needed to develop drug producing cyanobacteria with these techniques.

**CONFLICT OF INTERESTS**

The authors declare that there is no conflict of interests regarding the publication of this paper.

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