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Research Article

Molecular identification and toxicity effects of cyanobacteria species isolated from the Khoor-e-Khooran mangrove forest, Persian Gulf

Zaheri A.[1,2]; Bahador N.[2*]; Yousefzadi M.[3]; Arman M.[4]

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Abstract

The increasing incidence of the harmful cyanobacterial blooms in mangrove ecosystem is a potential threat for aquatic organisms and their consumers. In the present study, we have evaluated the biodiversity of cyanobacteria and monitored their cyanotoxins. We isolated 120 bacterial isolates using BG11 medium from water and sediment samples collected from 10 stations throughout of the Khoor-e-Khooran mangrove forest at august 2018. Biodiversity and distribution pattern based on morphological characteristics showed that 10 cyanobacterial genera were spread over the studied area. *Phormidium*, *Oscillatoria*, *Spirulina* and *Nostoc* genera were dominated with frequency percentages of 25%, 20%, 10%, and 10% respectively. Analysis of 16S rRNA sequences showed that the strains have high similarity with type strains in NCBI GenBank ranged from 98% to 100%. Phylogenetic analysis proposed the non-indigenous origin of *Microcystis* strains because of their phylogenetic divergence. We detected microcystin gene in *Microcystis* sp. strain KH 3, *Microcystis* sp. strain KH 4 and *Microcystis* sp. strain KH 11, while nodularin and cylindrospermopsin gene were not detected in all isolated cyanobacteria. The extracted metabolites from KH 3 and KH 4 strains showed cytotoxicity with LC_{50} of 139.3 and 225.8 µg/mL against *Artemia salina* respectively. Their LC_{50} were 231.3 and 211.2 µg/mL against shrimp larvae respectively. They inhibited the proliferation of HUVEC cell lines with IC_{50} of 11.13 and 13.29 µg/mL and HEPG2 with IC_{50} of 15.49 and 12.51 µg/mL, respectively. Our results represented diversity and distribution pattern of cyanobacteria and demonstrated the incidence of microcystin in the Khoor-e-Khooran mangrove forest.

Keywords: Marine cyanobacteria, Cyanotoxins, Mangrove forest, Persian Gulf, Shrimp

1- Department of Microbiology, College of Science, Science and Research Branch, Islamic Azad University, Fars, Iran
2- Department of Microbiology, College of Science, Agriculture and Modern Technology, Shiraz Branch, Islamic Azad University, Shiraz, Iran
3- Department of Marine Biology, Faculty of Marine Sciences and Technology, University of Hormozgan, Bandar Abbas, Iran.
4- Department of Biology, Payame Noor University, PO BOX 19395-3697, Tehran, Iran.
* Corresponding author's Email: bahador@iaushiraz.ac.ir

Introduction

Cyanobacteria are photoautotrophic oxygenic bacteria that have acquired special characteristics during evolution to adapt to biogeochemical and ecological changes (Huisman *et al.*, 2018; Barzkar *et al.*, 2021a). The ecological flexibility of cyanobacteria enables them to grow in diverse ecosystems and environmental conditions. However, their diversity pattern could be influenced by anthropogenic and climatic-induced changes (Paerl, 2018). The increasing occurrence of harmful cyanobacterial blooms (HCBs) is a consequence of these global environmental variations (Visser *et al.*, 2016). Increases in HCBs occurrences in susceptible ecosystems like mangrove habitats could lead to catastrophic outcomes for their fauna, flora and human health (Weber *et al.*,, 2020).

Mangrove forests are coastal ecosystems that are located in some tropical and subtropical areas (Gozari *et al.*, 2021). However, their plant diversity is poor, but they support a high diversity of animal species such as a variety of birds, mammals, amphibians, arthropods, fish and shellfish species (Friess *et al.*, 2019). This habitat plays a significant role in accumulation of carbon and nutrients, recovery of marine biomass and also functions as a nursery place for larval growth of some animals such as commercial fish and shellfish species (Malik *et al.*, 2017; Carrasquilla-Henao *et al.*, 2019; Pourmozaffar *et al.*, 2019b). Because of these natural functions, this ecosystem have ecological and economical importance (Anneboina and Kumar, 2017). The interactions of plants, animals and microorganisms are necessary to guaranty balanced food chains and survival of the mangrove ecosystem (Nelson *et al.*, 2019; Pourmozaffar *et al.*, 2019a).

Mangrove-inhabiting cyanobacteria tolerate to fluctuation of environmental conditions such as temperature, salinity, light intensity, osmotic pressure, ionic concentration and weathering in this ecosystem (Alvarenga *et al.*, 2015). Therefore, mangrove-inhabiting cyanobacteria are considered a biodiversity hotspot (Mariani *et al.*, 2015). However, the biodiversity of mangrove-inhabiting cyanobacteria is underestimated and a large number of them has not properly characterized (Barzkar *et al.*, 2021b; Nabout *et al.*, 2013). This is mainly because of the use of morphological features compared to molecular methods, which provided considerable accurate data for identification. Understanding of cyanobacterial diversity in mangrove forests can facilitate monitoring program and bloom modeling to control harmful cyanobacterial blooms (Song 2017; Ralston and Moore 2020). Several studies showed that mangrove-inhabiting cyanobacteria dominantly belong to *Oscillatoria*, *Phormidium*, *Spirulina*, *Lyngbya*, *Nostoc* and *Chroococcus* genera (Sakthivel and Kathiresan 2013; Ram and Shamina 2017; Gaysina *et al.*, 2019).

The most challenging properties of cyanobacteria are their potential to produce toxic metabolites nominated as cyanotoxins. Cyanotoxins categorized into hepatotoxins, dermatotoxins and neurotoxins (Meriluoto *et al.,* 2017). The deleterious effects of cyanotoxins on aquatic organisms and their potential for bioaccumulation and transferring through the food web can endanger biodiversity and aquatic consumer's health (McQuaid and Lee, 2019). Cyanotoxins have acute effects on aquatic animals such as reduce their survivorship, inhibit their feeding and cause paralysis. The most important chronic effects of cyanotoxins on aquatic animals are reduction of growth and fecundity, behavioral alterations and biochemical alterations in activity of important enzymes like phosphatases, glutathione-S-transferases, acetylcholinesterase and proteases (Ferrão-Filho and Kozlowsky-Suzuki, 2011). For instance, microcystins, which classified in hepatotoxins group, produce by *Microcystis* species. This genus is a bloom-forming cyanobacterium in estuarine and mangrove ecosystems and responsible for intoxication of aquatic organisms and their consumers (Pham and Utsumi, 2018). Concludingly, monitoring of cyanotoxins especially in mangrove ecosystems is essential for better understanding and predicting the emergence of HCBs and to ensure public safety (Jaramillo and O'Shea 2019; Roy-Lachapelle *et al.,* 2019). Detection of toxic genes and toxicity assays are robust approaches to monitor cyanotoxins.

Khoor-e-Khooran mangrove forests is a natural protected area that located near the Bandar-e-Khamir city on the northern coast of the Persian Gulf (Milani, 2018). This habitat harbors high density of mangrove plants and provided a focal point for the propagation of fish and shellfish species in the region (Tamadoni Jahromi *et al.,* 2021a and b). The aims of the present study were to monitor the toxigenic cyanobacteria in the Khoor-e-Khooran mangrove forests and to investigate cyanobacterial diversity in this natural protected area.

Materials and methods

Sample collection

Sampling was conducted in August 2018 in the Khoor-e-Khooran mangrove wetland located on the northern coast of the Persian Gulf, Bandar-e-Khamir, Iran. Water samples were collected from 10 selected stations from 30 cm below the surface in a 250 mL dark glass bottle. The sediment samples were collected from same stations by a grab sampler (Gozari *et al.,* 2019c). Sampling process was done in triplicate and all samples were taken to the laboratory immediately within 3 hours (Authority GBRMP , 2019).

Isolation of cyanobacteria

One hundred µL of each sample was inoculated on the BG 11 medium, which was prepared with natural seawater. The inoculated media were incubated at 28˚C under 12 hours light

and 12 hours darkness with light intensity 1500-2000 lux. After 2 weeks, the appeared colonies were investigated for belonging to cyanobacteria. Distinct colonies were selected according to morphological characteristic and were purified by subculturing on to BG11 medium (Ferris and Hirsch, 1991).

Characterization of cyanobacteria

The purified isolates were identified according to morphological characteristics based on the identification key in Standard Methods for Examination of Water and Wastewater and WHO guidelines (Lawton *et al.,* 1999; Carranzo 2012) .The micromorphological characters were observed with a light microscope (Nikon, Japan) with 40X magnification.

Detection of cyanotoxins genes

For genomic DNA extraction the purified isolates were inoculated in BG 11 medium and incubated in the appropriate condition, which was previously mentioned. The sufficient biomass (50-100 mg) of the isolates were harvested and DNA extraction was carried out by DNG^{TM} – Plus kit (Iranian Gene Fanavar Company). After DNA extraction, the cyanotoxins biomarkers, including microcystin, nodularine and cylindrospermopsin were detected using the Microcystin/nodularine gene PCR detection and Cylindrospermopsin gene PCR detection kits (Iranian Gene Fanavar Company) respectively according to company instruction. The results of experiments were detected by agarose gel electrophoresis (Emtyazjoo *et al.,* 2019)

Extraction of cyanotoxins

After the cultivation period, cells were harvested by centrifugation. Harvested cells were briefly washed with distilled water to remove salts. The cyanobacterial extracts were obtained by homogenization using methanol: chloroform (1:1 v/v) in the ratio of 1:10 (biomass: solvent) and kept at 25˚C for 24 h in a photo-incubator. Solutions were centrifuged at 10 000 rpm for 10 min, the supernatant recovered, and subsequently dried and stored at -20˚C (Nazemi *et al.,* 2017). A total of 50 mg of the cyanobacterial crude extracts was dissolved in 1mL of dimethyl sulfoxide (DMSO) for bioassays. (Maruthanayagam *et al.,* 2013).

Brine shrimp cytotoxicity assay

Toxicity of extracted metabolites from cyanobacterial isolates was assayed by Brine-shrimp microwell cytotoxicity method. One gram of the commercially available (INVE^{TM}) of brine shrimp cysts *Artemia salina* cultivated in 3% of saline water at 22-29 ˚C under white light for 48h hours. For cytotoxicity assay, 100 µL nauplii suspension containing 15 nauplii per 100 µL was transferred to 100 µL cyanobacterial extract (125, 250,500 and 1000 µL/mL) in a 96-well microplate and incubated at 25˚C for 24 hours (Gozari *et al.,* 2018; Gozari *et al.,* 2019a). The percent of mortality was recorded. The cytotoxic activity was calculated by the following formula and presented as LC_{50}.

Toxicity = ($N_{control}$ - N_{test}/$N_{control}$) ×100%

$N_{control}$ is the number of live nauplii in untreated well and N_{test} is the number of live nauplii in treated well.

Toxicity against shrimp larvae

Toxicity of extracted metabolites against *Penaeus vanammei* larvae was investigated and reported as LC_{50} value (Gozari *et al.,* 2016). The LC_{50} value is a concentration of an extract that could kill 50% of the whole treated larvae. The shrimp larvae at post larval stage 9 were provided from the hatchery center of the Persian Gulf and Oman Sea Ecological Research Institute. Then 10 larvae were transferred to 250 mL Erlenmeyer flask containing 200 mL seawater. The extracted metabolites were added to each flask at final concentrations 1000, 500, 250, 125 µg/mL. The mortality rates were recorded up to 6 days and LC_{50} value were calculated by following equation:

Toxicity = ($N_{control}$ - N_{test}/$N_{control}$) ×100%

$N_{control}$ is the number of live larvae in untreated flask and N_{test} is the number of live larvae in treated flask.

Cytotoxicity against human cell lines

The cytotoxicity of cyanobacterial extracted metabolites was evaluated in HUVECs (human umbilical vein endothelial cells) and HEPG2 (hepatocellular carcinoma) cell lines by MTT cell proliferation assay. The One hundred microliters of the HUVECs or HEPG2 cell suspensions in DMEM or RPMI media were transferred in 96-well Microplates. The cell density was subjected at 10^4 cells per well. After incubation at 37°C for 24 hours in CO_2 incubator, cell lines were treated with 100 µL of each extract at certain final concentrations (100, 50, 25, 12.5 µg/mL). After 36 hours additional incubation, 50 µL of the prepared MTT solution (5 mg/mL) was injected in to each well and kept in incubator for 4 hours. The MTT solution was removed and 100 µL of DMSO/ethanol solution (4:1) was added to each well. Then the 96- well plate was kept in a shaker to dissolve the formazan dye. Finally, the absorbance of each well was recorded at wavelength of 550 nm by microplate reader (Gozari *et al.,* 2019b). The survival rate of the cell lines was calculated by following formula:

$$\text{Cell viability (\%)} = [(OD_{test}) - (OD_{Blank}) / (OD_{control}) - (OD_{Blank})] \times 100$$

Molecular Identification of cyanobacteria

After the genomic DNA extraction that was previously described, the specific genes were detected by Cyanobacteria Specific PCR Detection Kit (Iranian Gene Fanavar Company). The PCR reaction was performed according to company‘s instruction. Amplification and visualization of the 487 bp fragment on agarose gel confirmed the cyanobacterial identity. For

phylogenetic analysis, we amplified the 16S rRNA gene using PCR reaction by universal primers including 9F and 1541R as described by Heuer (1997). The PCR products were purified with the Roche PCR Purification kit (Roche Applied Science) and sequenced by Macrogen Company (Seoul, Korea). The 16S rRNA gene sequences were analyzed by BLAST program at NCBI (National Centre for Biotechnology Information) (Madden, 2013) and submitted to the GenBank database with following accession numbers: MN864652, MN864653, MN864654, MN864655, MN864656, MN864657, MN864658, MN864659, MN837908, MN837909. Phylogenetic tree was constructed based on neighbor joining algorithm (Saitou and Nei, 1987) using MEGA X program (Kumar *et al.*, 2018).

Statistical analysis

All of the experiments were performed in triplicates. The statistical significance of the data was analyzed with one-way ANOVA followed by LSD using SPSS program (Version 24) and the significance level was set at $p<0.05$. The results of biodiversity of cyanobacteria were reported as percentage. The cytotoxicity results expressed as mean LC_{50}±standard error (SE). The LC_{50} values of the extracts were calculated using the linear regression between the final concentration of the extracts and respective cytotoxicity percent calculated from the previously mentioned equation by the software Graph Pad PRISM version 6 (Graph Pad Software, San Diego, CA). The statistical significance of resultant tree topology was evaluated by bootstrap analysis (Felsenstein, 1985) based on 1000 replicates.

Results

Isolation and Characterization of Cyanobacteria

One hundred isolates of cyanobacteria were obtained from the collected samples. Morphological properties showed that the isolated strains belonged to 10 different genera of cyanobacteria. *Phormidium*, *Oscillatoria*, *Spirulina* and *Nostoc* genera were dominated in collecting samples with 25%, 20%, 10%, and 10% respectively (Fig. 1). Molecular identification of 24 distinct isolates with specific primers confirmed that 10 strains belonged to cyanobacteria (Fig. 2).

Detection of cyanotoxins genes

Screening of microcystin, nodularin and cylindrospermopsin genes showed that microcystin/nodularin genes were existed in genomic DNA of the 3 out of 10 isolated strains (Fig. 3). While cylindrospermopsin genes were absent in all strains (Fig. 4).

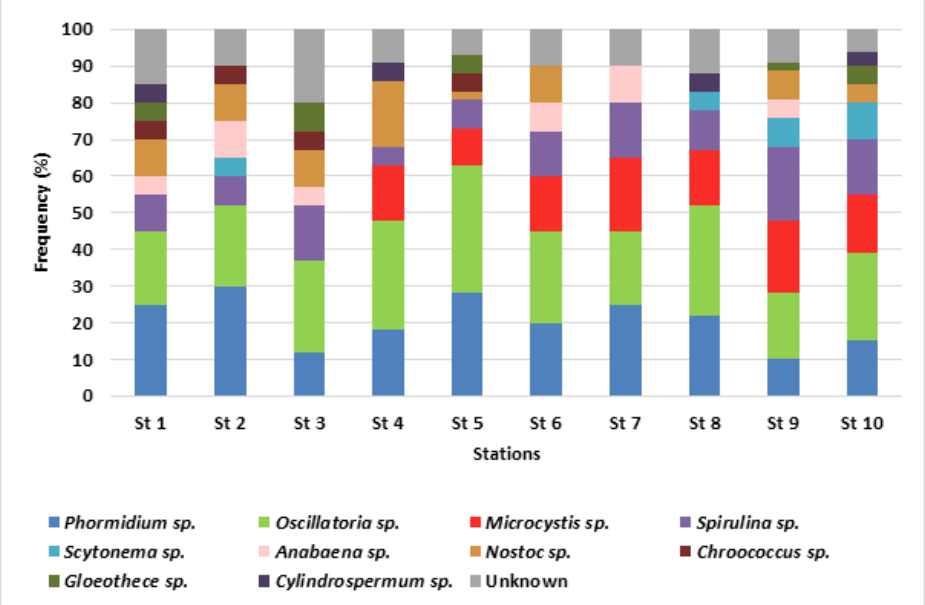

Figure 1: Biodiversity pattern of cyanobacteria in collected samples from the Khoor-e-Khooran wetland. The frequency of each genus was represented by different colors. Some genera were absent in some stations.

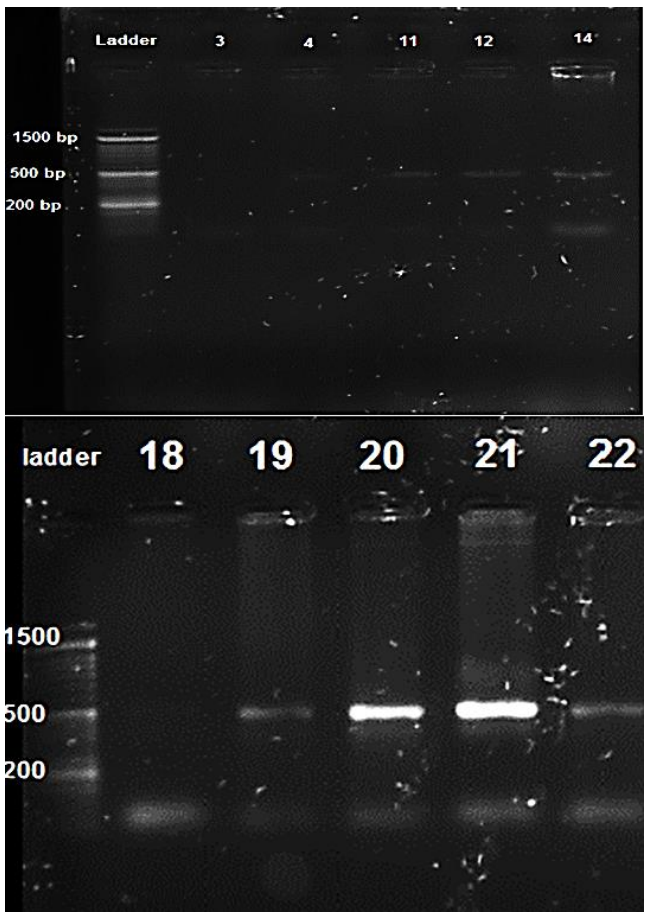

Figure 2: Agarose gel electrophoresis of the amplified cyanobacterial specific genes in the isolated strains. The first lane in each series was loaded by the gene ruler and displayed as ladder. The number of each isolate was shown above the lanes.

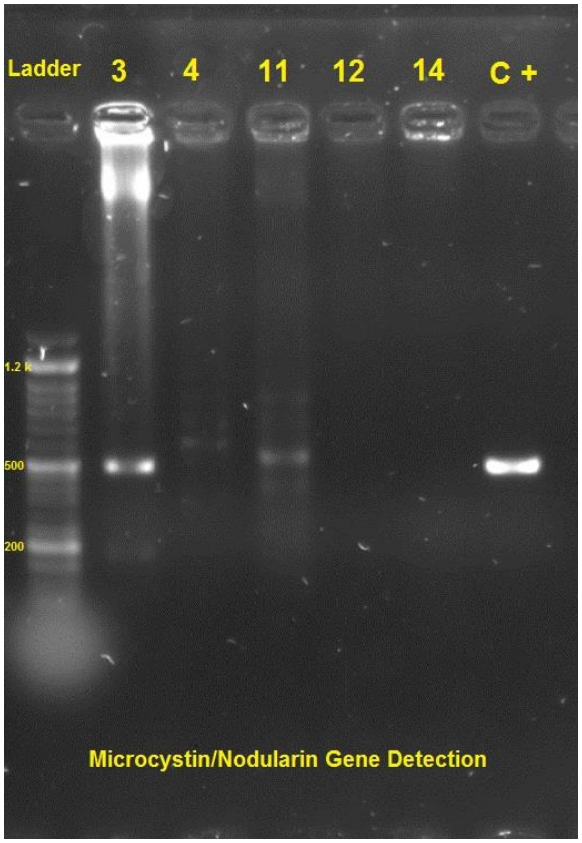

Figure 3: Agarose gel electrophoresis for detection of the amplified microcystin/nodularin gene in the isolated strains. The first lane was loaded by the gene ruler and displayed as ladder. The number of each isolate was shown above the lanes. Control positive test was shown as C^+.

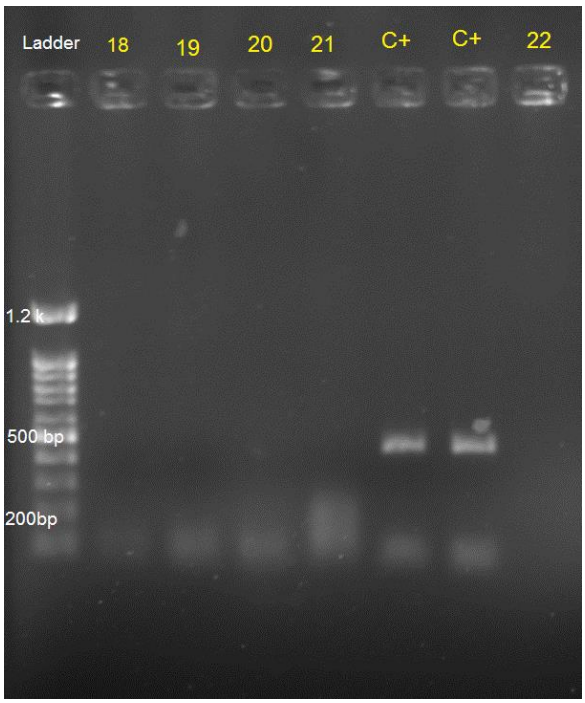

Figure 4: Agarose gel electrophoresis for detection of the amplified cylindrospermopsin gene in the isolated strains. The first lane was loaded by the gene ruler and displayed as ladder. The number of each isolate was shown above the lanes. Control positive test was shown as C^+.

Cytotoxicity assays

After the detection of cyanotoxins genes in the isolated strains, we assessed cytotoxicity of the three-cyanobacterial strains, which have toxic genes using three different cytotoxicity assays. In the first line of cytotoxicity assay, the extracted metabolites from two strains showed toxicity against Artemia larvae, while one strain did not show any toxicity. Dose- response curve showed that the toxicity effect followed a dose dependent trend. The LC_{50} value for extracted metabolites from strain 3 was equal to 139.3 µg/mL. While, strain 4 could kill 50% of Artemia cell at LC_{50} value equal to 225.8 µg/mL. However, strain 11 did not show significant toxicity against Artemia cells at the experimented doses (Fig. 5A).

In the second line of cytotoxicity assay, the extracted metabolites from strain 3 and strain 4 killed shrimp larvae, while strain 11 did not show any larval toxicity. The observed toxicity showed dose dependent curve (Fig. 5B). The toxicity of extracted metabolites from strain 3 was able to kill shrimp larvae at LC_{50} equal to 231.3 µg/mL. The toxicity of extracted metabolites from strains 4 was recorded at LC_{50} equal to 211.2 µg/mL while strain 11 could not kill the exposed larvae (Fig. 5B). In the third line of cytotoxicity assay, the results showed that the extracted metabolites from strain 3 and strain 4 exhibited high cytotoxicity against HUVEC and HEPG2 cell lines (Fig. 5 C, D). The evaluated extracts exhibited dose-dependent cytotoxicity toward the human tumor cell lines. The extracted metabolites from strain 3 exhibited highest IC_{50} values of 15.49 and 11.12 µg/mL against HUVEC and HEPG2 cell lines respectively. The extracted metabolites from strain 4 showed cytotoxic activity toward HUVEC and HEPG2 at IC_{50} values equal to 12.51 and 13.19 µg/mL respectively. While, the extracted metabolites from strain 11 did not show any significant activity against cell lines (Fig. 6).

Molecular Identification of cyanobacteria

Analysis of the partial sequences of 16S rRNA gene using BLAST software exhibited different similarity ranges between selected 10 cyanobacterial strains and the type strains in the NCBI database. *Phormidium* sp. strain KH 12 showed high similarity (99.51%) with *Phormidium sp.* MOR364. *Spirulina* sp. strain KH 14 represented 99.51% homology with *Spirulina subsalsa* SERB 25. The 16S rRNA of the *Microcystis* sp. strain KH 3 represented 98.64% similarity with *Microcystis aeruginosa* SERB 2. The similarity of 16S rRNA of *Microcystis* sp. strain KH 4, *Scytonema* sp. strain KH 19 and *Anabaena* sp. strain KH 22 showed 98.56%, 99.69%, and 99.30% with *Microcystis aeruginosa* SERB 2, *Scytonema myochrous* SERB 29 and *Anabaena cylindrica* SERB 19. However, The 16S rRNA of the *Oscillatoria* sp. strain KH 18, *Microcystis* sp. strain KH 11, *Nostoc* sp. strain KH 20 and *Chroococcus* sp. strain KH 21 showed full compliance

with *Oscillatoria acuminata* PCC 6304, *Microcystis aeruginosa* SERB 2, *Nostoc* sp. USMNA and *Chroococcus turgidus* SERB 26, respectively.

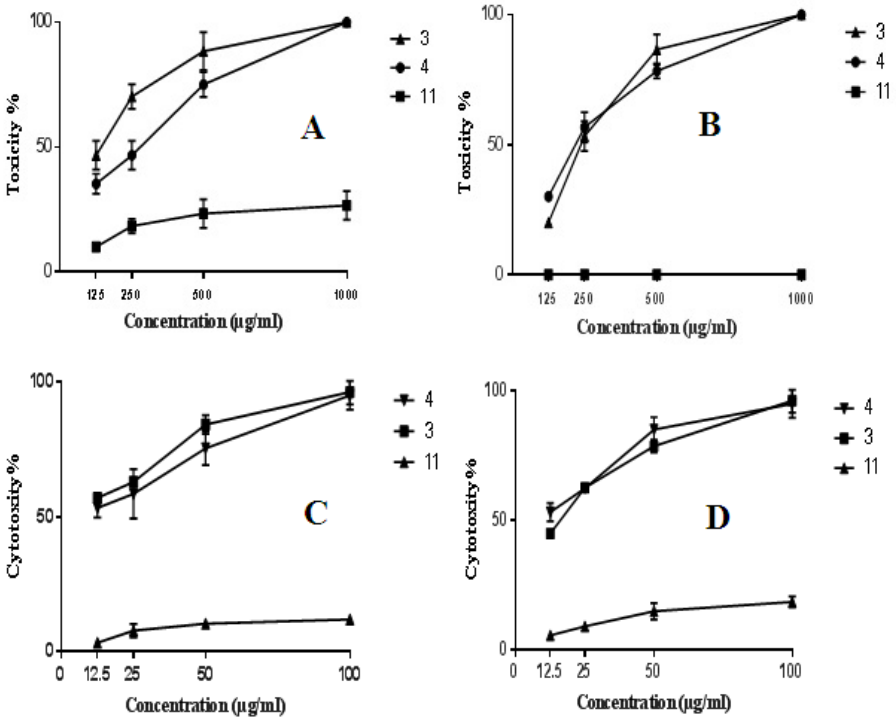

Figure 5: Cytotoxicity of extracted metabolites from the isolated cyanobacteria. A. cytotoxicity against *Artemia salina*, B. against *Penaeus vanammei* Larvae, C. against HepG2 cell line, D. against HUVEC cell line. 3: *Microcystis* sp. strain KH 3, 4: *Microcystis* sp. strain KH4, 11: *Microcystis* sp. strain KH 11.

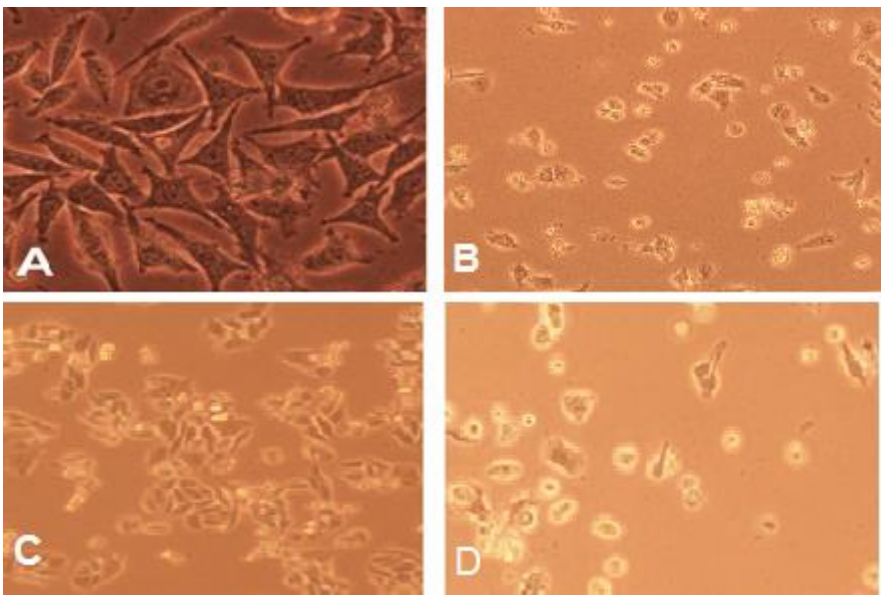

Figure 6: Cytotoxic activity of extracting metabolites against human cell lines. A. HUVEC cells in control well. B. HUVEC cells after treatment with cyanobacterial extract. C. HEPG2 cells in control well. D. HEPG2 cells after treatment with cyanobacterial extract.

Interpretation of the constructed phylogenetic tree based on 16S rRNA gene demonstrated that the cyanobacterial strains positioned in two different clades. *Microcystis* strains were located together into the same cluster although they were merged in subclades. *Phormidium* and *Oscillatoria* were located in a same clade while *Chroococcus* and *Spirulina* strains located in other clade but they have been derived from a common ancestor (Fig. 7). Other strains developed different lineages in a same clade. All isolated strains located with their closest type strains in a same cluster.

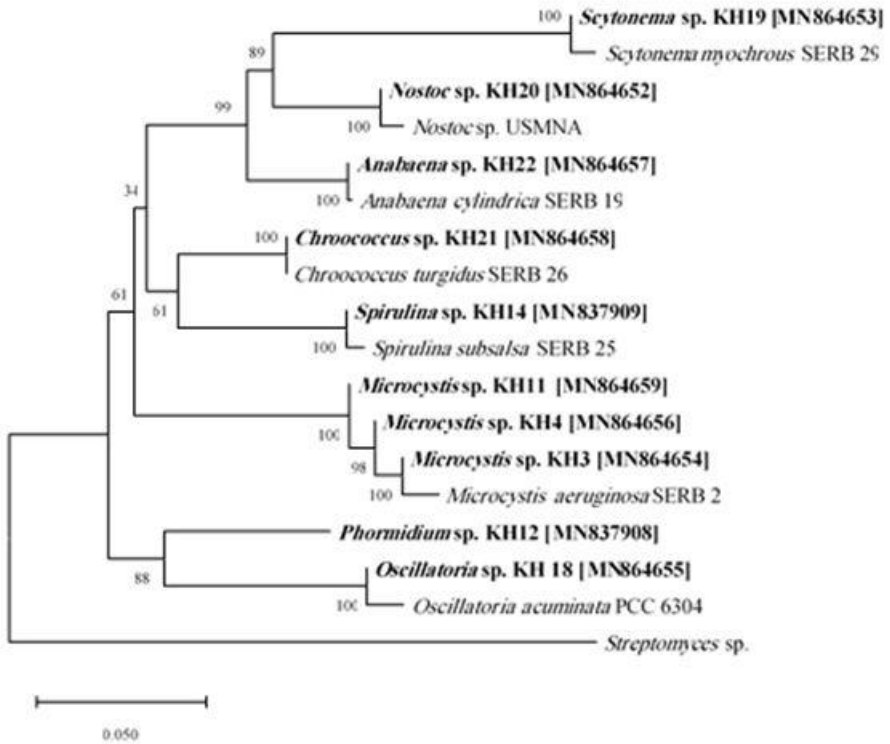

Figure 7: Phylogenetic tree based on 16S rRNA gene sequence analysis, reconstructed from evolutionary distances by using the neighbor-joining method, showing the phylogenetic position of the isolated cyanobacterial strains (Bold font) and type strain in NCBI GenBank. Bootstrap values are indicated at the relevant branching points. Numbers of branch node are bootstrap values based on 1000 resampling. Bar 0.02 substitutions per nucleotide position. *Streptomyces* sp. was used as an out-group.

Discussion

Monitoring of toxicogenic cyanobacteria in mangrove ecosystems is a necessary requirement because of the increasing occurrence of harmful cyanobacterial blooms due to climate change (Ramos *et al.*, 2017). We isolated 120 presumptive cyanobacterial isolates from water and sediment samples throughout the mangrove ecosystem in Khoor-e-Khooran wetland. The biodiversity pattern showed that *Phormidium*, *Spirulina*, *Oscillatoria* genera were dominated in all sample stations. Other studies also reported the dominance of these genera in mangrove ecosystems. For example, 17 species of cyanobacteria, which

dominantly belonged to *Oscillatoria* were isolated from Cardoso Island, Brazil (Branco *et al.,* 1994). In another study, Ram and colleagues (2017) showed that *Oscillatoria salina, Oscillatoria ornata* and *Oscillatoria vizagapatensis* dominated in seven mangrove ecosystems in the India. Mohamed and Al-Shehri (2015) , also identified 15 genera and 5 families of cyanobacteria from microbial mats of 3 mangrove ecosystems in the Red Sea. The isolated cyanobacteria dominantly belonged to *Oscillatoria* and *Phormidium* genera.

Several possible factors could determine the diversity pattern of cyanobacteria in the Khoor-e-Khooran wetland. In mangrove areas at Sao Paulo state, south-east Brazil, the first factor is the fluctuation of physicochemical conditions, including temperature, salinity, oxygen and light intensity (Alvarenga *et al.,* 2015). The second influencing factor is the urban and industrial wastewaters that flow up to the mangrove areas at south east coast of India (Balasubramanian *et al*., 2011). The ballast water of commercial cargo ships, which contained non-indigenous species might be another determining factor. Interestingly, an international sea route is passing near the Khoor-e-Khooran wetland.

Molecular identification of distinct isolates confirmed the morphological characterization of eight isolates, while the identities of two isolates were not confirmed. These results revealed that 14 isolates that we previously identified as cyanobacteria based on morphological properties did not belong to them. Inconsistency between morphological and molecular identification mainly because of their basic principles were reported in other studies. For instance the isolated cyanobacteria from the Sao Paulo were characterized as *Nostoc* and *Leptolyngbya* genera based on morphological properties, whereas molecular identification showed that these isolates were new taxa (Silva *et al.,* 2014). Comparison of 16S rRNA sequences of the cyanobacterial strains with closest strains in NCBI GenBank showed different homology ranged from 98 to 100 %. Mohamed and Al-Shehri (2015) also reported the high similarity of isolated cyanobacteria with their closest strain ranged from 67.6 to 97.7% (Mohamed and Al-Shehri, 2015). The phylogenetic analysis showed that *Microcystis* strains located in a different cluster and formed a different lineage during evolution. The *Microcystis* strains located in a same cluster with *Microcystis aeruginosa* SERB 2 that was isolated from the Andaman Island, India. Consequently, it could be hypothesized that the toxigenic *Microcystis* strains might be originated from non-indigenous species.

Detection of toxic genes showed that the microcystin/nodularine genes were existed in isolated cyanobacteria while cylindrospermopsin gene was not detected. Cyanotoxins genes also were investigated in other studies. For example Zare *et al.* (2015) detected the saxitoxin genes (sxtAF, sxtAR) in

isolated cyanobacteria from the Kor river. They proposed that molecular detection of toxins has been presented an alternative method for chemical analysis. Among the isolated strains, *Microcystis* sp. strain KH3 and *Microcystis* sp. strain KH4 exhibited high cytotoxic activity against *Artemia salina*, shrimp larvae and human cell lines while *Microcystis* sp. strain KH11 did not show any significant toxicity. The cytotoxicity value of the extracted metabolite from *Microcystis* sp. strain KH3 was recorded as 139.3, 231.3, 11.13 and 15.49 µg/mL against Artemia, shrimp larvae, HEPG2 and HUVEC cell lines respectively. These values were 225.8, 211.2, 13.19 and 12.51 µg/mL for the extracted metabolites from *Microcystis* sp. strain KH4. Mohamed and Al-Shehri (2015), reported that the extracted cyanotoxins from 9 cyanobacterial strains showed cytotoxicity against *Artemia salina* ranged from 0.3 to 5.1 mg/mL. Maruthanayagam *et al.* (2013) showed that the extracted cyanotoxins from *Geitlerinema* sp. CNP 1019 exhibited cytotoxic activity against MCF7 cell line with GI_{50} value equal to 25.7 µg/mL. Introduction of microcystin into food chains well studied in marine ecosystems. Studies showed that accumulation of microcystin in phytoplanktivorous fishes is 3.5 times more than zooplanktivorous fish. In addition, accumulation of microcystin in liver of *Osmerus eperlanus* was recorded as 874 µg/mL (Ibelings *et al.*, 2005). Filter-feeder coelenterates especially eatable ones such as oysters can also accumulate cyanotoxins and consequently be considered as potential danger to the consumer's health (Ferrão-Filho and Kozlowsky-Suzuki, 2011; Tamadoni Jahromi *et al.*, 2020). Fish cage-cultured farms also can be influenced from the cyanobacterial blooms in the Khoor-e-Khooran wetland. In conclusion, the existence of microcystins in Khoor-e-Khooran wetland, which is a major hatchery for shrimp's populations in the region, requires more monitoring efforts. These results also represented a new understanding of cyanobacterial diversity in the Khoor-e-Khooran mangrove wetland.

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