Molecular detection and phylogenetic analysis of Cyanobacteria species Case study (Libyan drinking water)

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Abstract

As the detection of toxins is vital in water quality management, a study was done by employing the molecular genetics approach to explore the genetic divergence, phylogenetic relationships and potential toxin-producing cyanobacteria. The molecular genetics approach employed in this study focuses on the analysis of cyanobacterial DNA strains isolated directly from water samples. Water samples were collected from MMR reservoirs in different locations, including municipal water supplies and private wells in south and west Tipoli. DNA were extracted directly for water samples and Polymerase Chain Reaction (PCR) was used to selectively amplify the 16S rRNA gene region. Primers specific to conserved regions of the 16S rRNA gene are used. We tested a set of oligonucleotide primers for the specific amplification of 16S rRNA gene segments. Six cyanobacteria-specific primers were designed. These primers bind successfully to the DNA and allow for selective amplification of the target region. The amplified DNA of PCR products was then subjected to DNA sequencing techniques. The obtained DNA sequences are then compared against a reference database containing known 16S rRNA gene sequences of cyanobacterial strains available in the Gene bank. The comparison performed using bioinformatics tools that align the obtained assembled 16S rRNA sequences were used both for a basic local alignment search tool (BLAST) analysis for genus-level identification and to generate a phylogenetic tree. Phylogenetic analysis involves comparing the sequences of different cyanobacterial strains to determine their evolutionary relatedness was carried out. The constructed phylogenetic tree provided insights into the relatedness of the isolated strains and their potential origins. Phylogenetic tree was inferred by NJ distance method the clusters are well supported by bootstrap analysis, phylogenies determined illustrate the evolutionary affiliations among 20 isolates, 16 isolates were classified as identified cyanobacteria. The results revealed the presence of diverse of non-toxic Cyanobacteria species in Libyan drinking water, with variations in abundance and distribution across different sampling sites and compared with several known toxin-producing Cyanobacteria genera, such as *Microcystis* ،*Nostoc* and Anabaena, were detected in Indonesian samples with more than 90% similarity irrespective of their toxicity which showed the suitability of the 16S rRNA gene for taxonomic orientation. A cluster of 10 strains from Indonesian water samples, tow strains of cyanobacteria, *Microcystis wesenbergii* VN451 and *Nostoc sp.* KVJF4 cluster were found associated with toxic bloom in Indonesian rivers and water reservoirs, that included hepatotoxic isolates identified as *Microcystis Wesenbergii* formed a monophyletic group. The *Microcystis* lineage was also distinct from the lineage containing the unicellular genus *Nostoc.* The secondary structure of a *Microcystis* 16s rRNA molecule was determined, and genus-specific sequence signatures were used to design primers that permitted identification of the potentially toxic cyanobacteria belonging to the toxic genus such as *Microcystis* and *Nostoc* via DNA amplification. The aim of this study was to detect the toxic cyanobacteria by using PCR and DNA sequences of 16srRNA supported by phylogenetic analysis and to ensure the safety of drinking water in Libya, it is imperative to regularly monitor Cyanobacteria populations and implement appropriate

mitigation strategies. The findings of this study contribute to the understanding of Cyanobacteria diversity in Libyan water sources and serve as a basis for formulating targeted approaches to manage and safeguard water quality. In summary, the study used 16S rRNA gene sequencing to analyze the microbial composition of drinking water samples from Libya and Indonesia. It found diverse non-toxic Cyanobacteria species in Libyan samples and detected toxin-producing Cyanobacteria in Indonesian samples. The high similarity in sequences, even for toxin-producing genera, demonstrated the utility of the 16S rRNA gene for taxonomic classification in microbial studies.

Introduction

The man-made river (MMR) is the primary of the water source in Libya and constantly the open reservoirs affected by different types of contamination including microorganism such as cyanobacteria. Cyanobacteria are photosynthetic microorganisms commonly found in various aquatic environments, including drinking water sources, while many species are harmless, some have the potential to produce harmful toxins, known as cyanotoxins. Toxic cyanobacteria metabolites, known as cyanotoxins, comprise a wide range of compounds, including cyclic peptides (microcystins, nodularin) and alkaloids (cylindrospermopsins, anatoxins, saxitoxins) that can be hepatotoxic, cytotoxic, genotoxic or neurotoxic., and can pose significant risks to human health and the environment (Brooks et al. 2016). In response to the risks associated with known cyanotoxins, the World Health Organization (WHO) has published guidelines for their monitoring and management of drinking water (Choru et al. 2021). In terms of their morphology and phylogenetics, Cyanobacteria are one of the most diverse groups of prokaryotes (Waterbury et al., 2006). Cyanobacteria are currently placed into five orders: *Chroococcales*, *Pleurocapsales*, *Oscillatoriales*, *Nostocales* and *Stigonematales* (Tomitani et al., 2006). The microcystin MCs belong to the most common cyanotoxins group found worldwide and are produced by multiple taxa (e.g., *Microcystis aeruginosa*, *Planktothrix* sp., *Anabaena*/*Dolichospermum*, *Os- cillatoria* and *Nostoc*).

Svirc˘ev et al., 2019). As a result, the detection and characterization of cyanobacterial species in drinking water have become crucial for ensuring the safety and quality of potable water supplies. Traditionally, cyanobacterial identification relied upon light microscopy to differentiate species and classify them according to their morphology have limitations in providing detailed information about the diversity and specific species present in water samples (Rippka et al.,1979). Due to their high biodiversity, however, many cyanobacteria have very similar morphology making them indistinguishable using microscopy techniques. Further, certain species cannot grow in laboratory conditions (uncultured cyanobacteria) (Ward et al., 1995) and therefore it is difficult to classify cyanobacteria into correct taxonomic groups in botanical nomenclature. As a solution, molecular techniques have emerged as powerful tools for the sensitive and specific detection of cyanobacteria and have revolutionized the field of microbial ecology and water quality assessment. Results from the monitoring of cyanobacterial blooms in lakes of Wallonia, Flanders and Brussels, Belgium, using LC-MS/MS, PCR and sequencing techniques, to assess the risks associated with recreational waters (Van Hassel et al., 2022). Nowadays, additional molecular analysis of the genes and internal transcribed spacer regions within the DNA is required for taxonomic identification (Komárek et al., 2014). A number of genetic markers genes have been utilized for phylogenetic analyses of cyanobacteria including 16S rRNA (Wu et al., 2011). The 16S rRNA gene sequence is the most widely applied strategy for assessing cyanobacterial biodiversity in nature (Ercolini et al., 2004). Because its existence in all bacteria, the 16S rRNA gene is a highly

conserved and universally present gene in prokaryotes, including cyanobacteria. the 16S rRNA gene is large enough for informatics purposes (Patel et al., 2001). An important characteristic that favors the use of the 16S rRNA gene in particular is the presence of multiple conserved/hypervariable regions that allow multiple options for PCR primer design (*Van de Peer* et al., 1996). A one-year monitoring study of Slovenian waterbodies using qPCR (mcyE, cyrJ, sxtA genes) and LC-MS/MS (microcystins, cylindrospermopsin, saxitoxin) was conducted by Zupancic et al. (*Zupanc˘ic* et al., 2021). Potentially toxic Microcystis and Planktothrix cells were detected by qPCR and microscopic analysis and a positive correlation between the numbers of mcyE gene copies and microcystin concentrations was observed. Cyanobacterial specific primers have been developed for the 16S rRNA gene (Valério et al., 2009). Specific primers designed to bind to conserved regions of the 16S rRNA gene enable the selective amplification of cyanobacterial DNA, even in the presence of other microbial populations. Several water microbiome studies based on 16S rRNA amplicon sequencing have described microbial composition, which differs according to the water source (e.g., river (Romero et al., 2021) lake [17] (Li et al., 2017), groundwater (Bruno et al., 2018) or seawater (Belila et al., 2016). To gain insights into the composition and diversity of cyanobacteria present in the drinking water samples, these PCR products undergo in-depth DNA sequencing analysis. High-throughput sequencing technologies, such as Illumina sequencing, allow for the simultaneous sequencing of numerous DNA fragments, providing a comprehensive view of the cyanobacterial community. The obtained 16S rRNA gene sequences provide valuable genetic information that can be used to determine the phylogenetic relationships between different cyanobacterial strains. Phylogenetic analysis involves the construction of evolutionary trees, which depict the relatedness and evolutionary history of the identified cyanobacterial species. Additionally, by comparing the sequences of toxin and non-toxin producing cyanobacteria, we can infer whether certain genetic markers are associated with toxin production, contributing to a better understanding of the potential health risks posed by specific cyanobacterial strains in drinking water. Molecular data have become a popular tool for phylogenetic analysis of cyanobacteria (Kulasooriya et al., 2011).

Phylogenetic analyses of cyanobacteria based on 16S rDNA were carried out by several groups (Garcia-Pichel et al., 2001), which indicated that *Chroococcales* (I) and *Oscillatoriales* (III) were polyphyletic. Other orders, *Nostocales* (IV) and *Stigonematales* (V), which form heterocysts, were shown to be monophyletic. Of the other orders, monophylicity was indicated for *Pleurocapsales* (II) which forms internal spores (baeocytes); however, only a few strains were phylogenetically analyzed. Thus, the present study was specifically focused on determining the cyanobacterial composition in different water reservoirs localities in west Libya with the partial sequences of the 16S rRNA gene, attempting to employ the 16S rRNA gene to explore phylogenetic relationships among cyanobacteria belonging to five orders of cyanobacteria isolated from different localities. In this paper, we identification of twenty cyanobacterial strains based on molecular genetics characterization using 16S rRNA gene sequencing, basic local alignment search tool (BLAST) analysis, and phylogenetic analysis. No studies so far have been conducted to identify the potential toxin producers cyanobactera in Libyan water bodies used for human consumption. By employing this comprehensive approach, we aim to gain a deeper understanding of the cyanobacterial community in Libyan drinking water sources, identify potential toxin-producing strains, and assess their phylogenetic relationships. This information is critical for devising appropriate management and mitigation strategies to safeguard the quality and safety of drinking water and protect public health

Material and methods

Site selection, sampling and cyanobacterial isolation:

(Table.1 and 2): Water samples were collected from the different reservoirs in the west of Libya (Table.1) and from Indonesian reservoirs (table 2).

Table.1

No of Samples Location	Source
Sample 1; Njila	Water direct from underground well
Sample 2; Tareeq Almatar	The sample from water storage tank, the source came from underground well
Sample 3; Janzour	The sample from water storage tank, the source came from underground well
Sample 4; Alserraj.	From the Man-Made River (MMR), Large reservoirs provide storage, and pumping stations control the flow into the cities as water public supply
Sample 5; Ghutshaal.	From the Man-Made River (MMR) Large reservoirs provide storage, and pumping stations control the flow into the cities.
Sample 6; Tajoura,	From the Man-Made River (MMR) Large reservoirs provide storage, and pumping stations control the flow into the cities.
Sample 7; Garapoli,	The sample from water storage tank, the source came from underground well
Sample 8; Qaser Bin Ghashir	From the Man-Made River (MMR) Large reservoirs provide storage, and pumping stations control the flow into the cities
Sample 9; Garyan,	Water taken from water storage tank water rain source
Sample 10; Sabratah	The sample was taken from water storage tank, the source came from underground well

Table 2

No of Samples Location	Source
Sample 11 Makassar city	From 8 m underground water well after the filtration
Sample 12 Makassar	From underground water well before the filter.
Sample 13 Makassar.	From water storage tank, Makassar
Sample 14 Makassar, public water supply	Water from tank (government filtration water):
Sample 15 near Batraya	Water from the river
Sample 16 (government filtration water)	Water from house before tank (government filtration water) :
Sample 17 UNHAS University	From river near UNHAS University
Sample 18 from the empty water after tank finish water and start come dirty from the tank and pipes	Sample 18 water after tank finish water and start come dirty from the tank and pipes (UNHAS University)
Sample 19 UNHAS University	Water of UNHAS University river
Sample 20 UNHAS University	Water UNHAS University river

Sampling was carried out in duplicates. Both surface and bottom (using a hand corer- Wildco 2424-B). Further, deep dug wells used as the source of potable water were selected in a random basis from the different area and water samples were collected into 250 mL brown glass bottles in duplicates. The collected water samples were concentrated using centrifugation using a Beckman-CP Centrifuge at 3500 rpm for 10 minutes, the resulting pellet was obtained. DNA extraction was carried out using Boom's method (Boom et al. in 1990. After DNA extraction. Five sets of primers were designed, used for PCR to detect the genetic material of 16s rRNA gene of cyanobacterial strains DNA.

Three sets of primers have been used for Libyan samples:

Primer 1: FP; CYA1F (5'CCAGCAGCCGCGGTAATACG-3')
RP; CYA1R (5' ATCGGCTACCTTGTTACGACTTC-3',
Primer 2: FR; CYA2F (1 GCGTGGGAACCTGCCTTTA19)
RP; CYA2R (1 TCGGAATTCCACTAACCTCTCC 22),
Primer 3: FR; CYA3F (1 GCATACGACCTGAGGGTGAAA21) and
RP; CYA3R (1 CCAGCCGTGCAGTCACAAAT 20).

Two sits of Primers for the Indonesian:

Primer 4: FP; CYA4F (1ATACGACCTGAGGGTGAAAGC21)
RP; CYA4R (1TCACCGGCAGTCCCATTAGA20)
Primer 5: FR; (CYA5F): (1GGAATTCCTGCAGCCCG17)
RP; (CYA5R): (1 GAGTCAGTGACCGAGGAAGC 20)

Polymerase Chain Reaction (PCR) was performed Purification of the amplified DNA was obtained. The purified DNA samples were subjected to DNA sequencing. Methods used for identifying the presence of cyanobacteria using the 16S rRNA gene region through PCR amplification and subsequent DNA sequencing. This process involved various steps, including PCR amplification, gel electrophoresis, gel extraction, DNA sequencing, and phylogenetic analysis. Amplification: The 16S rRNA gene region (approximately 991 bp) was amplified using PCR with modified protocols based on (Nübel et al. 1997). The resulting PCR products were separated using 1.5% agarose gels electrophoresis containing ethidium bromide using Lambda virus DNA/Hind III as Marker data not shown. Gel images were documented using a Gel Documentation system. Amplified fragments with the target DNA were excised from the gel and purified using a gel extraction kit with the gene Elute Gel Extraction Kit (SIGMA, USA) according to the manufacturer's instructions. The purified DNA fragments were sent to a commercial facility (Macrogen Inc., South Korea) for DNA sequencing using the DNA sequencing software programme BioEdit 7.0.9. The 16S rRNA PCR products were sequenced directly and DNA sequencing was performed on both strands with the Taq Dye Deoxy Terminator cycle-sequencing kit and an automated model 373A DNA sequencer (Applied Biosystems) according to the manufacturer's instructions.

Bioinformatics Analysis:

The obtained sequence data were analyzed using DNA sequencing software (Bio Edit 7.0.9) and compared against the NCBI database using BLAST (blastn) to verify the cyanobacterial origin. PCR products that showed reproducibility were selected for phylogenetic assessment. A neighbor-joining (NJ) analysis was performed using MEGA 4.0 analysis software to illustrate the relationship of the partial 16S rRNA gene among representative cyanobacteria. Accession Numbers: The nucleotide sequences from this study were deposited in GenBank under accession numbers.

Result

The results cover various aspects, including design of Primers, these primers targeted specific regions (V3, V6) of the 16S rRNA gene for PCR amplification. To ensure specific amplification of the 16S rRNA gene section, the target area for of the primers had to be exclusive for cyanobacteria. Cyanobacteria-specific primers separately targeting the V3 and V6 regions of the 16S rDNA, Fig.1.

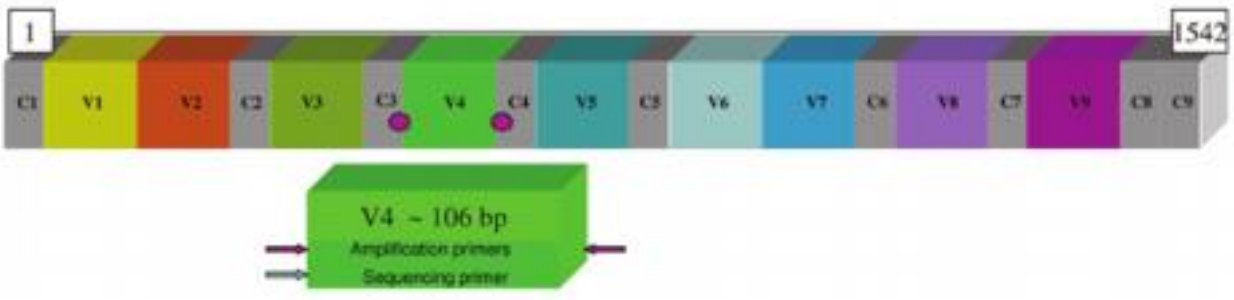


Figure 1. *Conserved and hypervariable regions in the 16S rRNA gene. The interspersed conserved regions (C1–C9) are shown in grey, and the hypervariable regions (V1–V9) are depicted in different colours. Also illustrated is an example of primer selection for DNA amplification and sequencing-based microbial identification (V4 sub region with pink circles and arrows representing primer-binding sites). Clinical Chemistry 55:5 (2009)*

PCR was used to amplify partial sections (ranging from 800 bp to 1.2 kb) of the cyanobacterial 16S rRNA gene extracted directly from water samples of Libyan and Indonesian locations yielding a PCR products**.**

In fig.2 the bands size were estimated, the GE analysis visualizes the bands that represented the water samples no. (1, 3 and 4). The strains in lane 1: *Starria zimbabweensis*, lane 2: no DNA detected, lane 3:, *Coleofasciculus sp*. SBK29 and lane 4: *Microcoleus sp*. *MMG-10*. The expected size of PCR fragment, 1, 3, 4 were 900 bp.

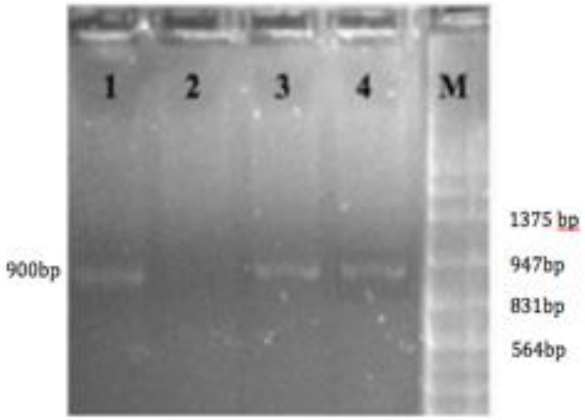


Fig 2. *Lane 1, Starria* Z (SAG74.90*; no 2 PCR product; Coleofasciculus sp.* SBK29*; no 4; Microcoleus sp. MMG-10Key to tracks: Lane: M. lamida 1kb DNA as molecular size marker.*

In Fig 3, the second primer CYA2F and CYA2R, specifically for Microcoleus sp. MMG-10 16S rRNA gene, partial sequence product length equal 478 bp. The sense forward primer (1GCATACGACCTGAGGGTGAAA 21) was taken from position 50 to 70 and the antisense reverse primer (5 CCAGCCGTGCAGTCACAAAT 3), was taken from position 527-508. PCR bands using template DNA represented the water samples no. 5 ,6, 7. and 8. Sample 5 was *Pseudanabaena sp. CAWBG530*, sample 6 was *Starria zimbabweensis SAG 74.90*, sample 7 was *Microcoleus sp*. MMG-10 and sample 8 was *Cyanothece sp*. MMG-1, (Lanes: 5, 6, 7, and 8). The expected size of PCR fragment, 5, 6, 7, 8 was 940.

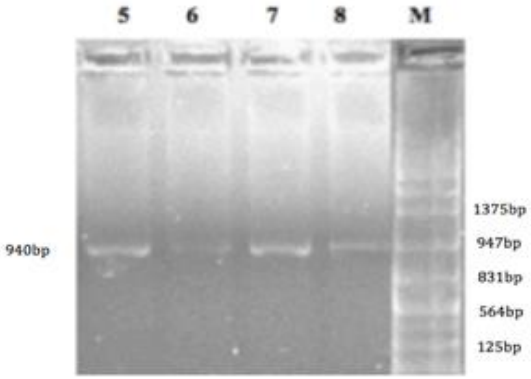


Fig 3. *Lanes: 5, Pseudanabaena sp. CAWBG530; 6, Starria zimbabweensis SAG 74.90 ; 8, Microcoleus sp. MMG-10; Key to tracks : Lane: M. λ ladder 1kb DNA as a molecular size marker*

In f**ig. 4 and 5** The third primer CYA3F and CYA3R of samples (9, 10, 15) specifically for the *Cyanothece sp*. MMG-11 16S ribosomal RNA gene, partial sequence product length equal 940bp. The sense forward primer (1ATACGACCTGAGGGTGAAAGC21) was taken from position 49 to 69 and the antisense reverse primer (5 TCACCGGCAGTCCCATTAGA 3) was taken from position 1032 to 1013. Represented the water samples no 9, 10 (Lane: 9, 10) and the expected size of PCR fragment 9, 10 (800bp).

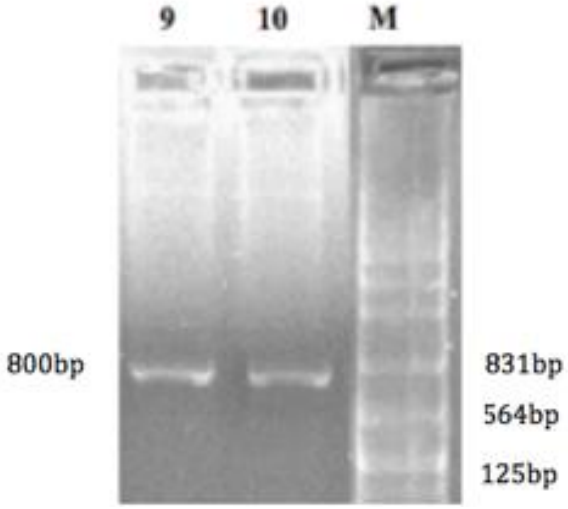


Figure *4. Lanes: 9, Cyanothece sp. MMG-11; 10, Cyanothece sp. MMG-1; Key to tracks :Lane: M. λ ladder 1kb DNA as a molecular size marker 1kb*

In general, the amplification yielded single band in different size approximately 900 a bp for CYA1F/R and 940 bp for CYA2A/R and 800bp for the CYA3F/R primers respectively, with the exception of only one strain not revealed the PCR products for 16s rRNA fig. 2, lane 2 the strain was isolated from TV reservoir.

Amplification of 16s rRNA gene by PCR of the Indonesian samples

Various of these primers established a PCR products DNA for each isolate from Indonesian samples. This analysis revealed unique band profiles among each of the different cyanobacterial genera examined, with the extent of this variation dependent on the primer combination used. The amplified PCR products ranged in size from approximately 800 to 1.2 kb. Fourth primer CYA5F and CYA5R, mainly designed for *Calothrix D253* genomic DNA (clone AG13) strain, product length equal 357. The sense forward primer (5 GGAATTCCTGCAGCCCG 3) was taken from position 57 to 73. 69 and the antisense reverse primer (GAGTCAGTGACCGAGGAAGC20) was taken from 394 to 395. In fig. 5 and 6 the amplified PCR products of 16S rDNA gene of selected cyanobacterial strain obtained from Indonesian samples: PCR amplification using forward primers CYA4F and reverse primer CYA4R used mainly for isolates *Calothrix D253* and *Cyanothece sp*. MMG-11. from samples number 11, 12, (Lane: 11, 12, and sample 14, 15, (Lane: 14, 15) respectively

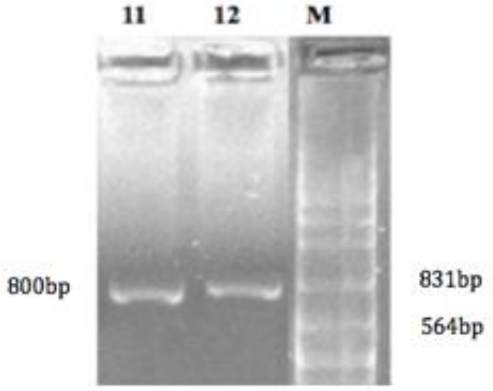


Figure 5. *Lanes: 11, Calothrix D253; 12, Calothrix D253. Lane: M. λ ladder 1kb DNA as a molecular size marker. The expected size of PCR fragment for 11 and 12 was 800bp).*

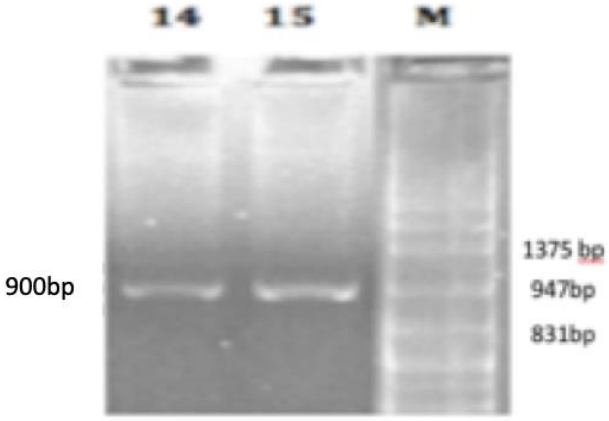


Figure 6. *Lanes: 14, Calothrix D253 ; 15, Cyanothece sp. MMG-11. Lane: M. λ ladder 1kb DNA as a molecular size marker. The expected size of PCR fragment for 11 and 12 was (900 bp).*

Sixth primer CYA6F and CYA6R for sample 18, specifically for *Microcystis wesenbergii* VN451 16S ribosomal RNA gene, partial sequence, product length equal 860. The sense forward primer (5 TAAGAATCTAACTTCAGGACGGGG 3) was taken from position 77 to 100 and the

antisense reverse primer:(5CCAGCTTTCACCAGGGTTCG3) was taken from position 936 to 91.7. The forward primer CYA5F and reverse primer CYA5R for cyanobacterial isolates number 18 and 19 was represented.

In fig. 7. PCR amplification bands using template DNA from cyanobacterial strains: *Microcystis wesenbergii* VN451 strain (Lane: 18), and *Cyanobium sp*. CENA138 (Lane: 19), The expected size of PCR fragment for 18 and 19 was (1200 bp) and 1000 pb respectively.

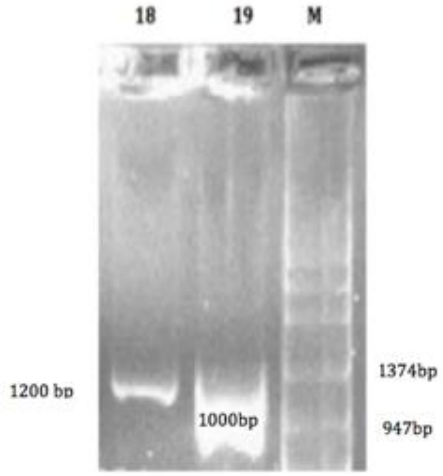
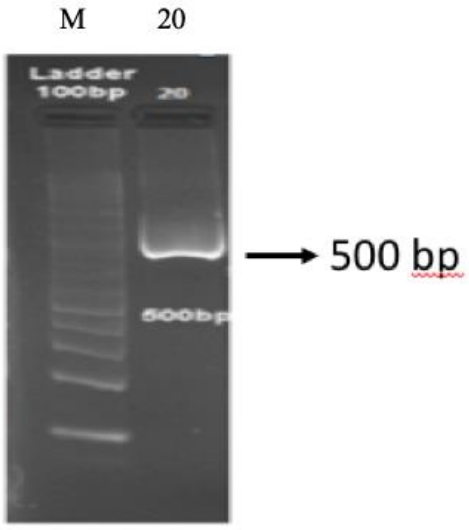


***Figure** 7. Lanes; 18, Microcystis wesenbergii VN451; 19; and Line 19, Cyanobium sp. CENA138 Lane: M. λ ladder 1kb DNA as a molecular size marker. Figure 4.9 Lanes: 20, Nostoc sp. KVJF4 16S ribosomal RNA gene,, partial sequence product length equal 898; amplicon size (500 bp)Lane: M. λ ladder 1kb DNA as a molecular size marker*

Finally in fig. 8 the sixth primer CYA7F and CYA7R for water sample 20 mainly for *Nostoc sp*. KVJF4 16S ribosomal RNA gene, partial sequence product length equal 898. The sense forward primer (5GTGGCGGACGGGTGAGTA 3) was taken from position 77 to 94, and the antisense reverse primer (5 TCCCGAAGG CACTCTTCCC 3) was taken from position 974 to 956.



***In fig. 8.** A PCR product of the expected size for 16S-rRNA amplicon (500 bp) was observed as indicated in lane 20*

16SrRNA gene segments selectively retrieved from cyanobacteria strains in non axenic cultures and directly sequenced. The number of PCR products and their sizes and relative intensities varied among the different genera. For all strains that were tested, 16SrRNA amplification yielded one band of different size: 500, 800, 940, and 1200bp. 16S primers successfully amplified DNA from all the strains that were tested, yielding for most genera PCR products ranging between 500 to 1200 bp. The GE gel electrophoresis demonstrate the possibility of separating PCR products obtained with the primer set targeted to 16S by using DNA from 16 selected cyanobacterial strains. The 16S rRNA PCR product of approximately ranged from 500 bp to 1.2 kp gave rise to one band in the GE gel occupying different positions for all strains. We have found that the primer CYAF and CYAR is very cyanobacterial specific, specially the primer CYA5F and CYA6R with Microcystis and Nostoc strains, preferentially amplifying cyanobacterial DNA, even in the presence of contaminating eubacterial DNA.

PCR products Sequences of Libyan water samples

Both strands of the PCR product from all isolates as templates were sequenced directly using the Applied Biosystems 373A automated sequencer. The sequencing was carried out for the 16 isolates with partial 16S rRNA gene. Sequences were determined by using the same primers as those used for PCR amplification of isolates. We adjusted the PCR conditions to optimize reactions, and our primer(s) are appropriately designed for automated sequencing, they have a Tm between 55 and 75. The primers enabled us to sequence both strands of the 16s rRNA gene with contiguous overlaps, both strands of the gene were sequenced in duplicate. Sequence data, therefore, can be generated without time-consuming molecular cloning procedures.

Data obtained from the Sequence 16S rRNA region of our isolates

To investigate the possibility that 16s rRNA gene existed in the genomes of at least some of our cyanobacterial species. 16 individual isolates were selected for automated sequencing. We sequenced the 16s rRNA regions from two isolates of the strains (SAG 74.90) and one isolate (SBK 29), two of MMG10_strain one of CAWBG530, four MMG-11, three D253, VN451and one CENA138 and KVJF4. This study presents a genetic characterization of 9 nontoxic cyanobacterial strains isolated from10 reservoirs located around in the Tripoli city. These strains belonged mainly to; 1. *Starria zimbabweensis SAG 74.90*; 3. *Uncultured Coleofasciculus sp*. *clone SBK29;* 4. *Microcoleus sp. MMG-10;* 5. *Pseudanabaena sp. CAWBG530;* 6. *Starria zimbabweensis SAG 74.90;* 7. *Microcoleus sp. MMG-10;* 8. *Cyanothece sp. MMG-11;* 9. *Cyanothece sp. MMG-11*; 10. *Cyanothece sp. MMG-11* as shown in Table 4.1. In fig 9. the partial nucleotide sequence of the PCR products generated from the 16s rRNA gene chromosomal DNA of the cyanobacterial *Starria zimbabweensis* SAG 74.90 isolates. And *Microcoleus sp*. MMG-10; *Cyanothece sp*. MMG 11; *Cyanothece sp*. MMG-11; *Cyanothece sp*. MMG-11 as data not shown.

Sample 6. Starria zimbabweensis SAG 74.90 16S ribosomal RNA gene, partial sequence

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4 GGTAGCNTGCCTCTCTTGCGATTTACCGCAAACCCTTGGGGAGAACCCAACTCCCGTGGTGT
GAGGGGCGGTGTGCAAAACGCGGGAAAACGTATTCCCCGGGGCGTGCTGCCCCGATTTTACGAG
AGTTTCCCCTCTCGTGCTCTAGATGTGCACAGAACAACCCGAAATGATAGGGTTTTTGGGGATT
AGCTCCTCCCCGGGGATGGGCTGCCCACTGTCCCCGCCATTGTAGACCGTGTGTACCCCACCCT
GAAAGGCCCATGAGAACTTGACTTCTTCCCCACTTCCCTCCGGTTTATCACCGGAGGTTTCCTT
AAAGTGCCCAACTGAATGATGGCAACTAAGGACGAGGGTTGCTCTCGTTGCGGGACTTAACCCA
CCATCTCACGACACGAGCTGACCACAGCCATGCAGCACCTGTCACCGCTTTCTCGAAAGGAACC
TTCCATCTCTGGAAACAGCCCGGGATGTCAAAGGCTGGTAAGGTTCTGCGCGTTGCATCTAATT
AAACCACATGCTCCACCGCTTGTGCAGGCCCCCGTCAATTCCTTTGAGTTTTAATCTTGCGACC
GTACTCCCCAGGCGGATAACTTAATGCGTTAGCTGCGCCACCCAAGAACCAAATGCCCGGACAG
CTAGTTATCTTCGTTTACGGGGTGNACTACCAGGTATCTAATCCTGTTTGCTCCCCACCCTTTC
NCACCTCANGTCTATACTTGTGCCAGTGAGTCTCCCTTCGCCACTGGGGTTCNTCTCGAATATC
TACGAATTTCACCTCTACACTCGNAAATCCACCTGACCTCTACCTANATCCANCNATCCAANTC
NAAAGGCGNTTCCGGGGTTGAAGCCCCGGG  864
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Fig. 9*. cyanobacterial Starria zimbabweensis SAG 74.90*

In fig. 10 and 11 the 16S rRNA gene of all bacteria in a sample is amplified in a PCR reaction. Direct sequence of the PCR fragment of the Indonesian water samples The 16s rRNA gene PCR products of *Microcystis wesenbergii* and *Nostoc* partially sequenced for both strands of the 16s rDNA and generated for the region from position 63 to position 963 for one Microcystis strains representing *M. wesenbergii* sample no. 18, 20] Other isolates, *Calothrix* D253 genomic DNA (clone AG13); *Calothrix* D253 genomic DNA (clone AG13); *Calothrix* D253 genomic DNA (clone AG13); *Cyanothece sp.* MMG-11; data not shown.

Sample 18. Microcystis wesenbergii VN451 gene for 16S ribosomal RNA, partial sequence

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63 CTTCTATGATGTGACGGGCGGTGTGTGCAAGGCCCGGGAACGTATTATCGCTGCTGGTGC
TGACCGGCGATTACTAGCGATTCCTCCTTCATGCAGGCGAGTTGCAGCCTGCAATCTGAACTG
AGGCCGGGTTTGCTGGGATTCGCTGGCTCTCGCGAGTTCGCTGCCCTTTGTCCCGACCATTGT
AGTACGTGTGTCGCCCAAGACGTAAGGGGCATGCTGACTTGACGTCATCCCCACCTTCCTCCG
GTTTGTCACCGGCAGTCTCCTTAGAGTCCCCAACTTAATGCTGGCAACTAAGAACGAGGGTTG
CGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCACCACCT
GTGTTCGCGCTCCCGAAGGCACCCCCAGCTTTCACCAGGGTTCGCGACATGTCAAGTCTTGGT
AAGGTTCTTCGCGTTGCATCGAATTAAACCACATACTCCACCGCTTGTGCGGGCCCCCGTCAA
TTCCTTTGAGTTTCACACTTGCGTGCGTACTCCCCAGGCGGGATACTTAACGCGTTAGCTTCG
GCACGGCTCGGGTCGATACAAGCCACGCCTAGTATCCATCGTTTACGGCTAGGACTACAGGGG
TATCTAATCCCTTTCGCTCCCCTAGCTTTCGTCCCTGAGTGTCAGATACAGCCCAGTAGCACG
CTTTCGCCACCGATGTTCTTCCCAATCTCTACGCATTTCACCGCTACACTGGGAATTCCTGCT
ACCCCTACTGCTCTCTAGTCTGCCAGTTTCCACCGCCTTTAGGTCGTTAAGCAACCTGATTTG
ACGACAGACTTGGCTGACCACCTGCGGACGCTTTACGCCCAATAATTCCGGATAACGCTTGCC
TCCCCCGTATACGGGCTGCTGG  963
```

Fig. 10. cyanobacterial *Microcystis wesenbergii*

Sample 20. Nostoc sp. KVJF4 16S ribosomal RNA gene, partial sequence

273 GGCGATGATGACTTCTCGTCATccccccTTCTTCCTCCTTTTCGCCGCCGGTCGCCTC
CTAATACCCCCCAATTTAATGCTGGCAAATAGGAAGGAGTGTTGCCCTCGCTGAGGGAATTAT
CCCAACATCTCACGAGACGAGCTGACGACAGCCATGGACCACCTGTGCTCGCGCTCGCGAAGG
CACCCCTATCTTTCATAGGGTTCTCGACATGTCAAGTCATGATAAGGTTCTTCGCGTTGCATC
GAATAAAAACATACTCTACCGCTTGTGCGCGCCCCCGCCTTTCCTTTGAGTTCACACTTGCAT
GCGTACTCCCGAGGAGTATACTTATCGCGTATCTCCGCACAGCTCGTGTCGATACACCCACGC
CTATATCATCGTTTACGGCTAGACTACCGGGTATCTATCTCTTCGCTCCCCAGCTTCGCCCTG
AGTGCAG 717

***Figure** 11 The partial nucleotide sequence of the PCR products generated from the 16s rRNA gene chromosomal DNA of the cyanobacterial of Nostoc sp. KVJF4 isolates. A*

BLAST search performed on these sequences revealed that the retrieved bands were all derived from cyanobacteria of various genera, including *Microcystis*, and *Nostoc.* Partial sequences for both strands of the 16s rDNA were generated for the region from position 63 to position 963 for *Microcystis* strains representing *M. wesenbergii.* We found striking variability in the sequencing of the 16s RNA region among the various species examined, the sizes of the individual 16S rRNA regions often differed tremendously. For example, while all species carry at least one copy of the 16S rRNA region, the size of the entire 16S region for the PCR products is 1200 bp, the number of the nucleotide sequencing of the partial sequencing of the isolates PCR products were not the same. in the other hand the size of the PCR products in the Indonesian strains were range between 13 bp and 963bp.

***Table 3** cyanobacterial strains isolated from10 reservoirs located around in the Tripoli city.*

Cyanobacteria strains	Resirevoure	Accession no	Amplicon size
Starria zimbabweensis SAG	Njila, water direct from underground well	gb|KM020013.1	18-606 bp
Uncultured Coleofasciculus sp. clone SBK29	Janzour; the sample from water storage tank, the source came from underground well	gb|KM108654.1|	30-56 6bp
Microcoleus sp. MMG-10	Alserraj; from the Man-Made River (MMR), Large reservoirs provide storage, and pumping	gb|KF157400.1|	26-568 bp
Pseudanabaena sp. CAWBG530	Ghutshaal, from the Man-Made River (MMR) Large reservoirs provide storage, and pumping stations control the flow into the cities.	gb|JX088101.1|	21-793 bp
Starria zimbabweensis SAG 74.90	Tajoura, from the Man-Made River (MMR) Large reservoirs provide storage, and pumping stations control the flow into the cities	gb|KM020013.1|	4-864 bp
Microcoleus sp. MMG-10	Garapoli, the sample from water storage tank, the source came from underground well	gb|KF157400.1	214-441bp
Cyanothece sp. MMG-11	Qaser Bin Ghashir, from the Man-Made River (MMR) Large reservoirs provide storage, and pumping stations control the flow into the cities.	gb|KF157401.1	4-710 bp
Cyanothece sp. MMG-11	Garyan ;home water storage tank from water rain	gb|KF157401.1	20-622 bp
Cyanothece sp. MMG-11	Sabratah , the sample from water storage tank, the source came from underground well	gb|KF157401.1	22-426 bp

This Indonesian study presents a genetic characterization of 7 nontoxic cyanobacterial strains isolated from 6 reservoirs located around in the Makassar city. These strains belonged mainly *Calothrix D253*, *Cyanothece sp. MMG-11 M. wesenbergii VN451 Cyanobium sp. CENA138 Nostoc sp. KVJF4 16S*, Table 4

Table 4 *cyanobacterial strains isolated from 6 reservoirs located around in the Makassar city*

Cyanobacteria strains	**Reservoir**	**Accession no**	**Amplicon size**
Calothrix D253	Sample 11. from 8m water well in our house in Makassar after the filter	emb|Z47172.1|	89-360 bp
Calothrix D253	Sample 12 from water well in our house in Makassar before the filter.	emb|Z47172.1|	107-394 bp
Calothrix D253	Sample 14 water after tank from the house (government filtration water):	emb|Z47172.1|	121-327 bp
Cyanothece sp. MMG-11	Sample 15 water from the river near (near batu raya):	gb|KF157401.1|	24- 563 bp
Microcystis wesenbergii VN451	Sample 18; water after tank finish water and start come dirty from the tank and pipes (UNHAS University)	dbj|AB666079.1|	63-963 bp
Cyanobium sp. CENA138	Sample 19; water of UNHAS University river	gb|KC695832.1|	13-854 bp
Nostoc sp. KVJF4 16S	Sample 20water UNHAS University river	|gb|EU022710.1|	273-717 bp

As we can see from the results of DNA sequences of PCR products that there was a difference in the size of the fragments obtained from the Indonesian strains, the largest size was from *M. wesenbergii* VN451 strain (963bp) and the smallest size from *Cyanothece sp*. MMG-11 was (111) and DNA sequences of the PCR products of the Libyan strains can be observed by comparing the alignment of the PCR products and the sequences in data base. The sequences size of the PCR fragments was from 144 bp to 375 bp, in the other hand the size of the PCR products in the Indonesian strains were range from the result, the direct sequence of this PCR fragment exhibits a very high level of identity. However, to investigate this further, it would be necessary to clone the PCR fragment and sequence this clone. Due to time limitation sequencing of the cloned PCR products was not undertaken.

Sequence Alignment and Investigation.

The PCR products sequences were analyzed by using the BLAST program for alignment with data base sequences to ensure that the sequences generated were cyanobacterial in origin. We used alignments of all available 16S rRNA sequences from cyanobacteria GenBank data bases. The alignments were searched upstream, starting from the 3′ end of the 16S rRNA gene, which resulted in the identification of nucleotide sequence that highly conserved in all phylogenetic groups of

cyanobacteria from which sequences were available. These alignments, containing most available cyanobacterial sequences, and an alignment of 16 strains
representative cyanobacterial sequences in were used to search for potential primer sites in the 16S rRNA gene. The 16s rRNA gene sequences determined were compared to each other, and to previously published almost complete 16s rRNA gene sequences for cyanobacteria and related organisms. These aligned sequences formed the basis for successive phylogenetic inferences.

DNA sequencing Alignment of Libyan sample

Databases showed that virtually all cyanobacteria had at least one operon containing a perfect match with the forward and reverse primer, which is targeted to the highly conserved 16s RNA gene. In fig. 12. Showed first sample, we aligned the 16s rRNA gene primary structures of the cyanobacterial strains described above from Libyan isolates such as *Starria zimbabweensis*, typical DNA sequence alignment and similarity analysis of the sequenced gene segment (458 bp) from Sample 6 *Starria zimbabweensis* SAG 74.9. Tajoura, (MMR). In fig 9. the partial nucleotide sequence alignment of the PCR products generated from the 16s rRNA gene chromosomal DNA of the isolates Microcoleus sp. MMG-10 A typical DNA sequence alignment and similarity analysis of the sequenced 16s rRNA gene segment (274 bp) Data not shown; Cyanothece sp. MMG 11; *Cyanothece sp.* MMG-11; Cyanothece sp. MMG-1 sequence alignment and similarity analysis of the sequenced 16s rRNA gene segment (296 bp), *Pseudanabaena sp. CAWBG530* from Ghutshaal, (MMR) reservoirs. A typical DNA sequence alignment and similarity analysis of the sequenced 16s rRNA gene segment (375 bp), data not shown

Score	Expect	Identities	Gaps	Strand
846bits(458)	0.0	734/872(84%)	21/872(2%)	Plus/Minus

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Query  4     GGTAGCNTGCCTCTCTTGCGATTTACCGCAAACCCTTGGGGAGAACCCAACTCCCGTGGT  63
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Sbjct  1388  GGTCGCCTGCCTCTCTTGCGAGTTAGCGCAACGCCTTCGGGTGAACCCAACTCCCATGGT  1329

Query  64    GTGAGGGGCGGTGTGCAAAACGCGGGAAAACGTATTCCCCGGGGCGTGCTGCCCCGATTT  123
             |||| |||||||||| | || ||  |  ||||||||| ||| ||| |||||  |||   |
Sbjct  1328  GTGACGGGCGGTGTGTACAAGGCCTGGGAACGTATTCACCGCGGCATGCTGATCCGCGAT  1269

Query  124   TACGAGAGTTTCC-CCTCTCGTGCTCTAGATGTGCACAGAACAACCCGAAATGATAGGGT  182
             ||| || | |||| ||| || ||| || ||  |||| || |||| ||||| ||| | ||
Sbjct  1268  TACTAGCGATTCCGCCT-TCATGCCCTCGAGTTGCAGAGGACAATCCGAACTGAGACGGC  1210

Query  183   TTTTGGGGATTAGCTCCTCCCCGGGGA-TGGGCTGCCCACTGTCCCCGCCATTGTAGACC  241
             |||||| |||||||||  |||  | || |  |||||||||||| ||||||||||  |
Sbjct  1209  TTTTGGAGATTAGCTC-ACCCTTGCGAGTTTGCTGCCCACTGTCACCGCCATTGTAGCAC  1151

Query  242   GTGTGTACCCCACCCTGAAAGGCCCATGAGAACTTGACTTCTTCCCCACTTCCCTCCGGT  301
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Sbjct  1150  GTGTGTAGCCCAGCCTGTAAGGGCCATGAGGACTTGACGTCATCCCCACCTTCCTCCGGC  1091

Query  302   TTATCACCGGAGGTTTCCTTAAAGTGCCCAACTGAATGATGGCAACTAAGGACGAGGGTT  361
             ||||||||||  |||||||||||||||||||| ||||||||||||||||||||||||||
Sbjct  1090  TTATCACCGGCAGTTTCCTTAAAGTGCCCAACTTAATGATGGCAACTAAGGACGAGGGTT  1031

Query  362   GCTCTCGTTGCGGGACTTAACCCACCATCTCACGACACGAGCTGACCACAGCCATGCAGC  421
             || ||||||||||||||||||||| ||||||||||||||||||||| ||||||||||||
Sbjct  1030  GCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCAGC  971

Query  422   ACCTGTCAC-CG-CTTTCTCGAAAGGAA-CCTTCCATCTCTGGAA-AC-AG-CCCGGGAT  475
             |||||||||  | |   | ||||  |||       ||||||  ||  |  | ||| ||||
Sbjct  970   ACCTGTCACTGGTCCAGC-CGAACTGAAGGAAAAGATCTCTCTAATCCGCGACCCAGGAT  912

Query  476   GTCAAAGGCTGGTAAGGTTCTGCGCGTTGCATCTAATTAAACCACATGCTCCACCGCTTG  535
             ||||||||||||||||||||||||||||||| || ||||||||||||||||||||||||||
Sbjct  911   GTCAAAGGCTGGTAAGGTTCTGCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTG  852

Query  536   TGCAGGCCCCCGTCAATTCCTTTGAGTTTTAATCTTGCGACCGTACTCCCCAGGCGGATA  595
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  851   TGCAGGCCCCCGTCAATTCCTTTGAGTTTTAATCTTGCGACCGTACTCCCCAGGCGGATA  792

Query  596   ACTTAATGCGTTAGCTGCGCCACCCAAGAACCAAATGCCCGGACAGCTAGTTATCTTCGT  655
             |||||||||||||||||||||||||||||  |||    |||||||||||||||||| ||||
Sbjct  791   ACTTAATGCGTTAGCTGCGCCACCCAAGTTCCATGAACCCGGACAGCTAGTTATCATCGT  732

Query  656   TTACGGGGTGNACTACCAGG-TATCTAATCCTGTTTGCTCCCCACCCTTTCNCACCTCAN  714
             |||||| ||| ||||||||| ||||||||||||||||||||||||||| ||||| |||||||
Sbjct  731   TTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTCAG  672

Query  715   -GTCTATACTTGTGCCAGTGAGTCTCCCTTCGCCACTGGGGTTCNTCTCGAATATCTACG  773
              ||| |||| ||| ||||| ||||  |||||||||||||| |||| || ||||||||||||
Sbjct  671   CGTCAATACCTGT-CCAGTTAGTCG-CCTTCGCCACTGGTGTTCTTC-CGAATATCTACG  615

Query  774   AATTTCACCTCTACACTCGNAAATCCACCTGACCTCTACCTANATCC-ANCNATCCAANT  832
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Sbjct  614   AATTTCACCTCTACACTCGGAATTCCAC-TAACCTCT-CCAGGATTCTAGCAATCTAGTT  557

Query  833   TCNAAAGGCGNTTCCGGGGTTGAAGCCCCGGG  864
             || ||||||  |||||||||||| ||||||||
Sbjct  556   TC-AAAGGCAGTTCCGGGGTTGA-GCCCCGGG  527
```

Figure 12 *16S ribosomal RNA gene of Starria zimbabweensis SAG 74.90, partial sequence Sequence ID: gb: KM020013.1 Length: 1462 Number of Matches: 1 Related Information Range 1: 527 to 1388*

DNA sequencing Alignment of Indonesian Samples

The BLAST results the homologous sequences found in Desmonostoc CENA386 than to those found in other cyanobacteria genera fig. (13) Only recently increasing evidence on the worldwide abundance of *Nostoc sp*. as a MC-producing organism has been reported. In Fig. 12. The BLAST results showed that the sample 18 Microcystis wesenbergii *VN451*, from the UNHAS University. A typical DNA sequence alignment and similarity analysis of the sequenced 16s rRNA gene segment (863 bp). *Microcystis wesenbergii VN451* was genetically more similar to the homologous sequences found in Microcystis aeruglhosa than to those found in other cyanobacteria genera. Sample 11 *Calothrix D253* from the river near (near baturaya).

Score	Expect	Identities	Gaps	Strand
1594 bits(863)	0.0	892/905(99%)	6/905(0%)	Plus/Minus

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Query  63    CTTCTATGATGTGACGGGCGGTGTGTGCAAGGCCCGGGAACGTATT-ATCGCTGCTGGTG  121
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Sbjct  1334  CTTCCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGAATTCACCGCCG-T-ATG  1277

Query  122   CTGACCGGCGATTACTAGCGATTCCTCCTTCATGCAGGCGAGTTGCAGCCTGCAATCTGA  181
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1276  CTGACCGGCGATTACTAGCGATTCCTCCTTCATGCAGGCGAGTTGCAGCCTGCAATCTGA  1217

Query  182   ACTGAGGCCGGGTTTGCTGGGATTCGCTGGCTCTCGCGAGTTCGCTGCCCTTTGTCCCGA  241
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1216  ACTGAGGCCGGGTTTGCTGGGATTCGCTGGCTCTCGCGAGTTCGCTGCCCTTTGTCCCGA  1157

Query  242   CCATTGTAGTACGTGTGTCGCCCAAGACGTAAGGGGCATGCTGACTTGACGTCATCCCCA  301
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1156  CCATTGTAGTACGTGTGTCGCCCAAGACGTAAGGGGCATGCTGACTTGACGTCATCCCCA  1097

Query  302   CCTTCCTCCGGTTTGTCACCGGCAGTCTCCTTAGAGTCCCCAACTTAATGCTGGCAACTA  361
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1096  CCTTCCTCCGGTTTGTCACCGGCAGTCTCCTTAGAGTCCCCAACTTAATGCTGGCAACTA  1037

Query  362   AGAACGAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGA  421
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1036  AGAACGAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGA  977

Query  422   CAGCCATGCACCACCTGTGTTCGCGCTCCCGAAGGCACCCCCAGCTTTCACCAGGGTTCG  481
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Sbjct  976   CAGCCATGCACCACCTGTGTTCGCGCTCCCGAAGGCACCCCCAGCTTTCACCAGGGTTCG  917

Query  482   CGACATGTCAAGTCTTGGTAAGGTTCTTCGCGTTGCATCGAATTAAACCACATACTCCAC  541
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Sbjct  916   CGACATGTCAAGTCTTGGTAAGGTTCTTCGCGTTGCATCGAATTAAACCACATACTCCAC  857

Query  542   CGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCACACTTGCGTGCGTACTCCCCAGG  601
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Sbjct  856   CGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCACACTTGCGTGCGTACTCCCCAGG  797

Query  602   CGGGATACTTAACGCGTTAGCTTCGGCACGGCTCGGGTCGATACAAGCCACGCCTAGTAT  661
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Sbjct  796   CGGGATACTTAACGCGTTAGCTTCGGCACGGCTCGGGTCGATACAAGCCACGCCTAGTAT  737

Query  662   CCATCGTTTACGGCTAGGACTACAGGGGTATCTAATCCCTTTCGCTCCCCTAGCTTTCGT  721
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  736   CCATCGTTTACGGCTAGGACTACAGGGGTATCTAATCCCTTTCGCTCCCCTAGCTTTCGT  677

Query  722   CCCTGAGTGTCAGATACAGCCCAGTAGCACGCTTTCGCCACCGATGTTCTTCCCAATCTC  781
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  676   CCCTGAGTGTCAGATACAGCCCAGTAGCACGCTTTCGCCACCGATGTTCTTCCCAATCTC  617

Query  782   TACGCATTTCACCGCTACACTGGGAATTCCTGCTACCCCTACTGCTCTCTAGTCTGCCAG  841
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  616   TACGCATTTCACCGCTACACTGGGAATTCCTGCTACCCCTACTGCTCTCTAGTCTGCCAG  557

Query  842   TTTCCACCGCCTTTAGGTCGTTAAGCAACCTGATTTGACGACAGACTTGGCTGACCACCT  901
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  556   TTTCCACCGCCTTTAGGTCGTTAAGCAACCTGATTTGACGACAGACTTGGCTGACCACCT  497

Query  902   GCGGACGCTTTACGCCCAATAATTCCGGATAACGCTTGCCTCCCCCGTAT-AC-G-GGCT  958
             |||||||||||||||||||||||||||||||||||||||||||||||||| || | ||||
Sbjct  496   GCGGACGCTTTACGCCCAATAATTCCGGATAACGCTTGCCTCCCCCGTATTACCGCGGCT  437

Query  959   GCTGG  963 0925003283
             |||||
Sbjct  436   GCTGG  432
```

Figure 13 *gene for 16S ribosomal RNA of Microcystis wesenbergii VN451, partial sequence Sequence ID: dbj\|AB666079.1\|Length: 1459Number of Matches: 1 Related Information Range 1: 432 to 1334 GenBankGraphics Next Match Previous Match.*

Sample 19 *Cyanobium sp. CENA138* from the water of UNHAS University river . A typical DNA sequence alignment and similarity analysis of the sequenced 16s rRNA gene segment (337 bp) data not shown. In fig. 14. Sample 20 *Nostoc sp. KVJF4*. from the water UNHAS University river. A typical DNA sequence alignment and similarity analysis of the sequenced16s rRNA gene segment (197 bp).

Score	Expect	Identities	Gaps	Strand
364 bits(197)	6e-99	379/464(82%)	24/464(5%)	Plus/Minus

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Query  273   GGCGATGATGACTTCTCGTCATccccccTTCTTCCTCCTTTTCGCCGCCGGTCGCCTCC  332
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Sbjct  1146  GGC-ATGCTGACTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTC-TCT-C  1090

Query  333   TAATACCCCCCAATTTAATGCTGGCAAATAGGAAGGAGTGTTGCCCTCGCTGAGGGAATT  392
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Sbjct  1089  T-AGAGTGCCCAACTTAATGCTGGCAACTAAAAACGAGGGTTGCGCTCGTTGCGGGAATT  1031

Query  393   ATCCCAACATCTCACGAGACGAGCTGACGACAGCCATGGACCACCTGTGCTCGCGCTCGC  452
             | ||||||||||||||| ||||||||||||||||||||||||||||||| ||||||||
Sbjct  1030  AACCCAACATCTCACGACACGAGCTGACGACAGCCATGGACCACCTGTGTTCGCGCTCCC  971

Query  453   GAAGGCACCCCTATCTTTCATAGG-G-TTCTCGACATGTCAAGTCATGATAAGGTTCTTC  510
             |||||||| | |  |||||| ||  | ||| |||||||||||| | || ||||||||||
Sbjct  970   GAAGGCACTCTTCCCTTTCA-AGAAGATTCGCGACATGTCAAGCCTTGGTAAGGTTCTTC  912

Query  511   GCGTTGCATCGAATAAAAC-ACATACTCTACCGCTTGTGCGCGCCCCCGCCT-TTCCTTT  568
             ||||||||||||||| |||| |||||||| |||||||||||| ||||||  |  |||||||
Sbjct  911   GCGTTGCATCGAATTAAACCACATACTCCACCGCTTGTGCGGGCCCCCCTCAATTCCTTT  852

Query  569   GAGTT-CACACTTGCATGCGTACTCCCGAGGAGT-ATACTTATCGCGT-ATCTCC-GCAC  624
             ||||| |||  ||||  |||||||||| ||| |  ||||||| ||||| | || | ||||
Sbjct  851   GAGTTTCACCGTTGCCGGCGTACTCCCCAGGCGGGATACTTAACGCGTTAGCTACGGCAC  792

Query  625   AGCTCGTGTCGATACAC-CCACGCCTA-TAT-CATCGTTTACGGCTAG-ACTACCGGG-T  679
              ||||| |||||||||  ||||||||| ||| ||||||||||||||| ||||| ||| |
Sbjct  791   GGCTCGGGTCGATACAAGCCACGCCTAGTATCCATCGTTTACGGCTAGGACTACTGGGGT  732

Query  680   ATCTA-TCTC-TTCGCTCCCC-AGCTT-CG-CCCTGAGTG-CAG  717
             ||||| || | |||||||||| ||||| || |||| |||| |||
Sbjct  731   ATCTAATCCCATTCGCTCCCCTAGCTTTCGTCCCTCAGTGTCAG  688
```

Figure 14 *16S ribosomal RNA gene of Nostoc sp. KVJF4, partial sequence Sequence ID: gb|EU022710.1|Length: 1481Number of Matches:1 Related Information Range 1: 688 to 1146GenBankGraphics Next Match Previous Match*

Phylogenetic analysis of the Cyanobacteria strains based on 16S rRNA gene sequences

To better understand the diversity of the cyanobacteria strains in drinking water supply collection, a phylogenetic analysis was carried out with the objective of providing an overview of the phylum cyanobacteria, using PCR products sequence amplified from purified chromosomal DNA, to confirm the presence of a nucleotide sequence in the cyanobacterial chromosomal DNA of these strains homologous to sequences of the 16s rRNA gene. The phylogenetic neighbour-joining tree generated from 16S rRNA sequences of the investigated strains revealed that the studied strains formed clearly defined clusters grouped according to the different cyanobacterial species. To elucidate the phylogenetic relationship between cyanobacterial strain in Libyan and various other morph-species described in Indonesia, a phylogenetic tree was constructed based on the alignment of the sequences of PCR products. Libyans cyanobacterial strains were classified into different

clusters based on their genetic similarities, *Pleurocapsales, Oscillatoriales*, *chroococcales* and *Synechococcales*. The tree is a sub-clade from a maximum likelihood analysis of all cyanobacterial genomes available from NCBI. After ambiguous characteristics were removed from the alignment, we using different methods of analysis, such as maximum parsimony and genetic distance metrics to obtained several phylogenetic trees. The input order of sequences into the phylogeny programs and the selection of outgroups used were also varied during this study.

Three major clades were revealed in Libyan samples

Clade A: Two of strain ***Starria zimbabweensis* SAG 74.90.** The closest relatives to *Starria zimbabweensis SAG* 74.90 strains |KM020013.1|with 2 *Uncultured bacterium* partial 16S rRNA gene, clone FD04D08 and *Altererythrobacter troitsensis* strain KMM6042 16S rRNA partial sequences and *Altererythrobacter dongtanensis* strain KCTC22672 complete sequence and *Altererythrobacter dongtanensis* strain JM27 16SrRNA gene partial sequence, representing two established genera and one new genus of the family *Microchaetaceae* Figure (15).

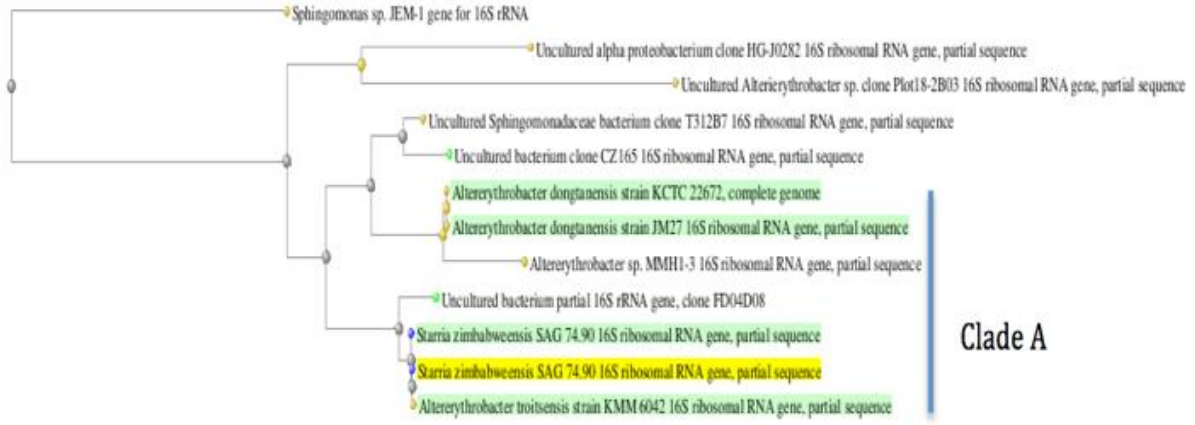

Figure 15*. Clade A, strain Starria zimbabweensis SAG 74.90. Phenogram based on the alignment of partial cyanobacterial 16S rRNA gene sequences and constructed tree of two of strain Starria zimbabweensis SAG 74.90sp were used as out-groups to root the presented phylogeny. Sequences for this strain were obtained by automated DNA sequencing methods.*

Clade B: strains includes Uncultured Coleofasciculus sp. clone SBK29 |KM108654.1|. The closest relatives to uncultured bacterium clone Prehnite 21 16S ribosomal RNA gene partial sequences and to the uncultured bacterium clone DM1-2, data not shown.

Clade C: strains Microcoleus sp. MMG-10 included two Microcoleus sp. Strains gb|KF157400.1|and four Cyanothece sp. strains MMG-11 gb|KF157401.1| with Uncultured Coleofasciculus sp. clone SBK29 |KM108654.1|) representing two established genera and one new genus of the family Microchaetaceae data not show.

Clade D: strains included Pseudanabaena sp. CAWBG530 gb|JX088101.1| with JF925326.1Calothrix sp CYN100 16S ribosomal RNA gene partial and KC211803.1 Uncultured Chroococcidiopsis sp clone Rib EI 16srRNA, Clade D Data not show.

Clade E: strains included. Four *Cyanothece* species. strains MMG 1gb|KF157401.1|. Uncultured with *Coleofasciculus* sp. clone SBK29 |KM108654.1|.Data not shown.

Phylogentic Analysis of Indonesian samples

The second cluster were isolated from Indonesia, divided in three clusters; *Nostocales, chroococcales* and *Synechococcales*. In addition, it was interesting that a distinct Indonesian group were different than Libyan strains only by two toxic -MC-producing isolates; (*Microcystis wesenbergii* VN451 and *Nostoc* sp. KVJF4). Clade F strains include three strains *Calothrix* D253 genomic DNA (clone AG13) emb|Z47172.1| with AP012495.1 and AP012495. Data not shown.

Clase G Phylogenetic analysis of the *M. wesenbergii* VN451 strains based on 16srRNA gene sequences.

An alignment of 1,334 nucleotides was used to calculate genetic distances M. wesenbergii. The phenogram was reconstructed from a pairwise distance matrix by using the neighbor-joining method. The strains identified as *M. wesenbergii* VN451 were located on the same terminal branch after a cladistics analysis of the sequence data was performed and were equally divergent from the cluster containing the remaining *Microcystis* strains, with average levels of similarity of 97 and 96%, respectively. The relationship of *M. wesenbergii* to the *M. aeruginosa* instead of other members of the genus *Microcystis*, was illustrated by the evolutionary distances and relative clustering show n in Fig. (16).

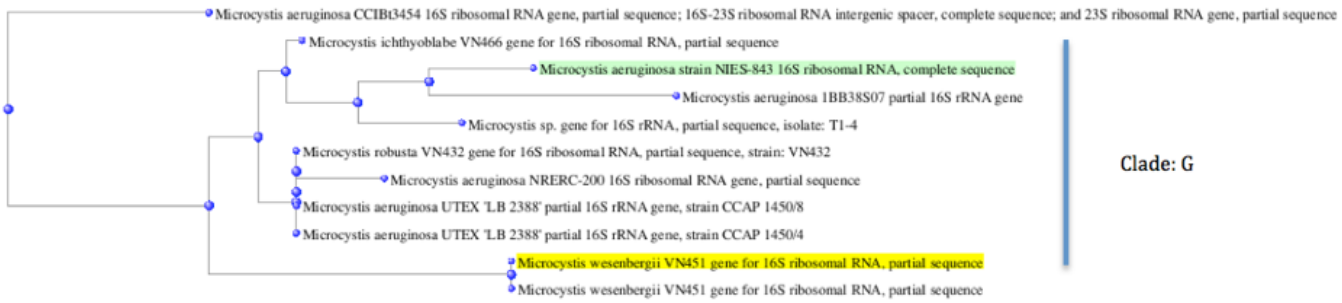

***Fig16**. Microcystis strains, derived from complete 16S rRNA gene sequences. The phenogram was reconstructed from a pairwise distance matrix by the neighbor-joining method. (Saitou, N., and M. Nei. 1987)(43).*

M. wesenbergii VN451 dbj|AB666079.1| with *Microcystis aeruginosa* strain NIES-843 16S ribosomal RNA complete sequence and *Microcystis ichethoblabe* VN466 gene for 16S ribosomal RNA partial sequence CLade G . The level of 16s rRNA gene sequence similarity to *Synechococcus sp,* one strains of this cluster, *M. wesenbergii* was isolated from Indonesian samples exhibited levels of sequence similarity ranging from 91 to 99%. The relationship of *M. wesenbergii* to the M. aeruginosa instead of other members of the genus *Microcystis,* was illustrated by the evolutionary distances and relative clustering. The strains identified as *M. wesenbergii* NIES107 were located on the same terminal branch after a cladistics analysis of the sequence data was performed and were equally divergent from the cluster containing the remaining *Microcystis* strains (Fig. 3.29), with average levels of similarity of 97 and 96%, respectively. *Cyanobium sp.* CENA138 with Uncultured_cyanobacterium_QSSC8L-3 16S ribosomal RNA AF448070.1_Synechococcus_sp-PS845_16SrRNA gene partial sequence, Phylogenetic affiliations between *Cyanobium sp* isolates and other cyanobacteria, derived from complete 16S

rRNA gene sequences. The phenogram was reconstructed from a pairwise distance matrix by the neighbor-joining method (43). Data not shown Fig (17)

Phylogenetic analysis of the Nostoc sp. KVJF4 strains based on 16srRNA gene sequences.
In this tree, one representing the toxic cyanobacterial strain was (Nostoc sp. KVJF4. Nostoc species were readily isolated from water samples, that were conspicuously taken from the water surface of UNHAS university river in Indonesia (site P) (Table 4.2). The diversity of 16S rRNA gene sequences obtained from the Nostoc strains isolated in laboratory is represented by the sequences from strains Nostoc sp. KVJF4 gb|EU022710.1|). Fig (17) Nostoc sp. KVJF4 gb|EU022710.1|With Nostoc sp Leptogium palmatumUK176 cyanobiont 16S ribosomal RNA gene, partial sequence Clade E. The 16S rRNA gene sequences from the Nostoc strains shared 96–100% similarity and all fell within Nostoc clade II as described. The strains of Nostoc sp. KVJF4 |formed a robust clade with strain Nostoc sp Leptogium palmatum UK176. However, Nostoc sp Leptogium palmatum UK176 was most closely related to sequences from Nostoc sp. in a large clade with other Nostoc species Fig (17)

Figure.17 *Clade H. Nostoc sp. KVJF4 strains 4.30 Maximum parsimony tree of 16S rRNA partial gene sequences showing the phylogenetic relationship of strains of Nostoc morphologically identified as two species isolated from the exterior of stone monuments in India (labelled with black dots) and other species of Nostoc and Desmonostoc isolated from different regions of the world .*

Results were obtained using neighbour-joining and maximum-parsimony trees. In particular, these are found in different branches within the cyanobacteria order; Pleurocapsales family has one strains *Starria zimbabweensis SAG 74.90*. The "oscillatoriales family" (two different strains *Uncultured Coleofasciculus sp.* clone SBK29 and. *Microcoleus sp. MMG-10*), and also within the *Synechococcales lineage* two strains and *Pseudanabaena sp. CAWBG530* and *Cyanobium sp. CENA138*. The Strains *Cyanothece sp. MMG-11* and *M. wesenbergii VN451* were placed within the *chroococcales lineages* and finally *Nostocales lineage* (two strains *Nostoc sp. KVJF4* and *Calothrix D253* genomic DNA (clone AG13). Toxic and nontoxic Cyanobacteria were found to be distributed throughout the cyanobacterial evolutionary tree in our samples as expected. Using phylogenetic analysis, we found that most of the cyanobacterial phylotypes within four distinct clades: I (*oscillatoriales*), II *(Synechococcales*), III (*Nostoc/Anabaena),* IV *(chroococcales*). In particular, these were found in different branches within the cyanobacteria order; Pleurocapsales family has two strain of *Starria zimbabweensis SAG 74.90.* Three strains *Calothrix D253* genomic DNA (clone AG13) emb|Z47172.1| with AP012495.1 and AP012495.1. The "oscillatoriales family" (two different strains *Uncultured Coleofasciculus sp. clone SBK29* and . *Microcoleus sp.*

MMG-10) and also within the Synechococcales lineage two strains *Pseudanabaena sp. CAWBG530* and *Cyanobium sp*. CENA138. The Strains *Cyanothece sp. MMG-11* and *Microcystis wesenbergii VN451* were placed within the chroococcales lineages and finally *Nostocales lineage* (two strains *Nostoc sp*. KVJF4 and *Calothrix D253* genomic DNA (clone AG13). *Cyanobium sp.* CENA138 gb|KC695832.1| with AF170763.1-*Uncultured_cyanobacterium*_QSSC8L-3_16S_ribosomal_RNA *Nostoc sp. KVJF4 gb|EU022710.1|*

Gen Bank Numbers

16s rRNA sequence data for species examined in this study were deposited with GenBank. Accession numbers are as follows: Two Starria zimbabweensis SAG 74.90strains |KM020013.1| Uncultured Coleofasciculus sp. clone SBK29 |KM108654.1|. Two Microcoleus sp. strains gb|KF157400.1| Pseudanabaena sp. CAWBG530 gb|JX088101.1| Four Cyanothece sp. strains MMG-11 gb|KF157401.1| Three strains Calothrix D253 genomic DNA (clone AG13) emb|Z47172.1| Microcystis wesenbergii VN451 dbj|AB666079.1| Cyanobium sp. CENA138 gb|KC695832.1| Nostoc sp. KVJF4 gb|EU022710.1|Length: 1481Number of Matches: 1.The phylogenetic tree obtained in this study was in agreement with the results of other studies that divided cyanobacteria into seven or eight major lineages (8, 10), except that group 2 (8) was here paraphyletic (Fig. 1). Genetic Diversity: The study revealed the genetic diversity of cyanobacterial strains isolated from different water sources in Libya and Indonesia. Presence of Toxic Strains: Some of the strains, such as Microcystis wesenbergii VN451 and Nostoc sp. KVJF4, were identified as toxic strains potentially capable of producing microcystins. GenBank Submission: The 16S rRNA gene sequences obtained from the study were deposited in GenBank under specific accession numbers. Overall, the study provided valuable insights into the genetic diversity and phylogenetic relationships of cyanobacterial strains in the studied regions. The results contribute to the understanding of cyanobacterial communities in water ecosystems and their potential implications for water quality and safety.

Discussion

The ability to place uncultured isolates of cyanobacteria strains on their taxonomic positions up to the order level using 16S rRNA sequences represents a significant advancement in microbial taxonomy and classification. This achievement is primarily facilitated by molecular techniques and bioinformatics tools that allow us to analyze the genetic material of cyanobacterial strains without the need for traditional cultivation methods. Traditionally, the classification and identification of cyanobacteria relied on morphological characteristics observed under a microscope, which could be challenging and limited in accuracy, especially for uncultured or morphologically similar strains. The use of 16S rRNA gene sequencing has revolutionized microbial taxonomy. The 16S rRNA gene is a highly conserved region in the bacterial genome but also contains variable regions that can be used for species differentiation. We amplify and sequence this gene from drinking water and environmental samples, containing uncultured microorganisms. By comparing the obtained sequences with a reference database of known sequences, researchers can infer the taxonomic position of the uncultured isolates. Assigning uncultured cyanobacteria into particular genera either by morphology or biochemically and understanding their toxicity is a difficult task. Study utilized molecular tools to overcome the above constrains. Sequence a PCR product directly can be generated without time-consuming molecular cloning procedures, allowing the rapid survey of a collection of strains for genetic diversity. To obtain high quality sequencing data, it is very important that the PCR reaction is specific and

strong. According to the results obtained in this study, it was evident that 16S rRNA gene is an effective tool in construing phylogenetic relationships between different genera within order level. The phylogenetic relationship arose from 16S rRNA gene sequence comparison supported the traditional classification of cyanobacteria which was based on morphological characters. All strains clearly demonstrated have cyanobacterial orders with few exceptions and therefore it showed the suitability of 16S rRNA gene for taxonomic differentiation According to the results, of the uncultured isolates, we are able to place cyanobacteria strains on their taxonomic positions up to order level with 16S rRNA sequences. This also supported in the work done by (Li et al. (2001). This phenomenon was also supportive with the previous studies done by several authors. Also, the 16S rRNA gene has been useful in identifying and classifying strains that belong to a single clade (Otsuka et al. 1999).

According to the phylogeny analysis, reservoirs and well waters in Tripol consisted rich cyanobacterial diversity which also have no potential to produce cyanotoxins. The partial 16S rRNA gene phylogeny of the order Oscillatoriales is depicted, it allowed the identification at species level of 3 sequenced isolates, Uncultured Coleofasciculus sp. clone SBK29, Microcoleus sp. MMG-10 and pseudoagardhii. The distribution of Oscillatoria and Lyngbya isolates in several distinct clades also reveals the polyphyletic nature of these genera. The partial 16S rRNA gene phylogeny of the order Oscillatoriales is depicted, it allowed the identification at species level of 3 sequenced isolates, Uncultured Coleofasciculus sp. clone SBK29, Microcoleus sp. MMG-10 and pseudoagardhii. The distribution of Oscillatoria and Lyngbya isolates in several distinct clades also reveals the polyphyletic nature of these genera. This is also the case with isolates, Cyanothece sp. MMG-11 chroococcales, Qaser Bin Ghashir Cyanothece sp. MMG-11 chroococcales Garyan Cyanothece sp. MMG-11 chroococcales, Sabratah Cyanothece sp. MMG-11 chroococcales bat raya Microcystis wesenbergii VN451 chroococcales. Isolates Pseudanabaena sp. CAWBG530 Synechococcales. From Ghutshaal and Starria zimbabweensis SAG 74.90. From Tajoura grouped in a cluster with Pleurocapsales are typical of Aphanizomenon gracile. It shows that the Nostocales form a monophyletic cluster. The order Pleurocapsales forms a monophyletic cluster, whereas the orders Chroococcales and Oscillatoriales are both divided into two distinct clades. **It's** important to note that while 16S rRNA sequencing is a powerful tool for taxonomic classification, it may have limitations in distinguishing closely related species or strains within a genus. In such cases, additional genetic markers or techniques may be necessary for more precise identification. In summary, the ability to use 16S rRNA gene sequencing to place uncultured cyanobacteria isolates on their taxonomic positions up to the order level represents a significant leap forward in our understanding of microbial diversity and ecosystem functioning. This approach has wide-ranging applications in ecology, environmental science, and biotechnology.

Conclusion

The present study provided DNA profiles, composed of phylogenetic characters, appropriate for the inference of relatedness and evolution of cyanobacterial taxa. These DNA profiles readily identify potential toxic and nontoxic species of cyanobacteria in west Tripoli reservoirs of drinking and recreational water supplies. Phylogeny by this molecular technique correlates with the grouping of cyanobacteria at the intergeneric level. This proposed tool for analyzing cyanobacterial systematics is based on a stable genetic character, the 16SrRNA gene locus. A phylogenomic of the 16SrRNA signature based approaches was used to characterize the major clades of cyanobacteria in drinking water collected from water samples in Libya and from Indonesia reservoris. The relationships among cyanobacteria, as indicated by tree topology, were

essentially identical by the genetic distance and parsimony methods for tree construction. Based on 16S rRNA results obtained from the present study, it was clearly acknowledged the significant diversity in cyanobacteria with potential microcystin (MC) and cylindrospermopsin (CYN) producers within the water in the Indonesian reservoirs. Among 20 isolates, 16 isolates were classified as unidentified cyanobacteria and considered as novel cyanobacterial genera. Identified isolates, were belonging to order Chroococcales,, Pleurocapsales, Oscillatoriales, Nostocales and Stigonematales. were identified up to species level. With 16S rRNA Phylogenetic results, eleven clusters clearly demonstrated have cyanobacterial orders with more than 90% similarity irrespective to their toxicity which showed the suitability of 16S rRNA gene for taxonomic differentiation. Sixteen isolates had the no potential to produce MC and two isolates to produce MC. Twenty uncultured cyanobacterial isolates were able to place on their taxonomic positions up to order level. Findings of the study concerm the rich cyanobacterial diversity and the divergence among the potential cyanotoxin producers in the Libyan water bodies and is an indication regarding the numerous health impacts faced by the people living in the Libyan country. Therefore, the study underlines a need of continuous monitoring programmed, the efficient management of water bodies and correct toxicity assessment by accurate identification of problematic cyanobacterial species is necessary for the waters used as sources of drinking water. This study aims to determine the phylogenetic affiliation of the cyanobacteria associated with using sequence data generated to the 16S rRNA with comparisons made to databases through the BLAST programme. We conclude that taxonomic identification of cyanobacterial species should be done by morphological analysis of wild and cultured material in combination with molecular techniques.

Reference

Belila, A.; El-Chakhtoura, J.; Otaibi, N.; Muyzer, G.; Gonzalez-Gil, G.; Saikaly, P.E.; van Loosdrecht, M.C.M.; Vrouwenvelder, J.S. Bacterial community structure and variation in a full-scale seawater desalination plant for drinking water production. Water Res. 2016, 94, 62–72.

Boom R, Sol C, Salimans M, Jansen C, Wertheim-van Dillen P, Van der Noordaa J (1990) Rapid and simple method for purification of nucleic acids. J Clin Microbiol 28(3):495–503.

Brooks, B.W.; Lazorchak, J.M.; Howard, M.D.; Johnson, M.-V.V.; Morton, S.L.; Perkins, D.A.; Reavie, E.D.; Scott, G.I.; Smith, S.A.; Steevens, J.A. Are harmful algal blooms becoming the greatest inland water quality threat to public health and aquatic ecosystems? Environ. Toxicol. Chem. 2016, 35, 6–13.

Bruno, A.; Sandionigi, A.; Bernasconi, M.; Panio, A.; Labra, M.; Casiraghi, M. Changes in the drinking water microbiome: Effects of water treatments along the flow of two drinking water treatment plants in a urbanized area, milan (Italy). Front. Microbiol. 2018, 9, 2557.

Castenholz R, Waterbury J (1989) Oxygenic photosynthetic bacteria. Group I. Cyanobacteria. Bergey's Man Syst Bacteriol 3:1710–1806.

Chorus, I.; Welker, M. (Eds.) Toxic Cyanobacteria in Water, 2nd ed.; CRC Press: Boca Raton, FL, USA; On Behalf of the World Health Organization: Geneva, Switzerland, 2021.

Ercolini D (2004) PCR-DGGE ngerprinting: novel strategies for detection of microbes in food. J Microbiol Methods 56(3):297–314.

Garcia-Pichel, F., Lopez-Cortes, A. and Nu«bel, U. (2001) Phyloge- netic and morphological diversity of cyanobacteria in soil desert crusts from the Colorado plateau. Appl. Environ. Microbiol. 67, 1902^1910.

Honda, D., A. Yokota, and J. Sugiyama. 1999. Detection of seven major evolutionary lineages in cyanobacteria based on the 16S rRNA gene sequence analysis with new sequences of five marine *Synechococcus* strains. J. Mol. Evol. 48:723-739.

Komárek, J.; Kaštovský, J.; Mares, J.; Johansen, J. Taxonomic Classification of Cyanoprokaryotes (Cyanobacterial Genera) Using a Polyphasic Approach. Preslia 2014, 86, 295– 335.

Kulasooriya S (2011) Cyanobacteria: pioneers of planet earth. Ceylon J Sci 40(2):71–88

Li, Q.; Yu, S.; Li, L.; Liu, G.; Gu, Z.; Liu, M.; Liu, Z.; Ye, Y.; Xia, Q.; Ren, L. Microbial Communities Shaped by Treatment Processes in a Drinking Water Treatment Plant and Their Contribution and Threat to Drinking Water Safety. Front. Microbiol. 2017, 8, 2465.

Li R, et al. 2001. First report of the cyanotoxins cylindrospermopsin and deoxycylindrospermopsin from Rhaphidiopsis curvata (Cyanobacteria). J. Phycol. 37:1121–1126.

Nübel U, Garcia-Pichel F, Muyzer G (1997) PCR primers to amplify 16s rRNA genes from cyanobacteria. Appl Environ Microbiol 63(8):3327–3332.

Otsuka S, Suda S, Li R, Watanabe M, Oyaizu H, Matsumoto S, et al. Phylogenetic relationships between toxic and non-toxic strains of the genus Microcystis based on 16s to 23s internal transcribed spacer sequence. FEMS Microbiol Lett. 1999;172(1):15–21.

Overall, this process allowed the researchers to identify and classify the cyanobacteria present in the samples and understand their taxonomic positions and potential toxicity.

Patel JB (2001) 16s rRNA gene sequencing for bacterial pathogen identi ca- tion in the clinical laboratory. Mol Diagn 6(4):313–322 .

.Rippka, R.; Stanier, R. Y.; Deruelles, J.; Herdman, M.; Waterbury, J. B. Generic Assignments, Strain Histories and Properties of Pure Cultures of Cyanobacteria. Microbiology 1979, 111, 1– 61.

Robertson, B. R., N. Tezuka, and M. M. Watanabe. 2001. Phylogenetic analyses of *Synechococcus* strains (cyanobacteria) using sequences of 16S rDNA and part of the phycocyanin operon reveal multiple evolutionary lines and reflect phycobilin content. Int. J. Syst. Evol. Microbiol. 51:861-871.

Shigeto Otsuka,* Shoichiro Suda,1 Renhui Li,2,† Satoshi Matsumoto, and Makoto M. Watanabe3 Morphological variability of colonies of Microcystis morphospecies in culture Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113–8657, Japan.

Svirc˘ev, Z.; Lalic´, D.; Bojadzija Savic, G.; Tokodi, N.; Drobac Backovic, D.; Chen, L.; Meriluoto, J.; Codd, G. Global Geographical and Historical Overview of Cyanotoxin Distribution and Cyanobacterial Poisonings. Arch. Toxicol. 2019, 93, 2429–2481.

Tamura K, Dudley J, Nei M, Kumar S (2004) Mega4: molecular evolution- ary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 24(8):1596–1599 .

Tomitani A, Knoll AH, Cavanaugh CM & Ohno T (2006) The evolutionary diversification of Cyanobacteria: molecular–phylogenetic and paleontological perspectives. Proc Natl Acad Sci USA 103: 5442–5447.

Van de Peer Y. A quantitative map of nucleotide substitution rates in bacterial rRNA. Nucleic Acids Res. 1996;24(17):3381–91.

Van Hassel, W.H.R.; Andjelkovic, M.; Durieu, B.; Marroquin, V.A.; Masquelier, J.; Huybrechts, B.; Wilmotte, A. A Summer of Cyanobacterial Blooms in Belgian Waterbodies: Microcystin Quantification and Molecular Characterizations. Toxins 2022, 14, 61.

Valério E, Chambel L, Paulino S, Faria N, Pereira P, Tenreiro R (2009) Molecular identi cation, typing and traceability of cyanobacteria from freshwater reservoirs. Microbiology 155(2):642–656.

Ward D, Ferris M, Bateson M, (1995) Organization of native populations within hot spring microbial mat communities: need for a more ecological approach. In: Abstract of papers, 7th symposium of the international society for microbial ecology, Santos, Brazil .

Waterbury JB (2006) The Cyanobacteria - isolation, purification and indentification. The Prokaryotes, Vol. 4 (Dworkin M, Falkow S, Rosenberg E, Schleifer K-H & Stackebrandt E, eds), pp. 1053–1073. Springer, New York.

Wu Z, Shi J, Xiao P, Liu Y, Li R (2011) Phylogenetic analysis of two cyanobacte- rial genera Cylindrospermopsis and Raphidiopsis based on multi-gene sequences. Harmful Algae 10(5):419–425.

Zupancˇicˇ, M.; Kogovšek, P.; Šter, T.; Rekar, R.; Cerasino, L.; Baebler, Š.; Klemencˇicˇ, A.K.; Eleršek, T. Potentially Toxic Planktic and Benthic Cyanobacteria in Slovenian Freshwater Bodies: Detection by Quantitative PCR. Toxins 2021, 13, 133.