Summary

The current study included the isolation and identification of five species of cyanobacteria (Cyanophyta) morphologically and molecularly from some aquatic habitats in Basrah Governorate, southern Iraq. These species were purified and cultured. Four of these species were locally identified for the first time, represented by the following species: *Chroococcidiopsis cubana*, *Cyanobacterium aponinum*, *Leptolyngbya halophile*, and *Oscillatoriales cyanobacterium*, in addition to algal *Cyanobacterium aponinum.* As for the fifth *cyanobacterium*, *Gloeocapsa calcarea*, its diagnostic gene sequence (*16S rRNA*) was globally recorded for the first time in the GenBank gene database, specifically within the National Center for Biotechnology Information (NCBI) website. The isolated species were assigned the following Accession numbers: OR269467.1, OR269464.1, OR269465.1, OR269463.1 and OR269466.1.

A phylogenetic tree was constructed for the isolated species based on their *16S rRNA* gene sequences, which revealed close relationships between the isolated strains and reference strains for each species. Furthermore, growth curves were plotted for the five isolated and purified species after cultivation in a liquid BG-11 medium. The growth constants (k), representing the rate of growth, were determined as 1.3, 1.216, 1.129, 1.278 and 1.144 respectively, for the isolated species. The generation times (G), representing the time taken for a Generation doubled, were calculated as 0.533, 0.570, 0.613, 0.542 and 0.605 days for the isolated species.

The capability of the five isolated and purified algal species to produce hepatotoxic known as MCs (Microcystins) was assessed. These toxins were isolated from the five species and quantified and characterized using two techniques, namely High-Performance Liquid Chromatography (HPLC). The results revealed statistically significant differences at a level of $P \leq 0.05$ among these species in their ability to produce hepatotoxic toxins. The algal *Ch. cubana* and *L. halophila* exhibited a high production

capacity for these toxins, with concentrations reaching 4.053 and 4.021 µg/l, respectively. Following them, the algal species *Cy. aponinum* produced these toxins at a concentration of 3.308 µg/l, while the species *O. cyanobacterium* and *Gl. calcarea* showed the lowest production of hepatotoxic compared to the other species under study, with concentrations of 1.291 and 1.565 µg/l.

The second method employed was the Enzyme enzyme-linked immuno Sorbent Assay (ELISA), which was similar to the results of the first method in that there were significant differences below the level of $P \leq 0.05$. The algal *Ch. cubana* demonstrated the highest propensity for producing these toxins using the ELISA technique. The concentration rate of these toxins was 4.034 µg/l for *Ch. cubana*, 3.793 µg/l for *L. halophila*, and 3.267 µg/l for *Cy. aponinum* Conversely, the algal *O. cyanobacterium* and *Gl. calcarea* exhibited the lowest hepatotoxic productivity among the studied species using the ELISA technique. The toxin concentration rates for these two species were 1.591 and 1.803 µg/l, respectively.

Statistical results indicate significant differences at the $P \leq 0.05$ level among the concentrations of hepatotoxic after their extraction and purification from the algal biomass and water at the five isolated sites in the current study using the Enzyme-Linked Immunosorbent Assay (ELISA) technique. The algal biomass isolated from Karama River exhibited a high production of hepatotoxic, with a concentration rate of 6.911 µg/l, followed by 6.556 µg/l in the algal biomass isolated from Al-Ashar River and 4.210 µg/l in the algal biomass isolated from Al-Khindak River, and 3.991 µg/l in the algal biomass at Shatt Al-Arab. In contrast, the algal biomass isolated from Shatt Al-Basrah showed the lowest production of hepatotoxic MCs compared to the other algal biomass in the current study, with a concentration rate of 1.934 µg/l. As for the water samples, the highest concentration of hepatotoxic was 1.418 µg/l in the Karama River, followed

by 0.779 µg/l in the Al-Ashar River and 0.663 µg/l in the Al-Khindak River. However, the concentrations of hepatotoxic in the water of Shatt Al-Arab and Shatt Al-Basrah were less than 0.15 µg/l.

This study relied on the Polymerase Chain Reaction (PCR) technique as a rapid and reliable method for detecting certain hepatotoxic-producing blue-green algae. The detection was based on the presence of specific genes related to microcystin synthesis, specifically the *mcyA* and *mcyE* genes. The results indicated that all the studied samples possessed these genes, except for the water samples from the river Shatt al-Arab and Shatt al-Basrah locations.

Quantitative Polymerase Chain Reaction (qPCR) was also employed to compare the cycle threshold (Ct) values and signal fluorescence produced by the amplified *mcyA* gene for each species of isolated and purified blue-green algae species, biomass and aquatic environment. The results revealed significant differences at the $P \leq 0.05$ level. Ch. cubana showed the highest efficiency in amplifying the *mcyA* gene, followed by the DNA of *L. halophila*, and then the DNA of *Cy. aponinum*. On the other hand, *O. cyanobacterium* and *Gl. calcarea* exhibited the least efficiency in amplifying the *mcyA* gene compared to the other studied species.

The extracted DNA from the algal biomass isolated from Karama River exhibited high efficiency in amplifying the *mcyA* gene, followed by the DNA from the biomass isolated from Al-Ashar River, and then the DNA from the biomass isolated from Al-Khindak River. In contrast, the sites of Shatt Al-Arab and Shatt Al-Basrah showed the lowest efficiency in amplifying the *mcyA* gene compared to the other studied locations.

The extracted DNA from the water of Karama River showed the highest efficiency in amplifying the *mcyA* gene, followed by the DNA from the water of Al-Ashar River and then the DNA from the water of Al-Khindak River. However, no amplification of the *mcyA* gene was observed in the DNA extracted from the water of Shatt Al-Arab and Shatt Al-Basrah.

It's worth noting that the molecular test results were consistent with the results from immune-based and chemical tests. Additionally, the diagnosis of the potential of isolated and purified blue-green algae in the current study to produce hepatotoxic MCs is the first of its kind locally and is rare globally.

The results indicated the presence of point mutations in the amplified region of the *16S rRNA* gene in the isolated strains compared to the reference strains, Transversion, Transition, Deletion and Insertion mutations. The strain *Cy. aponinum* OR269463.1 exhibited five mutations, while the strain *L. halophila* OR269464.1 also had five mutations in their nucleotide sequences. On the other hand, the strain *O. cyanobacterium* OR269465.1 had nine mutations in its sequence. The most increased number of mutations was in the strain *Ch. cubana* OR269467.1, with 21 mutations compared to the reference strains.

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