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Original Article

Molecular Detection of Cylindrospermopsin-Producing Cyanobacteria in the Coasts of Hormozgan Province, Iran

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Abstract

Background: In recent years, water and environmental pollution by cyanobacteria have been increasingly reported as a serious hazard to human health in the world. Cylindrospermopsin is one of the most important algal toxins produced by cyanobacteria that can cause damage to the liver, kidneys, thymus, and heart. The aim of this study was to detect cylindrospermopsin-producing cyanobacteria accurately and fast in the water resources of the Hormozgan province coast by molecular method.

Methods: We collected 20 water samples (2 L) from 20 sampling stations on the coasts of Hormozgan province in October 2017. The genomic DNA was extracted from water samples. Finally, molecular detection was conducted by the polymerase chain reaction (PCR) method.

Results: The optimized PCR successfully detected 20 cyanobacteria strains from all 20 samples. In addition, the presence of cylindrospermopsin-producing cyanobacteria was confirmed in 2 (10%) stations.

Conclusion: Generally, our study confirmed the risk of the presence of toxigenic cyanobacteria on the coasts of Hormozgan province and demonstrated that PCR assay is an accurate and fast method for early detection of cylindrospermopsin-producing cyanobacteria in water resources.

Keywords: Cyanobacteria, Cylindrospermopsin, Molecular detection, Province coasts

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Introduction

Cyanobacteria are photosynthetic prokaryotic blue-green algae that are present in warm, fresh, and eutrophic waters. The presence of cyanobacteria in local waters causes problems on a global scale by producing dangerous toxins called cyanotoxins (1). In addition, many of these species of cyanobacteria cause significant problems in rivers, freshwater lakes, oceans, and stored and drinking water (2). Therefore, in order to protect water consumers from exposure to cyanobacteria toxins, it is necessary to quantitatively and qualitatively investigate water sources for the presence of these dangerous toxins and to prevent possible risks caused by their consumption.

Two toxin types, cylindrospermopsin and microcystins, are the main causes of cyanobacterial poisoning (3). Both of these toxins can be produced by various cyanobacterial strains in the world (4); consequently, monitoring of cyanotoxins in water bodies has been increasing in recent years. Cylindrospermopsin was first detected in 1979 when 148 patients were hospitalized at the Australian Hospital in Palm Island with liver inflammation symptoms. The disease was associated with the presence of *Cylindrospermopsin raciborskii* in the source of drinking water (5). Cylindrospermopsin is an alkaloid toxin produced by a variety of cyanobacteria and uracil and hydroxyl-carbon-7 group play an important role in its toxicity (6). Cylindrospermopsin is also biologically active and inhibits several metabolic pathways, including the synthesis of glutathione and cytochrome P450 (7,8).

Since phenotypical and morphological investigations of cyanobacteria cannot be proper and accurate ways to detect toxigenic strains, methods with higher efficiency should be used. With the development of molecular genetics methods in recent years, molecular methods can be alternatively used to detect toxigenic cyanobacteria. Particularly, conventional polymerase chain reaction (PCR) methods for detection of toxigenic cyanobacterial strains have been confirmed as fast and inexpensive methods (9,10).

Persian Gulf region and Hormozgan province coasts in

the Middle East have been considered the most important channels of communication between continents, rich resources of oil and gas, and strategic locations. On the other hand, this area is important for the diversity of plants and animals (11). In addition, due to climatic conditions and the presence of effective factors, the coasts of Hormozgan province are susceptible to phenomena such as the accumulation of cylindrospermopsin-producing cyanobacteria (12).

Therefore, the aim of the present study was to investigate the efficiency of molecular PCR assay in detection of cyanobacteria and cylindrospermopsin-producing cyanobacteria in the water resources of Hormozgan province.

Materials and Methods

Sample Collection

In the present study, 20 water samples were collected from 20 stations on the coast of Hormozgan province in October 2017. For this purpose, we collected 2-L water samples in dark glass bottles from 100 m away from the coast. The sampling was performed in triplicate, and the obtained samples were transferred to the laboratory immediately in cold conditions within 3 hours. The coordinates of the sampling stations are presented in Figure 1. The water samples were centrifuged at 8000 rpm for 12 minutes, and the residual sediment was resuspended with 500 µL of deionized water.

Genomic DNA Extraction

The genomic DNA was extracted from the samples by a DNG-Plus extraction kit according to the manufacturer's instructions. The quantity and quality of extracted DNA samples were evaluated using a NanoDrop spectrophotometer and electrophoresis on 1% agarose gel, respectively. The obtained DNA samples were stored at -20°C until molecular detection.

PCR Amplification

Molecular detection of cyanobacteria and Cylindrospermopsin was performed by PCR method. For this purpose, PCR amplification was optimized by different concentrations of genomic DNA of *Microcystis aeruginosa* PCC 7806 strain. Universal primers were used for the detection of cyanobacteria, and specific primers were used for the detection of Cylindrospermopsin (Table 1). PCR amplification was performed in a total volume of 25 µL containing 0.5 µL of each primer (25 pmol), 1 µg of template DNA, 1.5 mmol/L of Mgcl2, 0.1 mmol of dNTP, 1.5 units of Taq DNA polymerase, and 12.5 µL of PCR buffer. The following thermal condition was used for the reaction: initial denaturation (1 cycle at 94 °C for 4 minutes), denaturation (40 cycles at 94 °C for 40 seconds), annealing (40 cycles at 50 °C for 30 seconds), extension (40 cycles at 72 °C for 25 seconds), and final extension (1 cycle at 72 °C for 5 minutes). The obtained PCR products were electrophoresed on 1.5% agarose gel, and the bands were visualized by a gel documentation system.

Results

Specificity of the PCR Test

In order to investigate the specificity of PCR test for the detection of cyanobacteria, DNA samples of *Staphylococcus aureus*, Hepatitis B virus, *Fusarium solani*, and *Saccharomyces cerevisiae* were used, and an optimized PCR test was evaluated with positive and negative control DNA samples. We also used DNA samples of *Microcystis aeruginosa*, *Nostoc, Anabaena*, and *Fischerella* to optimize detection of cylindrospermopsin. The obtained results indicated that PCR assay was able to detect cyanobacteria and cylindrospermopsin-producing cyanobacteria, whereas DNA samples of the other microorganisms were not detectable with the used primers. In addition, the limit of detection (LOD) of PCR was 100 copies per reaction for both cyanobacteria and cylindrospermopsin.

Frequency of Cyanobacteria

The frequency of cyanobacteria was investigated by universal primers in 20 water samples from different stations of Hormozgan province coasts. We detected cyanobacteria in all water samples (Figure 2). Moreover, the presence of cylindrospermopsin-producing cyanobacteria was observed in the samples of 2 stations (Figure 3).

Discussion

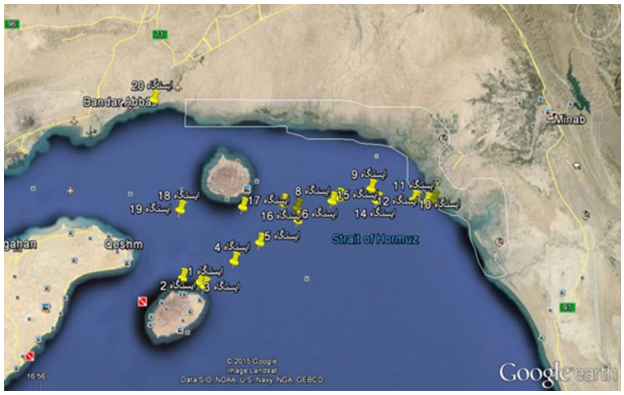

Figure 1. The Coordinates of the Sampling Stations on the Coasts of Hormozgan Province

Table 1. Primers Used to Detect Cyanobacteria and Cylindrospermopsin

Target Gene	Sequences Of Primers	Product Size	References
CYA359F CYA781R	5′-GGGGAATYYYCCGCAATGGG-3′ 5′-GACTACWGGGGTATCTAATCCCWTT-3′	487 bp	13
CynsulfF CylnamR	5′-ACTTCTCTCCTTTCCCTATC-3′ 5′-GAGTGAAAATGCGTAGAACTTG-3′	578 bp	14

The emergence of cyanobacteria is observed in tropical and subtropical areas, and usually in freshwater, brackish water, seawater, and eutrophic waters. On the other hand, some cyanobacteria species produce dangerous toxins causing a variety of diseases and even death (15). Considering that the Persian Gulf region and Hormozgan coasts are susceptible to such phenomenon, due to climatic conditions and the availability of effective factors, one of the concerns is the accumulation of cylindrospermopsin-producing cyanobacteria in the water resources of this region. Cylindrospermopsin can accumulate in tissues of aquatic animals and other organisms and play an important role in the prevalence of human poisoning and livestock losses (16). Since the morphological and microscopic methods cannot be suitable and accurate methods for the detection of toxigenic strains, other efficient methods should be developed. In this case, it is possible to develop high-performance analytical methods such as enzyme-linked immunosorbent assay (ELISA), protein phosphatase inhibition assay, high performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS), and solid phase adsorption toxin tracking (17,18).

In a previous study by Rasmussen et al, the real-time PCR method was used to detect the specific gene of *Cylindrospermopsin raciborskii*. They reported that real-time PCR is a rapid and sensitive test; however, because of the costly nature of this method, which requires the purchase of expensive instruments, it is commonly used in reference laboratories (19). In another study, Manali et al identified the presence of specific genes of cyanobacteria that encode cylindrospermopsin and microcystins, using multiplex PCR. They suggested that multiplex PCR provides low sensitivity, due to the competitive state of genes in this method (20). In another similar study, Gaget et al identified the presence of cylindrospermopsin and microcystins in Australian waters by ELISA, chromatography, real-time PCR, PCR, and LC-MS methods. They reported that PCR-based methods provide high sensitivity in the detection of cylindrospermopsin and microcystins (21). Liyanage et al detected cylindrospermopsin by amplification of 16S rRNA gene and reported that 16S rRNA gene is a suitable target for the detection of cylindrospermopsin (22). In addition, Barón-Sola et al identified the presence of cylindrospermopsin-producing cyanobacteria using the real-time PCR method by amplification of 6 target genes and reported that nTCA gene is a suitable target for detection of cylindrospermopsin (23). In this regard, we also found that PCR is a precise, fast, and highly sensitive method for the detection of cylindrospermopsin-producing cyanobacteria in water sources.

In this study, we detected 20 cyanobacterial isolates from the coasts of Hormozgan province by molecular method, 2 of which were cylindrospermopsin-producing cyanobacteria. A high level of cylindrospermopsin production can be observed in various regions of the world, but there is limited information on aquatic toxicity (24). This toxic cyanobacterium was a minor component of the mixed algal assemblage on coasts. However, cylindrospermopsin-producing *Kamptonema* may dominate the community composition in some areas (24). Previous studies have indicated that abiotic

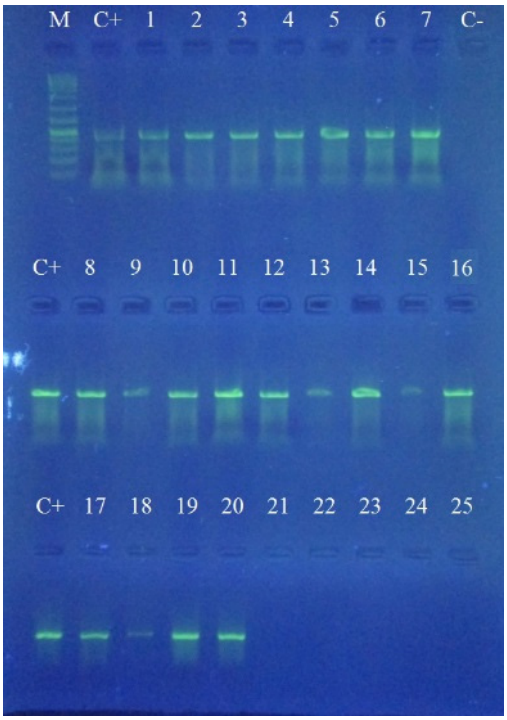

Figure 2. PCR Assay for Detection of Cyanobacteria with a Product Size of 487 bp. (M) 1 kb DNA Ladder; (C+) Positive Control of *Cylindrospermopsin Raciborskii* AWT205 Sample; (C-) Negative Control; (1-25) Positive Cyanobacteria Samples

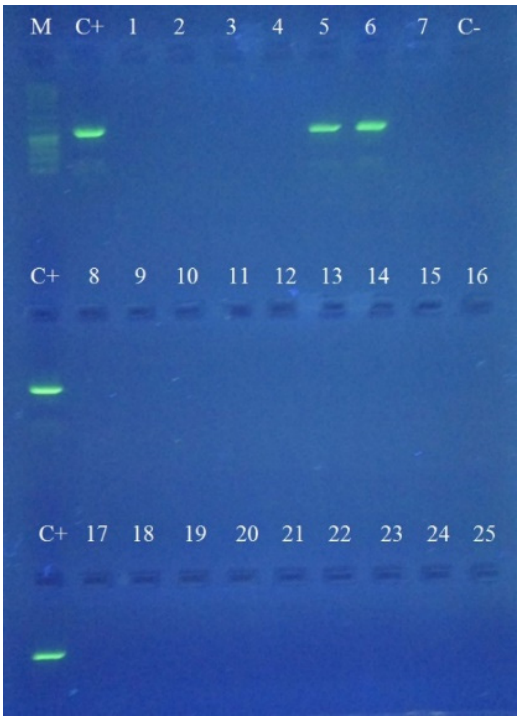

Figure 3. PCR Assay for Detection of cylindrospermopsin with a Product Size of 578 bp. (M) 1 kb DNA Ladder; (C+) Positive Control of *Cylindrospermopsin Raciborskii* AWT205 Sample; (C-) (1-25) Cyanobacteria Samples; (1-4 and 7-25) Negative Sample; (5 and 6) Positive Sample

factors influence the accumulation and release of cylindrospermopsin (25). Lower concentrations of phosphorus and nitrogen have been related to a higher accumulation of cylindrospermopsins in cyanobacterial cells (26,27). Lower concentrations of nutrients in the coasts of Hormozgan could trigger an increase in the detection of toxins such as cylindrospermopsin, and there is a constant need to monitor such changes in this environment. According to the World Health Organization, safe levels of cylindrospermopsin for lifetime drinking water, short-term drinking water, and recreational exposure are 0.7 µg/L, 3 µg/L, and 6 µg/L. However, environments are not well surveyed for the production of cyanobacterial toxins, and the microbial diversity of cyanobacteria is poorly understood.

In general, our study confirmed the presence of cylindrospermopsin-producing cyanobacteria in 10% of the stations in Hormozgan province by PCR. Accordingly, the PCR technique is proposed as a precise, rapid, inexpensive, and sensitive method for the detection of cylindrospermopsin-producing cyanobacteria in water sources.

Conclusion

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Acknowledgments

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Authors' Contributions

Conceptualization: MHSH.
Data collection: EEN.
Formal analysis: AD.
Funding acquisition: EEN.
Investigation: EEN.
Methodology: MHSH.
Project administration: MHSH.
Resources: EEN.
Software: AD.
Supervision: MHSH.
Validation: EEN, MHSH, AD.
Writing--original draft: EEN.

Competing Interests

The authors declare that there is no conflict of interests.

Ethical Approval

Not applicable.

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