Full Length Research Article

Genetic Relationships Among Local Oscillatoria Isolates from Freshwater Sources Using Inter-Simple Sequence Repeat (ISSR) Technique

https://doi.org/10.62940/als.v13i1.2194

Issue: Volume 13, Issue 1

Received: 16-12-2024

Revised: 18-01-2025

Accepted: 17-07-2025

Published online: 31-03-2026

Updated: 09-04-2026

Keywords: Genetic Diversity, Oscillatoria, Phylogenetic analysis, Cyanobacterial taxonomy, ISSR markers

Safa Ghassan Hameed[1],*, **Harith Kamil Buniya**[1]

1. Department of Biology, College of Education for Pure Sciences, University of Anbar, Ramadi, Iraq

* safaghassanbio@gmail.com

ABSTRACT

Background: Molecular characteristics are good markers for distinguishing between closely related species. Molecular taxonomy has been significantly improved by DNA-based strategies with increasing accuracy and efficiency in identifying species through the use of PCR-based methods. We used inter-simple sequence repeat (ISSR) markers in this research to compare and examine the genetic profiles of several *Oscillatoria* species from native freshwater environments.

Methods: Algal samples were taken from natural freshwater environments in the autumn season (September–November 2022). To reduce contamination, DNA was obtained from unialgal cultures of *Oscillatoria spp* . Then, ISSR analysis was done to assess genetic diversity and phylogenetic relationships between the isolated strains.

Results: Seven species were identified: *Oscillatoria acuta* (OS1), *O. princeps Vaucher* (OS2), *O. annae* (OS3), *O. margaritifera* (OS4), *O. proteus Skuja* (OS5), *Oscillatoria sp.* (OS6), and *O. sancta* (OS7). *O. annae* and *Oscillatoria sp.* were most similar and were found to have the greatest genetic similarity (index = 0.6598), indicating that a close evolutionary relationship exists. The two with the lowest similarity were *O. acuta* and *O. proteus Skuja* (index = 0.4330), indicating greater genetic divergence.

Conclusion: Our results support the use of ISSR markers in determining genetic diversity and phylogenetic relationships among *Oscillatoria spp*. This method is promising for improving the molecular taxonomy of cyanobacteria.

INTRODUCTION

Oscillatoria is a common and highly distributed genus of filamentous cyanobacteria (blue-green algae) that inhabits freshwater environments [1,2]. It has unbranched filaments made up of regular cells in one row, usually housed in a soft mucilaginous sheath. *Oscillatoria* is unlike most other cyanobacteria in that it does not form heterocysts or akinetes in regular conditions, although environmental stress will induce the production of one or the other [3,4]. One of its most distinguishing characteristics is its ability to perform anoxygenic photosynthesis — photosynthesis that does not produce oxygen—and to control its buoyancy through the use of gas vesicles to move vertically through the column of water in order to minimize shading by deeper cells [3].

Ecologically, *Oscillatoria* is an important primary producer in aquatic ecosystems, generating oxygen and cycling nutrients [4]. It is part of the food web as a major component, supporting several aquatic invertebrates and insect larvae. Specific species also play a role in nitrogen fixation, fixing nitrogen from the air and making it bioavailable, especially in nitrogen-deficient environments [5]. Yet, with excess nutrient loading, *Oscillatoria* will grow and reproduce in excess, producing so-called harmful algal blooms (HABs). These blooms cause a deterioration in the quality of the water, destabilize ecosystems, and are dangerous for aquatic life [5-7].

In addition to its ecological functions, *Oscillatoria* has been of interest due to its biotechnological potential. The organism has been used in wastewater treatment, biofuel production, and the biosynthesis of bioactive compounds. At the structural level, its filaments have regular cellular morphology with the terminal cells tending to have a characteristic cap-like thickening known as the calyptra. It reproduces by fragmentation that is brought about by necridia (dead cells with jelly-filled cavities) and specific hormogonia [5,8].

For the identification of genetic diversity in *Oscillatoria*, the current research employs Inter-Simple Sequence Repeat (ISSR) analysis, a molecular tool capable of detecting polymorphisms and investigating genetic diversity. ISSR is employed in genetic mapping, fingerprinting, and biodiversity due to its good reproducibility and power of discrimination [9,10]. ISSR markers have been used in very few studies from Iraq to examine the diversity of cyanobacteria [11]. Interestingly, Maulood *et al.* (2013) observed that there were 81 *Oscillatoria* species in Iraqi aquatic ecosystems, indicating the commonality of the genus in Iraq [12].

This study examined the genetic diversity and phylogenetic relationships of *Oscillatoria spp*. obtained from aquatic environments in Iraq's Anbar Governorate using ISSR markers. This study aimed to improve understanding of the taxonomy of cyanobacteria and ecological versatility in the underexplored region.

METHODS

Sample Collection

Fresh algal samples were obtained from freshwater sources in Ramadi, Anbar Governorate, western Iraq, during September– November 2022. Following sampling, the samples were transported directly to the Department of Biology laboratory to maintain viability. Laboratory maintenance involved incubation under conditions simulating natural light– dark cycles and a natural temperature regime.

Sample Preparation and Cultivation

Upon laboratory arrival, specimens underwent sequential rinsing with sterile distilled water to remove adherent particulate matter and external contaminants. Subsequently, cleaned samples were aseptically transferred to sterile 15 mL conical tubes containing fresh distilled water for preservation and further processing. A defined washing/ picking protocol was repeated until unialgal cultures were obtained. A serial dilution method was employed to establish unialgal cultures. These cultures were incubated under controlled light/dark conditions for two weeks.

Genomic DNA Extraction

Genomic DNA was extracted from the algal samples using a commercial genomic DNA extraction kit (Geneaid, Taiwan), following the manufacturer's protocol. The quality of DNA was

assessed via agarose gel electrophoresis using a 1% (w/v) agarose gel at a constant voltage of 65 V for 1 hour. DNA bands were visualized under ultraviolet (UV) light. DNA purity and concentration were measured using a NanoDrop spectrophotometer (Thermo Scientific), and DNA purity was evaluated using the A260/ A280 ratio, with purity values ranging between 1.6 and 2.0.

ISSR Amplification

ISSR- PCR reactions were conducted on DNA extracted from seven *Oscillatoria* species. The ISSR- PCR reactions were prepared according to the protocol described by Araújo et al. (2023) [13], using the following components:

1. PCR PreMix (commercially prepared).

2. ISSR primers (as listed in Table 1), supplied by Integrated DNA Technologies (IDT), Korea.

3. DNA template (25–50 ng per reaction).

PCR amplification was performed using primers with sequences and annealing temperatures listed in Table 1. The amplified products were analyzed using agarose gel electrophoresis, and banding patterns were documented for genetic analysis.

RESULTS

Genomic DNA Extraction

Electrophoresis performed on 1% (w/ v) agarose gel confirmed the successful extraction of genomic DNA from all seven *Oscillatoria* species. Each sample exhibited an intact high-molecular- weight genomic DNA band. DNA purity, as determined by a NanoDrop spectrophotometer (A260/ A280 ratio), ranged from 1.6 to 2.0, indicating acceptable quality for molecular analysis.

ISSR Amplification Results

Seven ISSR primers were utilized to assess the genetic variation among the *Oscillatoria* isolates. All primers generated bands, with polymorphism varying among primers, indicating high levels of genetic diversity. The amplification patterns revealed the number of binding sites (bands), their molecular size range, and the proportion of identical and polymorphic bands across the samples.

Primer SA5 produced the highest number of bands (47), with a molecular size range of 350–3000 bp, while SA1 yielded the fewest bands (33), ranging from 200– 2000 bp. Unique and absent bands were also observed across species, with primer-specific band variations detailed in Table 2 [13,14].

Overall, 280 total bands were recorded across all primers. Of these, 21 (7.5%) were identical, while 259 (92.5%) were polymorphic, highlighting substantial interspecies genetic variation.

Species-Specific ISSR Patterns

Species- wise analysis (Table 3) indicated that *Oscillatoria margaritifera* (OS4) displayed the highest number of bands (45), while *Oscillatoria acuta* (OS1) showed the fewest (33). The largest amplified product (3000 bp) appeared in OS2, OS4, and OS5, while the smallest fragments (200 bp) were identified in OS2, OS3, and OS4.

OS5 exhibited the highest number of unique ISSR bands (9), followed by OS4 (8), OS1 and OS6 (6 each), OS3 (4), OS2 (3), and OS7 (2). Absent bands were detected in OS4 (1), OS6 (2), and OS7 (1). These distinct banding profiles support the presence of genetic differentiation among the isolates.

Genetic Affinity Estimation

Genetic similarity among the species was calculated using the NTSYSpc 2.10e software based on the Nei similarity coefficient. The highest genetic similarity was recorded between OS3 (*O. annae*) and OS6 (*Oscillatoria sp.*) with a similarity index of 0.6598. The lowest similarity was observed between OS1 (*O. acuta*) and OS5 (*O. proteus Skuja*), with a similarity index of 0.4330.

Cluster analysis revealed two major clades: the first included OS3, OS6, and OS7, indicating closer genetic relationships among these species. The second clade comprised OS4 and OS5, which were more distantly related to OS1 (*Oscillatoria acuta)* and OS2 (*O. princeps Vaucher*). The dendrogram illustrating these genetic relationships based on ISSR data is shown in Figure 1 [15,16].

Figures

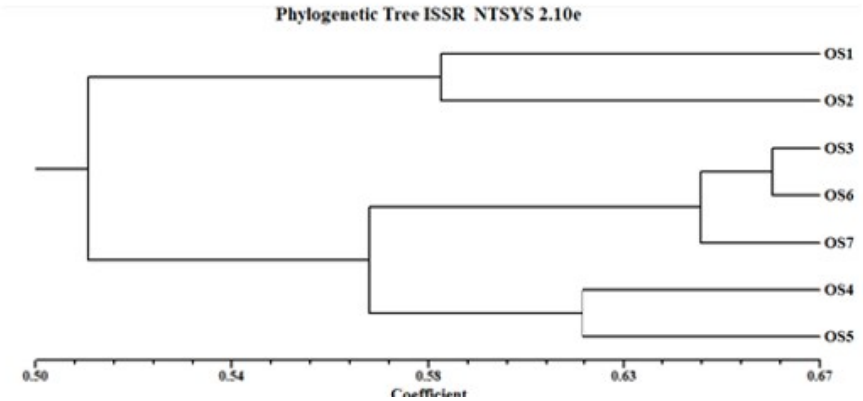

Figure 1: Genetic relationship between the studied species according to ISSR markers. Symbols represent the samples studied.

Tables

Primer	Primer sequence 5′→3′ *	Tm (°C) **
SA1	5′-CCGCAGCCAA-3′ (10-mer)	34 °C
SA2	5′-GCGATCCCCA-3′ (10-mer)	34 °C
SA3	5′-AACGCGCAAC-3′ (10-mer)	32 °C
SA4	5′-TGGATGCGA-3′ (9-mer)	32 °C
SA5	5′-GTGACGTAGG-3′ (10-mer)	32 °C
SA6	5′-CAATCGCCGT-3′ (10-mer)	32 °C
SA7	5′-CAGCACCCAC-3′ (10-mer)	34 °C

*Table 1: ISSR primer sequences along with the temperatures used for PCR. *mer: primer length, **Tm: melting temperature*

Location and size of unique bands (bp)*	Location and size of absent bands (bp)*	Number and proportion of dissimilar bands	Number and proportion of identical bands***	Lowest and highest molecular size (bp)	Number and proportion of bands**	Primer
2000 bp (OS1) 900 bp (OS2) 200 bp (OS2)	0	26 78.79 %	7 21.21 %	200 bp 2000 bp	33 11.78 %	SA1
0	0	24 63.16 %	14 36.84 %	200 bp 1500 bp	38 13.57 %	SA2
1800 bp (OS1) 1700 bp (OS5) 350 bp (OS5) 250 bp (OS7) 200 bp (OS2)	0	38 100 %	0	200 bp 2000 bp	38 13.57 %	SA3
1700 bp (OS1) 1200 bp (OS1) 550 bp (OS2) 450 bp (OS5) 200 bp (OS4)	0	44 100 %	0	200 bp 2000 bp	44 15.71 %	SA4
1500 bp (OS2) 1600 bp (OS5) 900 bp (OS6) 200 bp (OS2)	2000 bp 800 bp	47 100 %	0	350 bp 3000 bp	47 16.78 %	SA5
400 bp (OS3) 300 bp (OS7)	0	45 100 %	0	250 bp 2000 bp	45 16.07 %	SA6
1100 bp (OS5) 450 bp (OS5)	0	35 100 %	0	350 bp 2000 bp	35 12.5 %	SA7
Total	*N.A	259 92.5 %	21 7.5 %	*N.A	280	Total

*Table 2: ISSR primers and amplification results for the studied species. *bp number of base pairs *N.A not applicable **Number and proportion of bands with total percentage ***Variation in band size and band number, including the highest and lowest number of amplified sites for each primer and species*

Location and size of unique bands (bp)*	Location and size of absent bands (bp)	Number of bands detected	Number of identical bands***	Molecular size range (bp)	Number and proportion of bands**	Primer
1700 bp (SA4) 650 bp (SA4) 450 bp (SA6) 300 bp (SA1) 1800 bp (SA3) 1200 bp (SA4)	0	33 100 %	0	300 bp 2000 bp	33 11.78 %	OS1
3000 bp (SA5) 1100 bp (SA5) 550 bp (SA4)	0	41 100 %	0	200 bp 3000 bp	41 14.64 %	OS2
2500 bp (SA5) 1100 bp (SA3) 300 bp (SA1) 200 bp (SA2)	0	39 100 %	0	200 bp 2500 bp	39 13.92 %	OS3
2500 bp (SA5) 2200 bp (SA4) 1300 bp (SA4) 1100 bp (SA5) 900 bp (SA1) 450 bp (SA6) 250 bp (SA6) 200 bp (SA4)	800 bp (SA1)	45 100 %	0	200 bp 3000 bp	45 16.07 %	OS4
3000 bp (SA5) 2500 bp (SA5) 1800 bp (SA6) 1700 bp (SA3) 1600 bp (SA5) 1500 bp (SA4) 450 bp (SA7) 300 bp (SA1) 250 bp (SA6)	0	40 100 %	0	250 bp 3000 bp	40 14.29 %	OS5
2500 bp (SA5) 2200 bp (SA5) 1800 bp (SA6) 1700 bp (SA7) 400 bp (SA2) 200 bp (SA2)	1000 bp (SA7) 600 bp (SA7)	42 100 %	0	200 bp 2500 bp	42 15 %	OS6
1700 bp (SA7) 900 bp (SA4)	800 bp (SA1)	40 100 %	0	250 bp 2000 bp	40 14.29 %	OS7
Total	*N.A	259 92.5 %	21 7.5 %	*N.A	280 100 %	Total

*Table 3: Results of ISSR reactions according to the studied species. *bp number of base pairs *N.A not applicable **Number and proportion of bands with total percentage *** Variation in band size and band number, including the highest and lowest number of amplified sites for each primer and species*

DISCUSSION

The ISSR primers successfully amplified DNA sequences between simple sequence repeats, enabling discrimination among *Oscillatoria* species. All seven primers generated reproducible banding patterns, although variation among primers was observed, with certain bands absent in the relevant species reported in Table 3, likely due to the absence of complementary sequences in their DNA. Such absence is crucial in molecular taxonomy, as it highlights interspecific genetic variability [13].

A total of 280 bands were amplified, with 259 (92.5%) being polymorphic and 21 (7.5%) identical across species. Polymorphic bands provide valuable markers for distinguishing closely related taxa. Shared bands, observed predominantly with primers SA1 and SA2, indicate conserved loci identical across species, which can aid in the classification of unknown strains [14].

Among the primers, SA3 and SA4 produced the highest number of unique bands (5 each), while SA5 revealed two absent bands, further indicating its discriminatory potential. Primers SA6 and SA7 showed moderate variation, each producing two unique bands, and SA1 generated three. SA2 yielded no unique or absent bands, suggesting it targets highly conserved regions [14]

Species- wise, OS5 exhibited the highest number of unique bands (9), suggesting greater genomic divergence, followed by OS4 (8), OS1 and OS6 (6 each), OS3 (4), OS2 (3), and OS7 (2). Absent bands were detected in OS4 (1), OS6 (2), and OS7 (1), emphasizing their divergence at certain loci [14,15].

The dendrogram derived from ISSR data segregated the seven *Oscillatoria* species into two main clusters. Cluster I comprised OS3, OS6, and OS7, showing close genetic similarity. OS4 and OS5 were in Cluster II and were closer to each other than OS1 and OS2. The efficacy of ISSR in determining the relationships at the species level was further established by these patterns of clustering [15,16].

The patterns of observed genetic variability are consistent with previous observations by Mühlsteinová *et al.* (2018), where ITS sequencing revealed significant genetic diversity in *Oscillatoria* spp. The authors highlighted that application of morphology alone has its own shortcomings because genetically distinct strains tend to exhibit small phenotypic differences [17]. This trend highlights the need to use molecular protocols in the taxonomy of cyanobacteria.

Recent work by Gemmill *et al.* (2021) indicates ISSR markers possess greater resolving power to separate taxonomic boundaries, particularly in close genera with similar morphology [10]. The findings of the current work further support the view that ISSR analysis is effective in systematics research on cyanobacteria, complementing earlier work by Araújo *et al.* (2023) and

Unat (2022) [13,14]. Due to the comparative dearth of ISSR-based research on Iraqi freshwater cyanobacteria, these findings are valuable additions to the literature and highlight the need for further genomic research in this area.

The research emphasizes the necessity of multi- locus molecular techniques to elucidate relationships in the *Oscillatoria* ceae. Such research may establish the need for taxonomic revisions or the designation of new genera [17].

The current study effectively utilized ISSR markers to assess the genetic diversity between seven *Oscillatoria* strains from freshwater ecosystems in Anbar Governorate. Cluster analysis provided two distinct groupings: *O. annae* (OS3), *Oscillatoria sp.* (OS6), and *O. sancta* (OS7), with the most comparable genetic affinities. The second group contained *O. margaritifera* (OS4) and *O. proteus Skuja* (OS5), with closer similarity to one another than to *O. acuta* (OS1) or *O. princeps Vaucher* (OS2). These results not only validate the usefulness of ISSR markers in phylogenetic research but also provide valuable reference data that can guide subsequent studies to explore functional genes that are important in ecologically relevant processes, such as toxin biosynthesis and nitrogen metabolism, thereby further explaining the role of these organisms in the environment.

CONFLICT OF INTEREST

The authors have no conflicts of interest.

AUTHOR CONTRIBUTIONS

Conceptualization: Harith Kamil Buniya

Methodology, Software, Formal Analysis, Data Curation: Harith Kamil Buniya, Safa Ghassan Hameed

Writing of original draft: Safa Ghassan Hameed

Review, editing, and supervision: Harith Kamil Buniya

All authors have read and approved the final manuscript.

ACKNOWLEDGMENT

The authors express gratitude to the Department of Biology, College of Education for Pure Sciences, University of Anbar, for providing laboratory facilities to complete this research.

REFERENCES

1. Kaur M, Bhatia S, Gupta U, Decker E, Tak Y,
2. Nair S, Bhimba BV. Bioactive potency of cyanobacteria
3. Rani VU, Perumal UE, Palanivel S. Morphology and taxonomy of
4. Nesrullah Z, Al-Rubaiee G, Zaki NH. Evaluation of the effect of
5. Mulluye K, Bogale Y, Bayle D, Atnafu Y. Review on microalgae potential innovative biotechnological applications. Biosciences Biotechnology Research Asia, (2023); 20(1): 35–43.
6. Ayswaria R, Vijayan J, Nathan VK. Antimicrobial peptides derived from microalgae for combating antibiotic resistance: current status and prospects. Cell Biochemistry and Function, (2023); 41(2): 142–151.
7. Komárek J, Anagnostidis K. Cyanoprokaryota
8. Varalakshmi P, Arunkumar R, Chanthramohan L, Nagarajan M, Babu T. Study of anti-inflammatory, anti-diabetic, and analgesic activity of
9. Ragland A, Kumaresan V, Arumugam N. 2014; 1-712. Saras Publication
10. Gemmill CE, Grierson ER. Inter-Simple Sequence Repeats (ISSR), Microsatellite-Primed Genomic Profiling Using Universal Primers. Molecular Plant Taxonomy: Methods in Molecular Biology, (2021); 2222(2021): 249-262.
11. Kanaan A (2013) Analysis of the Genetic Diversity in Syrian Shami Goats. Damascus University.
12. Maulood BK, Hassan FM, Al-Lami AA, Toma JJ, Ismail AM. Checklist of algal flora in Iraq. 2013; 94. Ministry of Environment, Baghdad
13. Araújo FD, Bruno RD, Arriel NH, De Medeiros EP, De Lima LM,
14. Unat I. Different types and modifications of polymerase chain reaction. Journal of Molecular Virology and Immunology, (2022); 3(4): 159-176.
15. Holtzen S (2020) Genetic analysis of tarantulas in the genus Brachypelma using Inter-Simple Sequence Repeat (ISSR). Eastern Michigan University.
16. Nei M, Li WH. Mathematical model for studying genetic variation in terms of restriction

endonucleases. Proceedings of the National Academy of Sciences, (1979); 76(10): 5269–5273.
17. Mühlsteinová R, Hauer T, De Ley P, Pietrasiak N. Seeking the true