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Biosynthetic Gene Clusters and Anatoxin-a Detection in a Whole Genome of *Microcoleus* sp. HI-ES

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ABSTRACT

Cyanobacteria is a group of microorganisms that are known to produce a wide range of biologically active metabolites as well as a wide range of cyanotoxins such as anatoxins. In this study, the biosynthetic gene clusters (BGCs) that are responsible for bioactive secondary compounds production were genetically identified in the whole genome sequence of *Microcoleus* sp. HI-ES using antiSMASH 6.0 tool. Moreover, the gene sequence that is responsible for anatoxin production was also identified using both RAST tool and the NCBI database. The results have revealed that *Microcoleus* sp. HI-ES harbor 10 BGCs responsible for NRPS-like, NRPS, resorcinol, terpenes and T1PKS. The pharmaceutical impacts and the biological activities of these BGCs were also discussed. The anatoxin gene sequences detected in *Microcoleus* sp. HI-ES genome was closest homology to anatoxin gene sequences in *Oscillatoria nigroviridis* PCC 7112, *Planktothrix agardhii*, PCC 7805, *Anabaena cylindrica* PCC 7122, *Aphanizomenon flosaquae* KM1D3 and *Nostoc* sp. TCL240-02 with homology percentages of 93.71%, 81.55%, 75.60%, 74.21% and 74.73%, respectively. Other cyanobacteria genera that show query coverage and/or homology less than 70% were also reported. Finally, the phylogenetic tree based on anatoxin gene sequences between *Microcoleus* sp. HI-ES and cyanobacteria genera found in the NCBI was constructed using MEGA-X program with1000x replicates.

Keywords: Anatoxin, BGCs, *Microcoleus* sp. HI-ES, phylogenetic tree, Whole genome.

INTRODUCTION

Cyanobacteria are well-known to produce large varieties of bioactive secondary metabolites (Nandagopal *et al.*, 2021; Pooja, 2022). Cyanobacteria species isolated from different habitats have been studied for their produced bioactive compounds. For example, species belonging to *Lyngbya* such as *L. bouillonii* and *L. majuscula* which were isolated from marine environments have exhibited antifungal, anticancer, anti-molluscidal, antiproliferative and cytotoxic activities (Chang *et al.*, 2004; Ramaswamy *et al.*, 2007; Al-Hayani *et al*., 2020). Another example, are some strains belonging to the genus *Nostoc* which were isolated from terrestrial sources such as *Nostoc*. sp. GSV 224 and *Nostoc*. sp. ATCC 53789; and *N. calcicola* which is isolated from wastewater and have shown different capabilities including antitoxin, antifungal, and antibacterial effects besides inhibiting protease activities (Magarvey *et al.*, 2006; Gupta and Vyas, 2021). Other cyanobacterial species which belong to different genera such as *Hapalosiphon, Fischerella, Westiella, Microcystis, Planktothrix*, *Anabaena*, *Nodularia* and *Stigonema* have been also reported in many studied to produce diverse types of bioactive metabolites (Hillwig *et al.*, 2014; Nandagopal *et al.*, 2021). On the other hand, no studies have been carried out on other cyanobacterial genera such as *Microcoleus* which pollutes the fresh water. *Microcoleus* is a member genus of cyanobacteria in the family Microcoleaceae. The genus *Microcoleus* was proposed for the first time in 1892 by Gomont (Strunecký *et al.*, 2013). *Microcoleus* currently consists of 39 species with validly published and correct names and two subspecies (Parte *et al.*, 2020). The type species of *Microcoleus* is *M. vaginatus* and the GenBank accession number of its 16S rRNA gene is EF654072.1 (Siegesmund *et al.*, 2008). One of the most important characteristics of this genera is that it has been found to produce high concentrations of anatoxins (neurotoxins) including anatoxin-a (ATX), dihydroanatoxin-a (dhATX), homoanatoxin-a (HTX) and dihydrohomoanatoxin-a (dhHTX) (Kelly *et al.*, 2019; Puddick *et al.*, 2021). Nevertheless; non-toxin producing *Microcoleus* strains have also been reported (Heath *et al.*, 2016). Advance studies at the genomic level have revealed that cyanobacterial genomes are rich sources of biosynthetic gene clusters that function for the biosynthesis of biologically active compounds (Larsen *et al.*, 2021; Passi *et al.*, 2022). In this study, the complete secondary metabolite biosynthesis gene clusters in *Microcoleus* sp. HI-ES genome sequence, which was isolated from a freshwater sample in Iraq, were predicted using the antibiotics and Secondary Metabolite Analysis SHell (antiSMASH 6.0) database and tool version 6.0 (Blin *et al.*, 2021).

MATERIALS AND METHODS

Isolation and purification of *Microcoleus* sp. HI-ES

Microcoleus sp. HI-ES was successfully isolated and purified from a freshwater sample collected from the Mosul dam lake (approximately 60 km north of Nineveh Governorate, Iraq) according to Rippka *et al.* (1979) with some modifications. The modifications were involved by preparing agarose-plates (GB-11 liquid medium, 0.75% agarose) and by adding lysozyme (100 µg/ml), imipenem (100 µg/ml) and streptomycin (100 µg/ml) to eliminate the presence of contaminated bacteria; and cycloheximide (20 µg/ml) to eliminate the presence of eukaryotes. The purity of the culture was tested by spreading a loopful of *Microcoleus* sp. HI-ES filaments on nutrient agar plates and incubated at 37°C for 72 hours. The plates were checked constantly for the growth and were judged pure if no heterotrophic bacteria were observed.

DNA extraction for whole genome sequencing

To obtain high DNA yield, approximately 100 mg of *Microcoleus* sp. HI-ES biomass was suspended in 750 µl of Sodium Phosphate Buffer (10 mM), 200 µl of lysozyme (0.5 mg/ml) buffer, and 50 µl of Proteinase K (20 mg/ml) and incubated at 55°C for 2 hours. The suspension was freezed at -196ºC for 25-30 seconds and followed by 5 min of thawing at 37°C. This step was repeated five times (Bhardwaj *et al.*, 2019). Following the freeze-thaw cycles, the procedure of Presto™ Mini gDNA Bacteria Kit (Geneaid, Taiwan) was used for genomic DNA extraction of

Microcoleus sp. HI-ES. Ultimately, the genomic DNA was sent to Macrogen biotechnology company (South Korea) for whole genome sequencing.

Genome sequencing, assembly and annotation

To obtain the whole genome sequence, the sequence raw data reads obtained from the Macrogen biotechnology company were *de novo* assembled to the whole genome using SPAdes 3.5 (Bankevich *et al.*, 2012). The assembled whole genome was annotated using the RAST server (Overbeek *et al.*, 2014).

Biosynthetic gene clusters (BGCs) detection

The whole genome sequence of *Microcoleus* sp. HI-ES in GenBank format, which was annotated using the RAST server, was uploaded to the anti-SMASH 6.0 (antibiotics and Secondary Metabolites Analysis Shell) bacterial version database (Blin *et al.*, 2021). Before submitting, features of Known Cluster Blast, Cluster Blast, Sub Cluster Blast and MIBiG cluster comparison had been chosen for downstream analysis.

Anatoxin-a gene analysis

The full-length sequence of anatoxin gene was extracted from the annotated genome of *Microcoleus* sp. HI-ES. The RAST tool annotated 509 nucleotides of anatoxin-a. The BLASTn program was then used to search for homology of anatoxin-a gene in *Microcoleus* sp. HI-ES with entire sequences which are available on the NCBI GenBank database (Table 1). The sequences were retrieved and downloaded in FASTA format and aligned using MEGA-X software (Kumar *et al.*, 2018) with MUSCLE alignment algorithm. A Neighbor-Joining phylogenetic tree from 1000X replicates was constructed.

RESULTS AND DISCUSSION

Biosynthetic Gene Clusters (BGCs) Detection

In total, ten secondary biosynthetic gene clusters were predicted in *Microcoleus* sp. HI-ES, among which four NRPS-like (clusters 1, 7, 8 and 10), one NRPS (cluster 2), two resorcinol (clusters 3 and 5), two terpenes (cluster 4 and 9) and one T1PKS (cluster 6) Fig. (1). Pharmaceutical significance of non-ribosomal peptide and polyketide compounds is well-known and their pathways have received much attention (Komaki and Tamura, 2020; Iacovelli *et al.*, 2021).

Microcoleus

Region	Type	From	To
Region 1	NRPS-like	394,282	434,182
Region 2	NRPS	441,940	483,655
Region 3	resorcinol	813,549	853,543
Region 4	terpene	1,006,881	1,027,541
Region 5	resorcinol	2,216,655	2,257,650
Region 6	T1PKS	2,869,130	2,911,535
Region 7	NRPS-like	3,082,038	3,134,166
Region 8	NRPS-like	3,221,744	3,262,342
Region 9	terpene	3,600,861	3,621,790
Region 10	NRPS-like	4,209,974	4,250,678

Fig. 1: **Type of biosynthetic gene clusters with their location predicted in whole genome sequence of *Microcoleus* sp. HI-ES obtained from anti-SMASH 6.0 tool.**

Clusters 1, 2, 7 and 10 showed similarities to 1-nonadecene (100%), vioprolide (25%), puwainaphycin/minutissamide (44%) and anabaenopeptin (71%) known BGCs respectively Fig. (2). 1-nonadecene has been reported to have antimicrobial activities against some pathogens including *Staphylococcus aureus* and *Escherichia coli* (Smaoui *et al.*, 2012; Al-Rawi and Altaee, 2019). Vioprolides have been shown to be promising natural compounds with potent anticancer activities (Kirsch *et al.*, 2020). Puwainaphycin and minutissamide have been reported to have biological activities against human pathogenic fungi such as *Aspergillus fumigatus* (Hájek *et al.*, 2021). Anabaenopeptins (APs) are bioactive nonrobosomal peptides (NRPs) produced by diverse genera of cyanobacteria such as *Anabaena*, *Nostoc*, *Microcystis*, *Lyngbya*, *Planktothrix* and *Brasilonema* (Monteiro *et al.*, 2021). Studies have confirmed that APs can inhibit several proteolytic enzymes especially carboxypeptidase A (Spoof *et al.*, 2015) and protein phosphatases 1 and 2A (Mazur-Marzec *et al.*, 2015). HIV-1 transcription, cancer and cardiac hypertrophy were influenced by the inhibition effect of protein phosphatase 1 (McConnell and Wadzinski, 2009). Moreover, APs have been reported to cause harmful effects on some tested microorganisms such as the amoeba *Acanthamoeba castellanii* (Urrutia-Cordero *et al.*, 2013) and the nematode *Caenorhabditis elegans* (Lenz *et al.*, 2019).

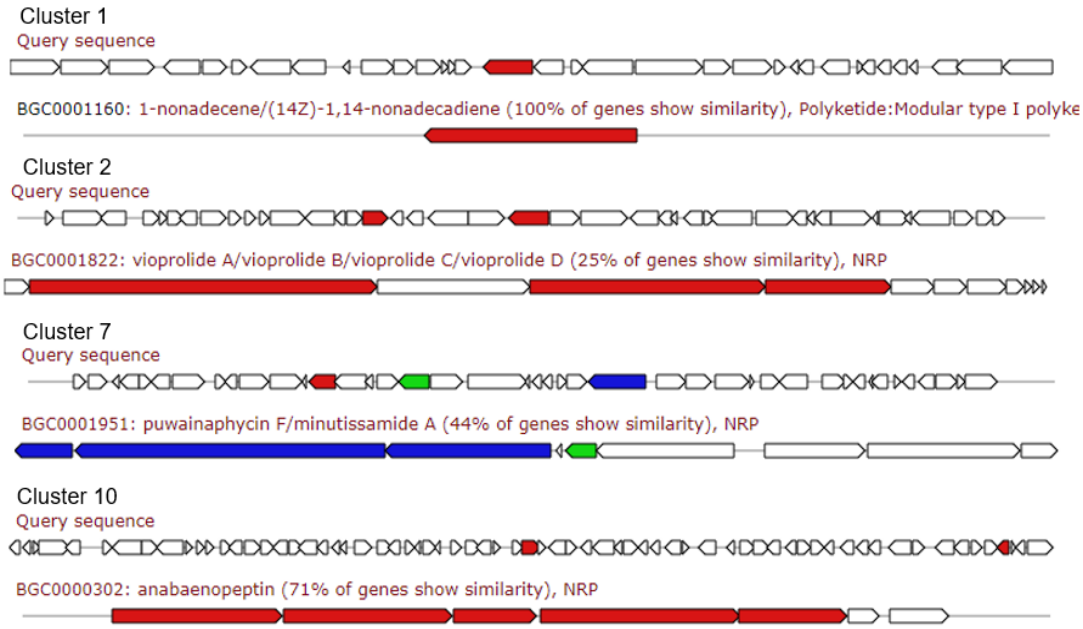

Fig. 2: **Known BGCs (clusters 1, 2, 7 and 10) output obtained from the antiSMASH 6.0 database. The percentage of genes in the query cluster that are present in the hit cluster is included as extra information.**

On the other hand, Terpene biosynthetic gene clusters are frequently identified in cyanobacteria members (Micallef *et al.*, 2015). They provide protection against extreme environmental stress such as high salt, pH and freezing/thawing (Agger *et al.*, 2008). Other BGCs in *Microcoleus* sp. HI-ES were found to share different low similarity in gene contents of unknown clusters which could potentially be sources for novel bioactive compounds.

Anatoxin-a gene detection

The results obtained from the NCBI have shown that *Microcoleus* sp. HI-ES contain an anatoxin-a gene in its genome that shared different homology with other cyanobacterial genera (Table 1). The results have displayed that anatoxin gene is found in seventeen cyanobacteria genera belonging to different families in cyanobacteria. The results of the constructed phylogenetic tree

Fig. (3) have revealed that anatoxin gene sequence of *Microcoleus* sp. HI-ES was closely related to *Oscillatoria nigroviridis* PCC 7112 (accession no. CP003614.1), *Planktothrix agardhii*, stain PCC 7805 (accession no. KU665242.1), *Anabaena cylindrica* PCC 7122 (accession no. AP018167.1), *Aphanizomenon flosaquae* KM1D3 (accession no. CP051528.1) and *Nostoc* sp. TCL240-02 (CP040094.1) with homology percentages of 93.71%, 81.55%, 75.60%, 74.21% and 74.73% respectively. Not surprising that anatoxin gene of *O. nigroviridis* PCC 7112 seems to be within the closest anatoxin gene to *Microcoleus* sp. HI-ES that clustered on the same clade Fig. (3). This is because *O. nigroviridis* PCC 7112 is known as *Microcoleus* sp. in Genome Taxonomy Database (https://gtdb.ecogenomic.org/) (Tee *et al.*, 2021). Moreover, *Microcoleus* and *Oscillatoria* are among the most common genera producing anatoxin-a that were reported in many studies (Bouma-Gregson *et al.*, 2019; Puddick *et al.*, 2021).

On the other hand, in addition to the genera mentioned in (Table 1), species of many other cyanobacterial genera such as *Cuspidothrix issatschenkoi*, *Cylindrospermum stagnale*, *Geitlerinema carotinum*, *Hydrocoleum lyngbyaceum* and *Lyngbya wollei* were also reported to produce wide ranges of anatoxins (Smith *et al.*, 2019; Wood *et al.*, 2020). However, the anatoxin gene sequences of these strains mentioned above have not been found in the NCBI GenBank database as the presence of their toxins were detected and analyzed using biochemical and immunological methods such as HPLC, LC-MS/MS and ELISA.

Table 1: Related anatoxin-a genes in different cyanobacteria species with their accession numbers that show homology with *Microcoleus sp.* HI-ES anatoxin genes retrieved from NCBI database

Cyanobacteria species	Query Coverage	Homology	Accession No.
*Oscillatoria nigro*viridis PCC 7112	100%	93.71%	CP003614.1
Planktothrix agardhii, stain PCC 7805	99%	81.55%	KU665242.1
Anabaena cylindrica PCC 7122	97%	75.60%	AP018167.1
Aphanizomenon flosaquae KM1D3	93%	74.21%	CP051528.1
Nostoc sp. TCL240-02	90%	74.73%	CP040094.1
*Calothri*x sp. PCC 7716	67%	69.34%	AP025018.1
Nodularia spumigena UHCC 0039	44%	71.62%	CP020114.1
Fremyella diplosiphon NIES-3275	62%	68.42%	AP018233.1
Microcystis aeruginosa FD4	60%	67.71%	CP046973.1
Sphaerospermopsis torquesreginae ITEP-024	93.3	67.38%	CP080598.1
Fischerella sp. NIES-4106	56%	69.07%	AP018298.1
Trichormus variabilis 0441	41%	68.90%	CP047242.1
Dolichospermum heterosporum TAC447	42%	68.21%	CP099464.1
Crinalium epipsammum PCC 9333	45%	67.67%	CP003620.1
Leptodesmis sichuanensis PKUAC	29%	69.80%	CP075171.1
Stanieria cyanosphaera PCC 7437	29%	69.54%	CP003653.1

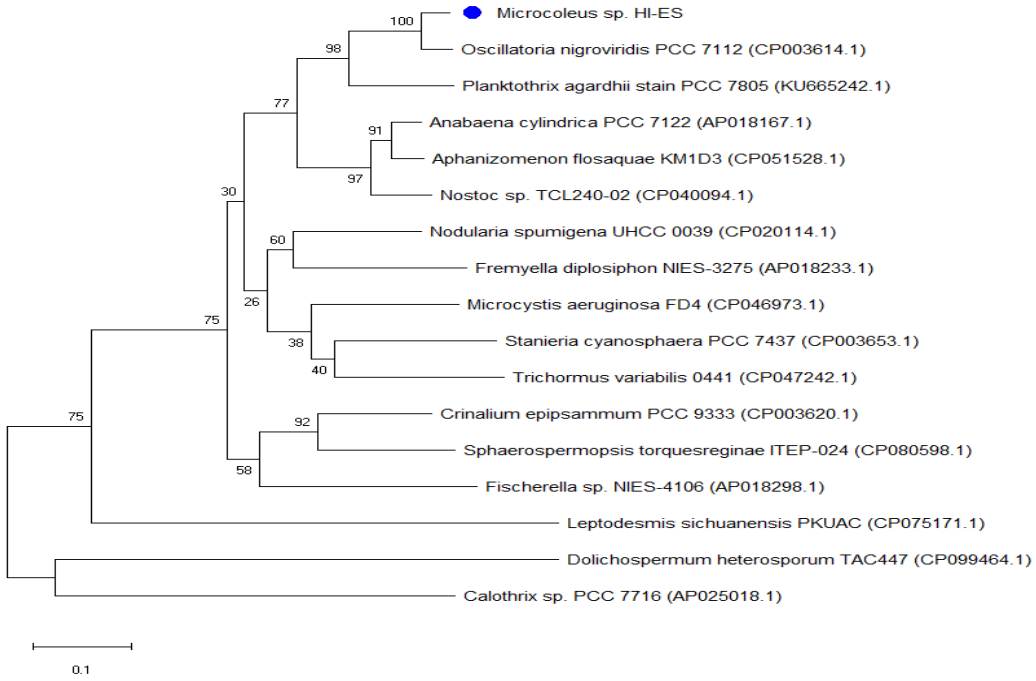

Fig. 3: **A Neighbor-Joining phylogenetic tree showing the homology between the anatoxin gene sequence of *Microcoleus* sp. HI-ES (indicated by blue circle) and the homology of anatoxin gene sequences in cyanobacteria genera found in NCBI. The tree was constructed using MEGA-X tool with1000x bootstraps. Sequence accession numbers are given in parentheses.**

CONCLUSIONS

This study has found the potential use of whole genome sequence and some specialized tools and databases for detection of BGCs and anatoxin gene sequences in a whole genome sequence. Moreover, the study revealed that local cyanobacteria strain *Microcoleus* sp. HI-ES harbor several BGCs that might produce biologically and medically active compounds. In addition, the genome analysis indicates that *Microcoleus* sp. HI-ES contains a gene sequence of anatoxin that shared high homology with other cyanobacterial genera.

REFERENCES

Agger, S.A.; Lopez-Gallego, F.; Hoye, T.R.; Schmidt-Dannert, C. (2008). Identification of sesquiterpene synthases from Nostoc punctiforme PCC 73102 and Nostoc sp. strain PCC 7120. *J. Bacteriol.*, **190**(18), 6084-6096. https://doi.org/10.1128/jb.00759-08

Al-Hayani, A.; Kannah, A.; Khaleel, H. (2020). Identifying the absorption range of Chara sp. Algae for different concentrations of cadmium. *Raf. J. Sci.*, **29**(1), 26-40. https://doi.org/10.33899/rjs.2020.164479

Al-Rawi, A.M.; Altaee, M.F. (2019). Contamination of domestic well water in Nineveh Governorate with some pathogenic bacteria and detection on its ability to biofilm formation. *Raf. J. Sci.*, **28**(3), 90-104.

Bankevich, A.; Nurk, S.; Antipov, D.; Gurevich, A.A.; Dvorkin, M.; Kulikov, A.S.; Pevzner, P.A. (2012). SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J. Comput. Biol.*, **19(**5), 455-477. https://doi.org/10.1089/cmb.2012.0021

Bhardwaj, B.; Agrawal, A.; Ledwani, L.; Vaiphei, S. T.; Jain, S. (2019). An efficient method for DNA extraction from cyanobacteria isolated from hypersaline and marine environments. *J. of Phyco..*, **55**(3), 733-737. https://doi.org/10.1111/jpy.12847

Blin, K.; Shaw, S.; Kloosterman, A.M.; Charlop-Powers, Z.; Van Wezel, G.P.; Medema, M.H.; Weber, T. (2021). antiSMASH 6.0: improving cluster detection and comparison capabilities. *Nucleic Acids Res*., **49**(W1), W29-W35. https://doi.org/10.1093/nar/gkab335

Bouma-Gregson, K.; Olm, M.R.; Probst, A.J.; Anantharaman, K.; Power, M.E.; Banfield, J.F. (2019). Impacts of microbial assemblage and environmental conditions on the distribution of anatoxin-a producing cyanobacteria within a river network. *The ISME J.*, **13**(6), 1618-1634. https://doi.org/10.1038/s41396-019-0374-3

Chang, Z.; Sitachitta, N.; Rossi, J.V.; Roberts, M.A.; Flatt, P.M.; Jia, J.; Gerwick, W. H. (2004). Biosynthetic pathway and gene cluster analysis of Curacin A, an Antitubulin natural product from the tropical Marine Cyanobacterium Lyngbya m ajuscula. *J. Nat. Prod.*, **67**(8), 1356-1367. https://doi.org/10.1021/np0499261

Gupta, V.; Vyas, D. (2021). Antimicrobial effect of a cyclic peptide Nostophycin isolated from wastewater cyanobacteria, Nostoc calcicola. *Curr. Bot.*, **12**, 94-101. Doi: 10.25081/cb.2021.v12.6612

Hájek, J.; Bieringer, S.; Voráčová, K.; Macho, M.; Saurav, K.; Delawská, K.; Hrouzek, P. (2021). Semi-synthetic puwainaphycin/minutissamide cyclic lipopeptides with improved antifungal activity and limited cytotoxicity. *RSC advances.*, **11**(49), 30873-30886. https://doi.org/10.1039/D1RA04882A

Heath, M.; Wood, S.A.; Young, R.G.; Ryan, K.G. (2016). The role of nitrogen and phosphorus in regulating Phormidium sp.(cyanobacteria) growth and anatoxin production. *FEMS Microbiol. Ecology.*, **92**(3), fiw021. https://doi.org/10.1093/femsec/fiw021

Hillwig, M.L.; Zhu, Q.; Liu, X. (2014). Biosynthesis of ambiguine indole alkaloids in cyanobacterium Fischerella ambiguate. *ACS Chem. Biol.*, **9**(2), 372-377. https://doi.org/10.1021/cb400681n https://doi.org/10.1371/journal.pone.0220422.

Iacovelli, R.; Bovenberg, R.A.; Driessen, A.J. (2021). Nonribosomal peptide synthetases and their biotechnological potential in Penicillium rubens. *J. Ind. Microbiol. Biotechnol.*, **48**(7-8), kuab045. https://Doi.org/10.1093/jimb/kuab045

Kelly, L. T.; Bouma-Gregson, K.; Puddick, J.; Fadness, R.; Ryan, K.G.; Davis, T.W.; Wood, S.A. (2019). Multiple cyanotoxin congeners produced by sub-dominant cyanobacterial taxa in riverine cyanobacterial and algal mats. PloS one, 14(12),e0220422.

Kirsch, V.C.; Orgler, C.; Braig, S.; Jeremias, I.; Auerbach, D.; Müller, R.; Sieber, S.A. (2020). The cytotoxic natural product vioprolide A targets nucleolar protein 14, which is essential for ribosome biogenesis. *Angew. Chem. Int. Ed.*, **59**(4), 1595-1600. https://doi.org/10.1002/anie.201911158

Komaki, H.; Tamura, T. (2020). Polyketide synthase and nonribosomal peptide synthetase gene clusters in type strains of the genus Phytohabitans. *Life.*, **10**(11), 257. https://doi.org/10.3390/life10110257

Kumar, S.; Stecher, G.; Li, M.; Knyaz, C.; Tamura, K. (2018). MEGA X: molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution, 35(6): 1547. https://doi.org/10.1093/molbev/msy096 .

Larsen, J.S.; Pearson, L.A.; Neilan, B. A. (2021). Genome mining and evolutionary analysis reveal diverse type III polyketide synthase pathways in cyanobacteria. *Genome Biol. Evol.*, **13**(4), evab056. Doi:10.1093/gbe/evab056

Lenz, K.A.; Miller, T.R.; Ma, H. (2019). Anabaenopeptins and cyanopeptolins induce systemic toxicity effects in a model organism the nematode Caenorhabditis elegans. *Chemosphere.*, **214**, 60-69. https://Doi.org/10.1016/j.chemosphere.2018.09.076

Magarvey, N.A.; Beck, Z.Q.; Golakoti, T.; Ding, Y.; Huber, U.; Hemscheidt, T.K.; Sherman, D. H. (2006). Biosynthetic characterization and chemoenzymatic assembly of the cryptophycins. Potent anticancer agents from Nostoc cyanobionts. *ACS Chem. Biol.*, **1**(12), 766-779. https://doi.org/10.1021/cb6004307

Mazur-Marzec, H.; Błaszczyk, A.; Felczykowska, A.; Hohlfeld, N.; Kobos, J.; Toruńska-Sitarz, A.; Węgrzyn, G. (2015). Baltic cyanobacteria–a source of biologically active compounds. *Eur. J. Phycol.*, **50**(3), 343-360. https://doi.org/10.1080/09670262.2015.1062563

McConnell, J.L.; Wadzinski, B.E. (2009). Targeting protein serine/threonine phosphatases for drug development. *Mol. Pharmacol.*, **75**(6), 1249-1261. Doi: https://doi.org/10.1124/mol.108.053140

Micallef, M.L.; D'Agostino, P.M.; Sharma, D.; Viswanathan, R.; Moffitt, M. C. (2015). Genome mining for natural product biosynthetic gene clusters in the Subsection V cyanobacteria. *BMC genomics.*, **16**(1), 1-20. https://doi.org/10.1186/s12864-015-1855-z

Monteiro, P.R.; do Amaral, S.C.; Siqueira, A.S.; Xavier, L.P.; Santos, A.V. (2021). Anabaenopeptins: What we know so far. *Toxins.*, **13**(8), 522. https://doi.org/10.3390/toxins13080522

Nandagopal, P.; Steven, A.N.; Chan, L.W.; Rahmat, Z.; Jamaluddin, H.; Mohd Noh, N. I. (2021). Bioactive metabolites produced by cyanobacteria for growth adaptation and their pharmacological properties. *Biology.*, **10**(10), 1061. https://doi.org/10.3390/biology10101061

Overbeek, R.; Olson, R.; Pusch, G.D.; Olsen, G.J.; Davis, J.J.; Disz, T.; Stevens, R. (2014). The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). *Nucleic Acids Res.*, **42**(D1), D206-D214. https://doi.org/10.1093/nar/gkt1226

Parte, A.C.; Carbasse, J.S.; Meier-Kolthoff, J.P.; Reimer, L.C.; Göker, M. (2020). List of Prokaryotic names with Standing in Nomenclature (LPSN) moves to the DSMZ. *Int. J. Syst. Evol. Microbiol.*, **70**(11), 5607. https://doi.org/10.1099/ijsem.0.004332

Passi, A.; Tibocha-Bonilla, J.D.; Kumar, M.; Tec-Campos, D.; Zengler, K.; Zuniga, C. (2022). Genome-scale metabolic modeling enables in-depth understanding of big data. *Metabolites.*, **12**(1), 14. https://doi.org/10.3390/metabo12010014

Pooja, S. (2022). Insight into the potential cyanobacterial metabolites and their screening strategies. *Biosc. Biotechnol. Res. Asia.*, **19**(1), 255. DOI:10.13005/bbra/2983

Puddick, J.; van Ginkel, R.; Page, C.D.; Murray, J.S.; Greenhough, H.E.; Bowater, J.; Finch, S.C. (2021). Acute toxicity of dihydroanatoxin-a from Microcoleus autumnalis in comparison to anatoxin-a. *Chemosphere.*, **263**,127937. https://doi.org/10.1016/j.chemosphere.2020.127937

Ramaswamy, A.V.; Sorrels, C.M.; Gerwick, W.H. (2007). Cloning and biochemical characterization of the hectochlorin biosynthetic gene cluster from the marine cyanobacterium Lyngbya majuscula. *J. Nat. Prod.*, **70**(12), 1977-1986. https://doi.org/10.1021/np0704250

Rippka, R.; Deruelles, J.; Waterbury, J.B.; Herdman, M.; Stanier, R.Y. (1979). Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *Microbiol.*, **111**(1), 1-61. https://doi.org/10.1099/00221287-111-1-1

Siegesmund, M.A.; Johansen, J.R.; Karsten, U.; Friedl, T. (2008). Coleofasciculus gen. nov. (Cyanobacteria): Morphological and Molecular Criteria for Revision of the Genus microcoleus gomont 1. *J. Phycol.*, **44**(6), 1572-1585. https://doi.org/10.1111/j.1529-8817.2008.00604.x

Smaoui, S.; Mathieu, F.; Elleuch, L.; Coppel, Y.; Merlina, G.; Karray-Rebai, I.; Mellouli, L. (2012). Taxonomy, purification and chemical characterization of four bioactive compounds from new Streptomyces sp. TN256 strain. *World J. Microbiol. Biotechnol.*, **28**, 793-804. https://doi.org/10.1007/s11274-011-0872-6

Smith, Z.J.; Martin, R.M.; Wei, B.; Wilhelm, S.W.; Boyer, G.L. (2019). Spatial and temporal variation in paralytic shellfish toxin production by benthic Microseira (Lyngbya) wollei in a freshwater New York Lake. *Toxins.*, **11**(1), 44. https://doi.org/10.3390/toxins11010044

Spoof, L.; Błaszczyk, A.; Meriluoto, J.; Cegłowska, M.; Mazur-Marzec, H. (2015). Structures and activity of new anabaenopeptins produced by Baltic Sea cyanobacteria. *Marine Drugs.*, **14**(1), 8. https://doi.org/10.3390/md14010008

Strunecký, O.; Komárek, J.; Johansen, J.; Lukešová, A.; Elster, J. (2013). Molecular and morphological criteria for revision of the genus M icrocoleus (O scillatoriales, C yanobacteria). *J. Phycol.*, **49**(6), 1167-1180. https://doi.org/10.3390/md14010008

Tee, H.S.; Wood, S.A.; Bouma-Gregson, K.; Lear, G.; Handley, K.M. (2021). Genome streamlining, plasticity, and metabolic versatility distinguish co-occurring toxic and nontoxic cyanobacterial strains of Microcoleus. *Mbio.*, **12(**5), e02235-21. https://doi.org/10.1128/mbio.02235-21

Urrutia-Cordero, P.; Agha, R., Cirés, S.; Lezcano, M.Á.; Sánchez-Contreras, M.; Waara, K.O.; Quesada, A. (2013). Effects of harmful cyanobacteria on the freshwater pathogenic free-living amoeba

Acanthamoeba castellanii. *Aquat. Toxicol.*, **130**, 9-17. https://doi.org/10.1016/j.aquatox.2012.12.019

Wood, S.A.; Kelly, L.; Bouma-Gregson, K.; Humbert, J.F.; Laughinghouse H.D.; Lazorchak, J.; Davis, T.W. (2020). Toxic benthic freshwater cyanobacterial proliferations: Challenges and solutions for enhancing knowledge and improving monitoring and mitigation. *Freshw. Biol.*, **65**(10), 1824. https://doi.org/10.1111%2Ffwb.13532

التحري عن مجموعات الجينات الحيوية والذيفان نوع a في كامل الجينوم البكتيري *Microcoleus* sp. HI- ES

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الملخص

البكتريا الخضراء المزرقة هي مجموعة من الكائنات الحية الدقيقة والمعروفة في انتاجها لمدى واسع من المركبات الفعالة حيويا وايضا انتاجها للسموم . توصلت الدراسة الى الكشف وتشخيص مجاميع الجينات الحيوية وكذلك الجين المسؤول عن انتاج الذيفان نوع a في بكتريا *Microcoleus* sp. HI-ES باستخدام برامج متخصصة مثل antiSMASH و RAST وبنك الجنات العالمي NCBI. اضافة الى ذلك ان بكتريا *Microcoleus* sp. HI-ES تحتوي على عشرة تجمعات جينية حيوية مختلفة مسؤولة عن انتاج مركبات فعالة حيويا تعود الى مجاميع فعالة مختلفة مع مناقشة الاهمية الصيدلانية والفعالية الحيوية لها. بينت الدراسة ايضا ان الذيفان الموجود في بكتريا *Microcoleus* sp. HI-ES كان أكثر قربا وتطابق في الانواع البكتيرية الاتية *Oscillatoria nigroviridis* PCC 7112 *Planktothrix agardhii*, PCC 7805, *Anabaena cylindrica* PCC 7122, *Aphanizomenon flosaquae* KM1D3 و *Nostoc* sp. TCL240-02 وبنسبة تشابة 93.71%، 81.55%،75.60%، 74.21 %, 74.73% على التوالي. تم ايضا رسم الشجرة الوراثية اعتمادا على تتابع جين الذيفان المشخص في بكتريا *Microcoleus* sp. HI-ES وتتابع جينات الذيفان لأنواع بكتريا الخضراء المزرقة الموجودة في بنك الجينات العالمي. في هذه الدراسة تم التعرف جينيا على مجموعات الجينات التخليقية الحيوية المسؤولة عن انتاج المركبات الثانوية (BGCs) النشطة بيولوجيًا في تسلسل الجينوم الكامل لـ *Microcoleus* sp. HI-ES باستخدام antiSMASH 6.0، علاوة على ذلك تم تحديد تسلسل الجين المسؤول عن إنتاج anatoxin باستخدام RAST.

الكلمات الدالة: الذيفان –a، الجينات الحيوية، الجينوم الكلي، بكتريا *Microcoleus* sp. HI-ES، شجرة وراثية.