Genome mining of Oscillatoria nigro-viridis genome reveals mycosporine like amino acids genes

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ABSTRACT

Mycosporine-like amino acids (MAA) are small molecules that contain a central cyclohexenone or cyclohexenimine ring and a wide variety of substitutions. MAAs absorb UV light that can be destructive to biological molecules like DNA, RNA, Proteins, etc. MAAs are widespread in the microbial world and have been reported in many microorganisms including eubacteria, cyanobacteria, micro- and macro-algae, as well as some multicellular organisms. In the present investigation we analyzed the genome of cyanobacteria Oscillatoria nigro-viridis to identify the possible set of genes that might be involved in the biosynthesis of these compounds. Genome mining identified a combination of genes, a predicted DHQ synthase and O-methyltransferase. The present study provides an insight into the genes of O. nigro-viridis involved in MAA biosynthesis and thus widens the field of research for these evolutionary and industrially important compounds.

KEYWORDS
Mycosporine like amino acids; Cyanobacteria; Genome mining; UV protection.

INTRODUCTION

Depletion of the ozone layer has increased the harmful ultraviolet radiation with wavelengths between 280–400 nm reaching Earth. These radiations, UV-A (wavelengths 315–400 nm) and particularly UV-B (wavelengths 315-400 nm) have most damaging effects on planktonic organisms, particularly on their DNA and other cellular components\(^1\). These organisms have evolved various strategies to cope with the harmful radiations\(^2\). One of the strategies is synthesizing or accumulating a variety of photo protective compounds, such as pigments melanin, carotenoids, etc or mycosporine-like amino acids (MAAs), that directly or indirectly absorb the energy of the solar radiation. MAAs are intracellular, colorless water-soluble compounds, having their maximum absorption between 309 and 360 nm, which is in the range of the damaging UV-B and UV-A wavelengths\(^8\). Chromophores responsible for the UVR absorbance in MAAs are derived from the shikimic pathway, which is used in phenylalanine synthesis. The synthesis of MAAs has been reported to occur in bacteria\(^1\), cyanobacteria\(^4\), phytoplankton and macro-algae\(^6\) but not in animals, where these compounds are supposed to be accumulated either via the food chain or synthesized by their symbiotic algal partner due to the lack of the shikimate pathway. Some MAAs also protect cells from reactive oxygen species.
Reactive oxygen species can be created during photosynthesis; further supporting the idea that MAAs provide protection from UV light. Mycosporine-glycine is a MAA that provides antioxidant protection even before oxidative stress response genes and antioxidant enzymes are induced\(^\text{7,9,11}\). MAAs from marine organisms are imine derivatives of mycosporines which contain an amino-cyclohexenimine ring linked to an amino acid, amino alcohol or amino group having absorption maxima between 320 and 360 nm\(^\text{2,8}\).

Many studies have been conducted regarding the biosynthetic route of these ecologically, evolutionarily and industrially important compounds\(^\text{3}\). However very few studies have been conducted to identify the genes involved in the biosynthesis of these compounds. In the present investigation we conducted a bioinformatics study to identify the possible genes involved in MAA biosynthesis by analyzing the genome of a photosynthetic cyanobacterium, *Oscillatoria nigro-viridis*. We compared the identified predicted genes to genes of MAA-synthesizing and non-synthesizing Cyanobacteria. The five Cyanobacteria used for comparison are: *Anabaena variabilis* PCC 7937, *Anabaena* sp. PCC 7120, *Synechocystis* sp. PCC 6803, *Synechococcus* sp. PCC 6301 and *Nostoc punctiforme*. The genomes of these bacteria have been sequenced.

**EXPERIMENTAL METHODS**

**Experimental organisms and genome**

*O. nigro-viridis* was used in this study. Whole-genome shotgun sequencing of *O. nigro-viridis* was performed using the 454 technology (GATC, Konstanz, Germany), giving 3,865,494 bp in 66 contigs reads. Automatic annotation was carried out using the MaGe pipeline.

**Genome mining**

The cyanobacteria used in this study are fully sequenced and available at Comprehensive Microbial Resource (CMR; http://cmr.jcvi.org/tigr-scripts/CMR/CmrHomePage.cgi) and Joint genome Institute (JGI; http://www.jgi.doe.gov/). Comparison of genomic regions was performed using the service provided by CMR under gene-specific tools (http://cmr.jcvi.org/cgi-bin/CMR/CmrManual.cgi). The nucleotide sequences of promising genes were translated into amino acid sequences using the facility of the Open reading frame (ORF) finder at NCBI (http://www.ncbi.nlm.nih.gov/gorf/gorf.html) and similarity searches for the proteins were performed using the BLAST service at NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Alignments were constructed using a progressive alignment algorithm.

**RESULTS AND DISCUSSION**

Genome mining revealed that *O. nigro-viridis* possesses the 3-dehydroquininate synthase (DHQS) gene. Genomic region analysis revealed that the DHQ gene has an O-methyltransferase gene downstream to it. Further, we compared the genomic region of *O. nigro-viridis* to *A. variabilis*, *Anabaena* sp. PCC 7120, *Synechocystis* sp. PCC 6803 and *Synechococcus* sp. PCC 6301 for DHQ and O-methyltransferase genes. The results from the genomic region comparison revealed that DHQ gene has similarity to NP_485964, NP_441388 and YP_171706 of *Anabaena* sp. PCC 7120, *Synechocystis* sp. PCC 6803 and *Synechococcus* sp. PCC 6301, respectively, based on the P values. The genomic region of *O. nigro-viridis* was also compared for O-methyltransferase with other cyanobacteria. These results show that the DHQS gene and O-methyltransferase are unique and present in one reading frame.

The nucleotide sequence of these two genes was translated into the amino acid sequence and the predicted amino acid sequences were used for a BLAST search. The result revealed the presence of putative conserved domains for the DHQS super family and identified the number of sequences for predicted DHQS from cyanobacteria sequences were not similar to fungi, dinoflagellates and metazoans.

**CONCLUSION**

This study revealed a set of genes in genome of *O. nigro-viridis* which are involved in biosynthesis of MAAs. This study will provide foundation for further studies of MAAs in other photosynthetic and extremely and moderately halophilic bacteria.
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REFERENCES


