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Scytonemin synthesis in *Oscillatoria nigro-viridis*

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ABSTRACT

Scytonemin absorbs UV light that can be destructive to biological molecules like DNA, RNA, Proteins, etc. Scytonemin is widespread in the microbial world and has been reported in many microorganisms including eubacteria, cyanobacteria, micro-and macro-algae, as well as some multi-cellular organisms. In the present study we investigatedcyanobacteriumOscillatoria nigro-viridis isolated from solar lakes often exposed to UV stress, for production of scytonemin. The cyanobacterium was found to synthesize scytonemin (absorption maximum at 250 nm) when isolated andpurified,under UV stress.

The present study provides a first insight into scytonemin biosynthesis in genus Oscillatoriaeand thus widens the field of research for molecular analysis of these evolutionary and industrially important compounds.

INTRODUCTION

Cyanobacteria, including genus Oscillatoriae, are notoriously hardy organisms that can be found living almost anywhere, from the polar and alpine regions to salt and freshwater in temperate and tropical regions, and even desert soil[1-5]. The earliest cyanobacteria, before the appearance of the ozone layer, may have benefitted from the buildup of thin layers of amorphous silica around their filaments that preferentially blocked UV-C, UV-B, and UV-A in that order[6]. The ozone layer today blocks all of the UV-C and much of the UV-B of the solar spectrum, but photosynthetic organisms must still contend with UV-A and some UV-B.[6]Cyanobacteria are known to migrate deeper into the microbial mat in response to high levels of UV, but doing so also decreases their photosynthetic efficiency[7]. Scytonemin, mycosporine-like amino acids (MAAs), and biopterin glucoside are some compounds thatmembers of the Oscillatoria genus of cyanobacteria can synthesize to preferentially block UV light[8-10]. MAAs and scytonemin together are a common occurrence because scytonemin screens UV-A particularly well while MAAs are highly effective at screening UV-B[9,11]. Previous studies have demonstrated that other Oscillatoriaspecies produce UV protectant compounds when exposed to UV light and that their concentration is related to the length of UV exposure[8,9,10].These compounds have also been shown to act as radical scavengers, acting as antioxidants to reduce damage to nucleic acids and photosynthetic pigments[12,13].

Bioinformatic analysis of the O. nigro-viridis genome showed that it has genes for the production of scytonemin. These genes, first identified in Nostoc. punctiforme, were named NpR1271, NpR1272, NpR1273, NpR1274, NpR1275, and NpR1276 and code for proteins named ScyF, ScyE, ScyD, ScyC, ScyB, and ScyA, respectively[14]. Some of these genes are homologous to known enzymes that are utilized in a proposed mechanism for the synthesis of scytonemin from amino acid precursors[15].This current investigation

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seeks to discover if O. nigro-viridis synthesizes scytonemin in response to UV exposure in a similar fashion to its relatives.

EXPERIMENTAL

500 mL of growth medium BG11 was prepared by adding 0.5 g lactic acid, 0.5 g oxalic acid, 0.5 g of amino acid mixture, 1 g glycerol, 6 g sodium bicarbonate, 2 g maleic acid, 10 g yeast extract, 10 g NaCl, and 500 mL deionized water. 200 mL of growth medium was inoculated with O. nigro-viridis and incubated at 42°C for one week. A 20 mL sample of the cyanobacterial biomass was centrifuged for 20 minutes before discarding the supernatant and rinsing the pellet with deionized water and centrifuging for an additional 20 minutes. The supernatant was once again discarded and the pellet suspended in 100 mL of 20% v/v methanol solution and incubated overnight at 42°C. This solution was then centrifuged for 20 minutes before filtering the supernatant and the filtrate's absorbance at 670 nm was measured against a blank of the methanol solution. This same extraction procedure was repeated on samples of bacteria that had been exposed to UV light for 2 hours, 4 hours, 8 hours, and 12 hours.

Further purification of the filtrate was performed by HPLC using a LiCrospher RP 18 column with precolumn (5 micrometer packing; 250 x 4 mm I.D.) A mobile phase of 0.02% acetic acid with a flow rate of 1.0 mL/min was used for this purification. UV-Vis spectrometry was used to record the absorbance peaks of the different fractions so that the identity of the compound of interest could be determined by the location of its absorbance maxima.

RESULTS AND DISCUSSION

The absorbance of the samples at 670 nm increased following exposure to the UV lamp, but the concentration increase was inconsistent with respect to the length of exposure, suggesting that another factor such as the incubation time in the methanol solutionwas having a greater impact on the concentration of scytonemin. Due to time constraints, 8 days passed between the start of the incubation for the 8 hour sample and that of the 4 hour sample in the aqueous methanol solution. This may have had a greater impact than we anticipated.

TABLE 1

Exposure time (hours)	Absorbance (blank corrected)
0	0.004
2	0.124
4	0.715
8	0.025
12	0.417

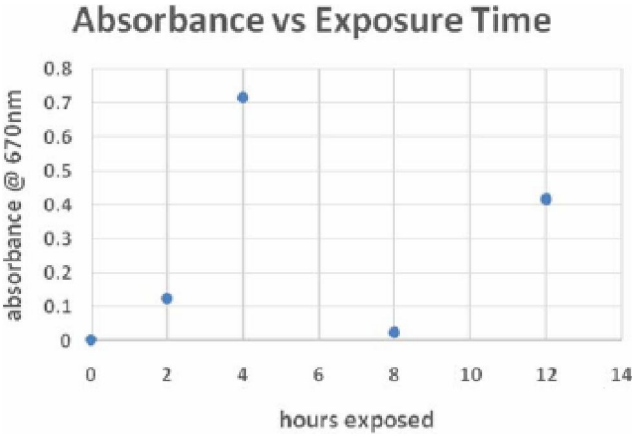

Figure 1

The primary absorbance peak of the compound of interest was near 255 nm in wavelength. The literature value of the scytonemin peak is 250 nm, which suggests that the identity of the compound is scytonemin, as hypothesized.

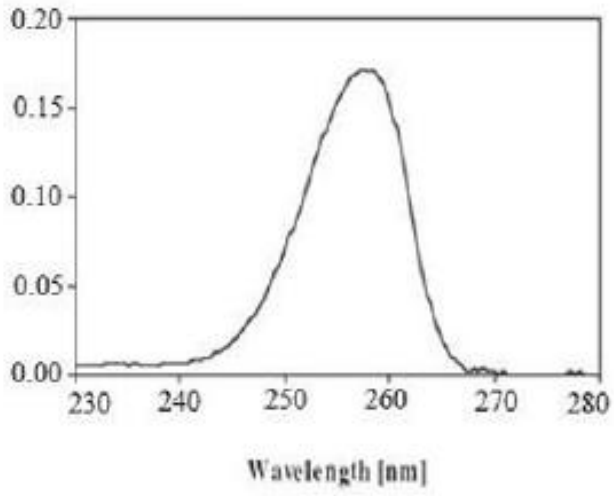

Figure 2

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