The response regulator NpF1278 is associated with sunscreen biosynthesis in the cyanobacterium Nostoc punctiforme ATCC 29133

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Abstract
Under exposure to long-wavelength ultraviolet radiation (UVA), some cyanobacteria can produce scytonemin, a yellow to brown, lipid-soluble, non-fluorescent stable sunscreen compound. A genomic region associated with scytonemin biosynthesis has been identified in the filamentous cyanobacterium Nostoc punctiforme ATCC 29133 that contains 18 adjacent genes transcribed in a single direction. Most of the genes in the upstream region of the cluster code for unique proteins involved directly in scytonemin biosynthesis. Further genomic characterization of this gene cluster in N. punctiforme has revealed a conserved putative two-component regulatory system (TCRS; NpF1277 and NpF1278) upstream and adjacent to the biosynthetic cluster that is likely involved in scytonemin regulation. In this study, it is hypothesized that if NpF1278, the putative response regulator, regulates scytonemin biosynthesis in N. punctiforme, there will be a change in the production of scytonemin following UVA radiation when the NpF1278 gene is deleted. To knock out NpF1278 from N. punctiforme, an in-frame marker-less deletion fragment complementary to the 5' and 3' ends of NpF1278 which lacks the internal sequence of the gene was constructed and was inserted into N. punctiforme through conjugation. The mutant strain was found incapable of producing scytonemin following UVA radiation as assessed using spectroscopy. To further study this system, the interaction of the NpF1278 protein with the promoter region of the scytonemin biosynthetic genes is being assessed using electrophoretic mobility shift assays (EMSA). If NpF1278 is associated with the production of scytonemin, it is expected to bind to the promoter region of the gene cluster. Thus far binding has not been observed. However, failure to bind to the promoter region can occur due to various factors which are being tested. Understanding these regulatory elements may enable future genetic manipulation to artificially control the amount of sunscreen produced for biomedical and industrial purposes.

Introduction and Objectives
Cyanobacteria are photosynthetic prokaryotes which can be found in most environments exposed to sunlight. As an adaptation to life under solar radiation, some cyanobacteria can produce the sunscreen pigment scytonemin (Fig. 1) in response to UVA radiation. Although the UVA sensing mechanism is currently unknown. Upstream from the scytonemin gene cluster in the model organism Nostoc punctiforme, there is a putative two-component regulatory system (TCRS; Figs. 2 and 3) that is conserved in over ten strains of cyanobacteria. Thus, it is hypothesized that this TCRS regulates the scytonemin biosynthetic gene cluster.

One mutant strain of N. punctiforme, NpF1278-, was obtained which lacked the scytonemin phenotype (Figs. 5-7).

Purified NpF1278 protein was obtained (Fig. 8) and preliminary EMSA binding assays suggest a shift may occur based on the presence of a high-molecular weight product following DNA staining (Fig. 9a). However, products detected after protein staining do not appear to be of the same size (Fig. 9b).

To test this hypothesis the objectives of this research were to 1) construct a mutant strain of N. punctiforme lacking NpF1278, the response regulator (RR) of this TCRS, and assay for its ability to produce scytonemin and 2) perform electrophoretic mobility shift assays (EMSA)s to determine if NpF1278 regulates scytonemin biosynthesis by binding to the promoter region of the scytonemin gene cluster.

Methods
An in-frame, marker-less deletion fragment of NpF1278 and the flanking regions was generated using fusion PCR (Fig. 4) and cloned into pPK278 for conjugation into N. punctiforme. Mutant colonies were screened for the ability to produce scytonemin and confirmed through PCR and sequencing. EMSAs were assessed on native polyacrylamide gels following binding reactions between the purified NpF1278 protein and a 199 bp DNA probe targeting the scytonemin gene cluster promoter region. Gels were stained with SYBR Green to visualize DNA and then washed and stained with SYPRO Ruby to visualize the protein.

Results
One mutant strain of N. punctiforme, NpF1278-, was obtained which lacked the scytonemin phenotype (Figs. 5-7).

Purified NpF1278 protein was obtained (Fig. 8) and preliminary EMSA binding assays suggest a shift may occur based on the presence of a high-molecular weight product following DNA staining (Fig. 9a). However, products detected after protein staining do not appear to be of the same size (Fig. 9b).

Conclusions and Significance
Based on the phenotype of the NpF1278 mutant strain, it appears as though the RR NpF1278 regulates scytonemin biosynthesis in N. punctiforme. While binding of NpF1278 with the promoter region has not been confirmed, preliminary data suggests that binding may occur. EMSAs are currently being modified to optimize binding conditions. Understanding these regulatory elements may enable future genetic manipulation to artificially control the amount of sunscreen produced for biomedical and industrial purposes.

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Works Cited