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Analysis of a Putative Histidine Kinase Associated with Sunscreen Biosynthesis in the Cyanobacterium Nostoc punctiforme ATCC 29133

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Analysis of a putative histidine kinase associated with sunscreen biosynthesis in the cyanobacterium *Nostoc punctiforme* ATCC 29133

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Abstract:

As phototrophic bacteria, cyanobacteria are continually exposed to ultraviolet radiation as they harvest solar energy. In particular, long-wavelength ultraviolet radiation (UVA) damages living cells by releasing reactive oxygen species. In phototrophs, this leads to harmful photosensitized proteins and pigments. To mitigate damage to the cell, some cyanobacteria produce a UVA-absorbing pigment in the extracellular sheath, known as scytonemin. Scytonemin is a heterocyclic, dimeric molecule that is only produced upon induction by UVA. It is suspected that it is regulated by a putative two component regulatory system (TCRS). In the cyanobacterium, *Nostoc punctiforme* ATCC29133, the putative sensor kinase, NpF1277, is found upstream from the genes for scytonemin biosynthesis and hypothesized to regulate their induction by sensing UVA. For this project, we are inactivating NpF1277 through an in-frame gene knockout in *N. punctiforme* to determine the effects on scytonemin production. NpF1277 was truncated by fusion PCR and ligated into plasmid vector, pRL278. This plasmid has been transformed into *E. coli* UC585 and will be transferred into *N. punctiforme* through biparental conjugation. The mutant will be verified by colony PCR and the ability to produce scytonemin under UVA will be assessed. Furthermore, the expression of the TCRS, both NpF1277 (sensor kinase) and NpF1278 (response regulator), will be evaluated in response to a variety of environmental conditions, including UVA, oxidative stress, and salinity.

Introduction:

Since cyanobacteria must absorb sunlight for photosynthesis they must be able to defend themselves against harmful ultraviolet radiation. The cyanobacterium *Nostoc punctiforme* (ATCC 29133), manages long-wavelength ultraviolet radiation (UVA) stress by releasing a yellow-brown sunscreen pigment known as scytonemin into the extracellular sheath. Based on comparative genomics of the scytonemin gene cluster, the production of scytonemin is hypothesized to be controlled by a putative two-component regulatory system (TCRS), NpF1277 and NpF1278 in *N. punctiforme*. It is suspected that this TCRS signals the activation of scytonemin biosynthetic genes when stimulated by UVA (Fig. 1). The putative sensor kinase for this TCRS (NpF1277) likely resides in the cell membrane and is thought to sense UVA.

Objectives:

1. To knockout the putative sensor kinase gene, NpF1277, in *N. punctiforme* and assess the effects on scytonemin production.

2. To measure the expression of NpF1277 and NpF1278 in *N. punctiforme* following UVA, oxidative, and salt stress.

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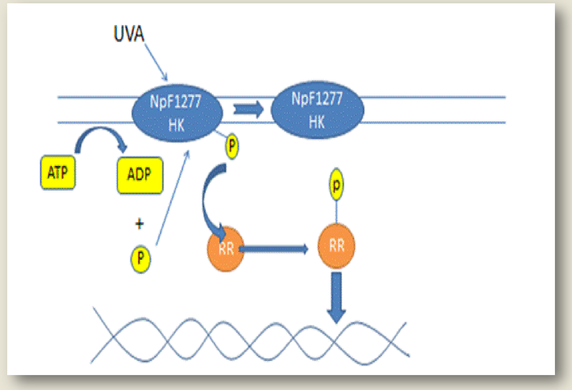

Fig. 1. Putative two-component regulatory system for scytonemin biosynthesis in *N. punctiforme.*

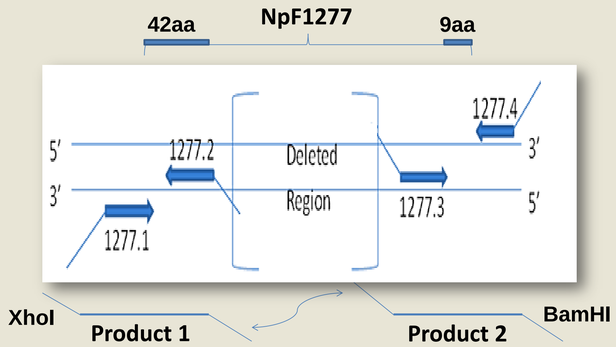

Fig. 2. Fusion PCR**.**

Materials and Methods:

NpF1277 will be knocked out in *N. punctiforme* through the insertion of a truncated, in-frame gene product generated through fusion PCR (Fig. 2). This was completed by fusing two shortened in-frame fragments from the 5' and 3' ends of NpF1277 with a complementary overhang using PCR. After the fused PCR product's size was confirmed, the truncated gene was cloned into pGEM-T (Promega). Plasmids from positive clones were purified and digested with Bam*HI* and Xho*I* in order to obtain the truncated fused gene with the complementary ends for cloning into the conjugal plasmid, pRL278. This construct was then inserted into *N. punctiforme* through biparental conjugation (Fig. 3)

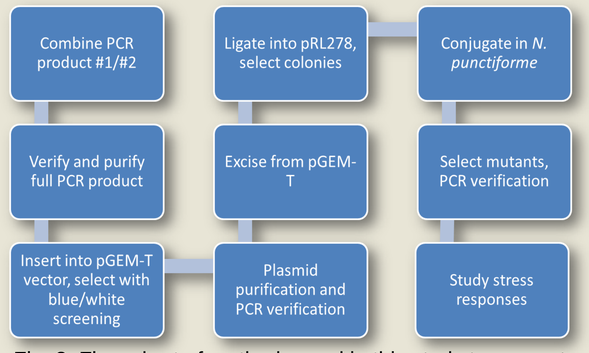

Fig. 3. Flow-chart of methods used in this study to generate knockout mutants.

Expression levels of the TCRS genes NpF1277 and NpF1278 will be measured by quantitative PCR (qPCR) following UVA, salt, and oxidative stress. Previous research did not find differential expression of these genes after 24 hrs of UVA stress (Soule et al. 2009). *N. punctiforme* will be filtered and grown in glass petri plates floating on top of AA media. After six days of white light acclimation and biomass accumulation, half of the cells will be exposed to the stress for 1 hr while the control group will not experience the stress. Cells will then be harvested immediately and frozen in liquid N for RNA isolation. Crude RNA will be treated with DNase, purified, and converted to cDNA. The cDNA will then be used in qPCR with SYBR green to measure the relative expression of stressed and unstressed cells.

Results and Discussion:

Successful completion at this point has yielded the plasmid construct pRL278 with a truncated NpF1277 insert. Currently we are waiting on the emergence of exconjugants. Once confirmed, the mutant strain will be assessed for its ability to produce scytonemin following exposure to UVA. It is hypothesized that the deletion of NpF1277 will limit scytonemin production when the mutant is exposed to UVA because it will be inhibited in its ability to sense UVA. qPCR assays are current and ongoing.

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Literature Cited:

Soule, T., Garcia-Pichel, F., & Stout, V. (2009). Gene expression patterns associated with the biosynthesis of the sunscreen scytonemin in *Nostoc punctiforme* ATCC 29133 in response to UVA radiation. *Journal of Bacteriology, 191*(14), 4639-4646. doi: 10.1128/jb.00134-09