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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 cyanobacterium N. punctiforme, isolated, and constitutive gene expression of NpF1277 with a 3’ 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 BamH1 and XhoI to obtain the truncated gene with the complementary ends for cloning into the conjugal plasmid, pRL278. This construct was then inserted into N. punctiforme through biparental conjugation.

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.

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 overlap 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 BamH1 and XhoI to obtain the truncated 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).

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 in 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.

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 assayed 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: