Design Brief

The application of cyanobacterial pigments to construct an environmentally friendly sunscreen*

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Reviewed on 4 May 2024; Accepted on 10 June 2024; Published on 26 October 2024

Ultraviolet (UV) radiation plays a crucial role in the development of skin cancer. The sun emits electromagnetic waves in the form of UV radiation, penetrating the human skin layers and contributing to skin burning, wrinkling, and aging. Overexposure to UV radiation can also produce reactive oxygen species (ROS), byproducts of oxidative metabolism, which cause untreatable cell damage and increase the risk of skin cancer. Sunscreen blocks UV rays from the skin. Even low-protection sunscreen reduces non-melanoma skin cancer by approximately 40% and lowers the risk of melanoma skin cancer by 50%. However, many of the active ingredients found in sunscreen are harsh chemicals that further skin damage. In 2021, 14 of the 16 FDA-allowed sunscreen chemicals underwent scrutinization for their lack of effectiveness and safety concerns. For example, two chemicals, oxybenzone and octinoxate, cause coral reef bleaching, ultimately killing the coral. Dramatic reductions in coral reef populations can disrupt the ecological balance and seriously impact the ecosystem. This project is an innovative method to extract two sunscreen pigments from marine bacteria, cyanobacteria, offering the opportunity to replace the traditional active ingredients in chemical sunscreens with nonhazardous pigments. This photosynthetic microbe produces two environmentally friendly pigments, scytonemin and mycosporine-like amino acids (MAAs), which shield the cell from 90% of UV-A light and absorb UV-B radiation, respectively. We will directly induce the production of scytonemin in the cyanobacterium Nostoc punctiforme and engineer Escherichia coli to overexpress the gene required for the biosynthesis of MAAs, synthesized mycosporine-glycine gene (MysC). Combining these pigments with inactive but beneficial ingredients commonly found in other sunscreens produces an environmentally friendly sunscreen that will benefit both the ecosystem and humans.

Keywords: Sunscreen, ultraviolet radiation, scytonemin, mycosporine-like amino acids (MAAs), cyanobacteria

Human skin is composed of two main layers: the epidermis, the visible outermost layer, and the dermis, the layer underneath it ("Epidermis," 2017). Ultraviolet (UV) radiation is part of the electromagnetic radiation that reaches the Earth from the sun and can penetrate the layers of human skin based on the UV radiation's wavelength (Hölzle & Hönigsmann, 2005). These wavelengths are

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classified into three types (Figure 1). UV-A (315–400 nm) has the longest wavelength among the three types and accounts for 95% of the UV radiation reaching the Earth's surface (*Radiation: Ultraviolet*, 2016). It can reach the deeper layer of the skin, the dermis, and is responsible for skin burning and wrinkling (*Radiation: Ultraviolet*, 2016). UV-B (280–315 nm) has a medium wavelength. Though it does not penetrate the skin layers as deeply as UV-A, it contributes to the thickening of the epidermis, which is potentially cancerous ("Hyperplasia," n.d.).

UV-C (100–280 nm) has the shortest wavelength among the three but is the most harmful type of UV radiation and can cause severe burns and eye injuries (Radiation: Ultraviolet, 2016). Fortunately, UV-C is completely filtered by the ozone layer in the Earth's atmosphere [Ultraviolet (UV), 2020].

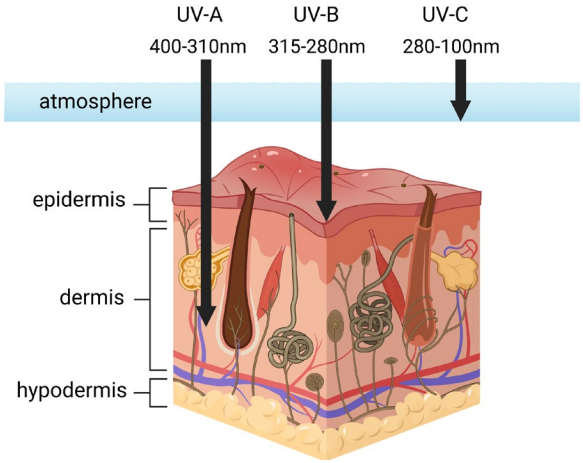

Figure 1. Different types of UV rays penetrating the skin.

Since UV-C and 90–95% of UV-B radiation are absorbed by the stratospheric ozone layer and do not reach the ground, the UV radiation types humans come in contact with are mainly UV-A and a small amount of UV-B (Singh et al., 2021).

Excessive exposure to UV radiation is a major risk factor for developing skin cancer. UV radiation can damage the basic building blocks of skin cells, including DNA and proteins. The damage can result in genetic defects, mutations, and premature aging. If left unrepaired, this cellular damage may ultimately lead to skin cancer. ("UV Radiation," n.d.).

Sunscreen is an effective protective agent against UV radiation ("How Do I Protect," n.d.). All sunscreens are composed of two types of ingredients: active and inactive. Active ingredients are responsible for the protection of the skin, while inactive ingredients contribute to the consistency and texture of the sunscreen ("Sunscreen: How to Help," n.d.). Sunscreens are categorized into two types based on their active ingredients: physical blockers (also referred to as mineral sunscreens) and chemical absorbers. The key difference between the two is that physical sunscreen stays on the skin and shields UV rays away, while chemical sunscreen sinks into the skin and absorbs UV radiation (Bramlet, 2016).

While sunscreen products on the market protect human skin, many of the active ingredients contained in the product damage the marine ecosystem. Popular active ingredients, such as oxybenzone and octinoxate, were banned in several regions, including Hawaii, Florida's Key West, and the western Pacific nation of Palau, due to their harmful effects on coral reefs and marine life ("Coalition Petitions," 2021). When harmful active ingredients such as oxybenzone and octinoxate enter the marine ecosystem, the coral's ability to defend itself from bleaching is inhibited (*Coral Bleaching*, 2015; *Is Your*, 2018). In addition to limiting corals' defense against bleaching, the chemicals have also been found to kill coral larvae and create phototoxins, substances that become toxic in the presence of light, when absorbed by mature coral (Bennett, 2022).

Although sunscreen application protects the skin from UV radiation damage, current research shows that harsh chemicals in modern sunscreens can also cause skin damage (Ruszkiewicz et al., 2017). In 2021, the FDA reviewed previously approved sunscreen ingredients, revealing that 14 out of 16 of the allowed chemicals were ineffective and posed safety concerns. Specifically, aminobenzoic acid (PABA) and trolamine salicylate were banned in the European Union due to their harmful effects on skin (*FDA Proposes*, n.d.). Zinc oxide and titanium dioxide were identified as the only harmless ingredients (*FDA Proposes*, n.d.). Although these ingredients were not banned,

their permissible concentrations and combinations were adjusted to ensure sunscreen effectiveness.

In light of these issues, this study will rely on the use of cyanobacteria to create our final sunscreen product. Cyanobacteria are photosynthetic microbes that flourish near the surface of inland waters (Vincent, 2009). Due to their location on the surfaces of lakes and ponds, cyanobacteria are exposed to harsh sunlight during their lifetime. To protect themselves from UV rays, many strains of cyanobacteria produce two pigments: scytonemin and mycosporine-like amino acids (MAAs). Unique to cyanobacteria, the butterfly-shaped yellowish-brown scytonemin pigment shields the cell from 90% of UV-A light (Figure 2) (Wada et al., 2013; Lewis, 2020; Assunção et al., 2022). Once isolated and placed in a solvent, scytonemin absorbs radiation at a maximum wavelength of 384 nm (Wada et al., 2013). MAAs are water-soluble pigments produced by cyanobacteria and algae (Figure 3) (Wada et al., 2015). MAAs, such as shinorine and mycosporine-glycine, have been shown to possess increased absorption of UV-B radiation while having maximum absorption of 334 nm and 331 nm, respectively (Shick et al., 1992; Whitehead & Vernet, 2000). Abiotic stressors such as pH and temperature have a limited effect on MAAs, which provide versatility and durability for sunscreen when MAAs are used as sunscreen ingredients (Kogej et al., 2006).

The antioxidative properties of scytonemin and MAAs are necessary to protect biological molecules against damage induced by UV radiation, along with their harmless effect on the marine environment (Klisch & Häder, 2008). This study aims to design a safe sunscreen for the consumer and

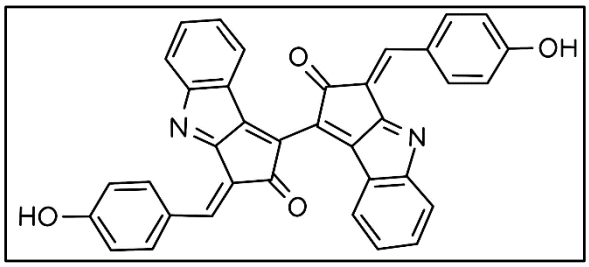

Figure 2. Chemical Structure of Scytonemin.

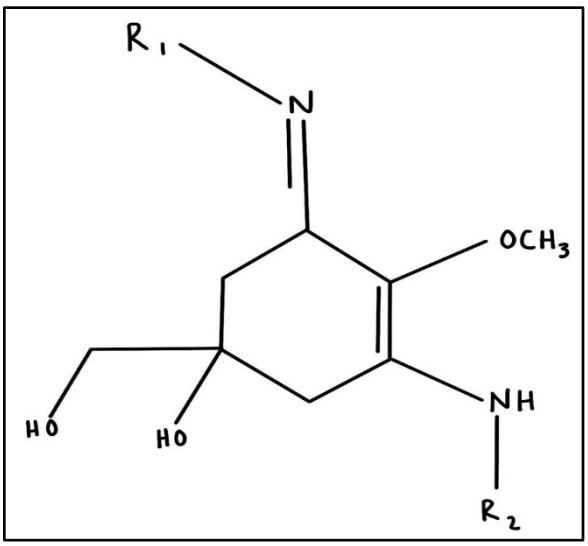

Figure 3. Chemical Structure of MAA.

the marine environment. After conducting more research, we found our previously devised restriction cloning method for the scytonemin gene cluster had been unsuccessfully attempted by other scientists due to the final dimerization step remaining an obstacle. This step involves the formation of a C-C bond between two monomer units of scytonemin. However, the exact mechanism and enzymes involved in this dimerization process are not well understood. Further research is needed to elucidate the mechanism and identify the enzymes or factors involved. Therefore, we proposed a new way to mass-produce scytonemin. Instead of inserting the gene cluster of scytonemin into a plasmid and transforming the plasmid into *Escherichia coli*, we will directly induce the expression of scytonemin biosynthesis genes in the cyanobacterium *Nostoc flagelliforme.* Though the current design will focus on the production of MAAs, the ultimate goal is to bioengineer both MAAs and scytonemin for safer

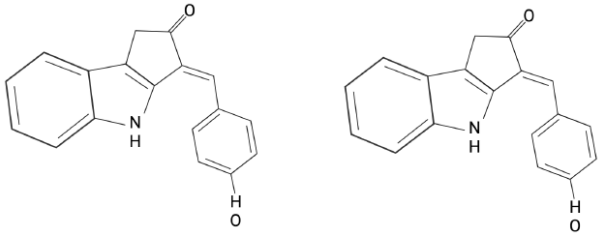

Figure 4. Two Monomers of Scytonemin.

sunscreen.

Systems level

This design utilizes pigments found in cyanobacteria, one of the largest and most diverse groups of bacteria populating inland waters, to substitute for active ingredients in modern chemical sunscreens. The chosen system involves the insertion of genes involved in the biosynthesis of MAAs into T7 Express *E. coli* by subcloning it into the pET-28a(+) plasmid and subsequent transformation. The pET-28a(+) plasmid contains a T7 promoter, a ribosome binding site (RBS), a T7 terminator, a gene that encodes for kanamycin resistance, an N-terminal his-tag, and a thrombin tag (Figure 5). After inserting the MAA gene (MysC) into the plasmid, we will transform it into T7 Express *E. coli* and grow it on LB agar plates with kanamycin. The kanamycin resistance gene in the plasmid will allow only successfully transformed bacteria to grow. After successfully culturing the desired bacteria, we will use high performance liquid chromatography (HPLC) in the MAA purification process to extract the desired MAA pigment for the final sunscreen product. The HPLC columns have an internal diameter of 4.6 mm and a length of 250 mm, with flow rates set between 1 and 2 min/mL (Cross, 2019).

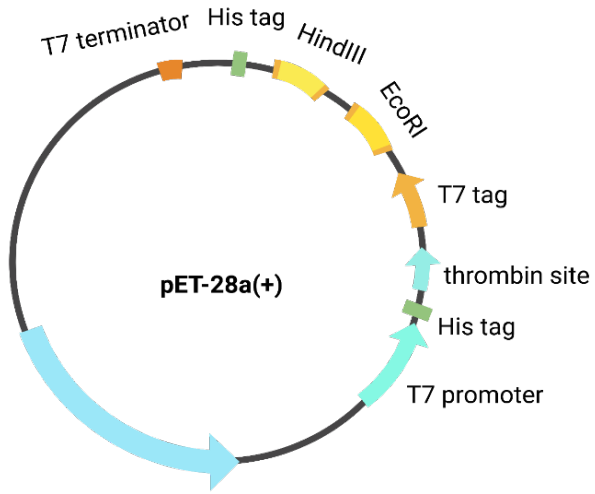

Figure 5. Plasmid pET-28a(+) Map.

Device level

The *E. coli* strain acts as a chassis to facilitate the synthesis of this project's intended protein and metabolite. T7 Express *E. coli* was chosen due to its compatibility for expression from the T7 promoter found in our plasmid and for protein production. The MysC gene was synthesized from the cyanobacterium sp. HL-69 bacteria. Our plasmid, pET-28a(+), includes a T7 promoter, an RBS, a T7 terminator, kanamycin resistance, an N-terminal his-tag, and a thrombin tag. A T7 is a strong promoter with a high transcription rate optimal for protein production.

We will insert the gene MysC that is required for the biosynthesis of MAAs into the pET-28a(+) plasmid. The gene is responsible for the encoding of ATP-grasp domain-containing protein (Chen et al., 2021). The ATP-grasp enzyme produces MAAs by conjugating glycine to 4-deoxygadusol(4-DG) (Jin et al., 2023).

Parts level

Our team chose a plasmid backbone pET-28a(+) because of its high expression level and its wide application to a range of bacterial expression systems. pET-28(+) is a bacterial expression vector with a T7 promoter, an RBS, an N-terminal his-tag, a T7 terminator, and a gene coding for kanamycin resistance. The T7 promoter along with an inducer enables the user to have control over the clone of the genes of interest with high selectivity. The RBS is a segment of an mRNA that is responsible for accuracy during the initiation of protein synthesis. The N-terminal his-tag enables purification via an immobilized metal ion affinity chromatography (IMAC) column. The T7 terminator is a naturally occurring terminator that initiates the release of the newly formed RNA. The gene that codes for kanamycin resistance acts as a selectable marker for the users to discern the successfully transformed bacteria since only the desired bacteria containing the plasmid will survive on the agar with kanamycin. The MAA biosynthetic genes are inserted into the plasmids using the

HindIII and EcoRI restriction enzyme sites. HindIII and EcoRI were chosen because their corresponding restriction sites exist within the plasmid's multiple cloning site but would not cut their respective target sequences.

So far, we found the gene sequence of MysC and the DNA sequence of the pET-28a(+) plasmid. We imported them into Benchling and inserted MysC into the plasmid through the restriction enzyme site HindIII and EcoRI. The purchase of the edited sequence will be completed through Twist Bioscience.

Safety

E. coli has been a primary model organism in scientific research for many decades. T7 Express *E. coli* is a Biosafety Level 1 organism. In order to ensure safety, personal protective equipment such as disposable gloves and an organic vapor respirator will be required to be worn during experiments, and proper protocol should be followed according to the guidelines set out by the manufacturer.

Cyanobacteria represent a large and diverse group of bacteria, each with varying biohazard safety considerations. In general, a small amount of cyanobacteria exposure is safe, but overgrowth of cyanobacteria is known to cause harmful algal blooms (HABs). These algal blooms release toxins into the environment that cannot be consumed by humans and cause the death of marine life. Cyanobacteria cultures must be properly disposed of when used in a lab to prevent them from affecting local waterways. Next, tie the upper third of the bag into a knot and affix a heat-sensitive indicator tape nearby the knot. Then, operate the autoclave for 30 minutes at 121°C and 15 psi. Once finished, dispose of the bag in the designated, red-lidded totes for laboratory waste. The bacteria culture plates should be directly collected into autoclavable bags ("Guidelines for Biological," n.d.). Although concerns exist regarding the safety of its use, the use of any species of cyanobacteria is not necessary within our study design since the study only requires cyanobacteria pigments.

Our design includes the use of the MAA gene MysC and scytonemin. Through a variety of studies, MAA and scytonemin have been shown to be ecologically and dermatologically safe (Sen & Mallick, 2021).

Discussions

In future experiments, it will be necessary to determine the specific proportion of active ingredients and inactive ingredients required for our final sunscreen formulation, as well as the appropriate inactive ingredients. The final product will be a sunscreen that combines scytonemin and MAAs as active ingredients, with glycerin (used as a natural moisturizer), dimethicone (which smooths the skin and forms a protective barrier), niacinamide (used to treat acne and protect from environmental stress), and dicaprylyl carbonate (which helps the absorption of other chemicals on the skin) as inactive ingredients. The absorption range of MAAs varies between 310 nm and 362 nm, depending on the specific type of MAA used, as different types of MAAs have varying UV radiation absorption abilities. The ability of MAAs to absorb different wavelengths of UV radiation depends on the extent of amino acid substitution, which is the replacement of one or more amino acids in a protein with another amino acid (Wada et al., 2013). Since MAAs are hydrophilic compounds, water-based formulation ingredients in sunscreen may lead to the degradation of MAAs and cause them to lose their absorption efficiency (Klisch & Häder, 2008). In the experimental test section, animal testing will be replaced with silicon models and *in vitro* methods to examine the effectiveness of the pigments' radiation-absorbing ability, which will be compared to industry-leading sunscreens. We additionally plan to conduct assessments to confirm the sunscreen's environmental harmlessness. Should the experimental outcomes validate our design's efficacy, we aspire to partner with cosmetic brands to advocate for eco-conscious sunscreen utilization and to mass-market our "cyano" sunscreen as a greener alternative to current market options. The synthesized scytonemin and MAAs might also find applications across the broader skincare industry, not limited to sun protection products.

Next steps

We discovered an implication regarding the overexpression of scytonemin in *N. flagelliforme*. However, the precise ratio and dosage of sodium bicarbonate and tryptophan used for supplementation should be thoroughly examined and determined (Gao et al., 2023). Assessing the ultimate product's ecological impacts is crucial but given the constraints of our high school's synthetic biology laboratory, we are not equipped to execute an exhaustive environmental impact evaluation presently. Nevertheless, we can experiment with the UV-shielding efficacy of our sunscreen on a preliminary basis, utilizing UV-sensitive stickers against established sunscreen brands. Moreover, we need to delve deeper into analyzing the specific components of the sunscreen, active and inert alike, and their respective proportions to achieve the desired stability, texture, and moisturizing effect. Looking ahead, we will be focused on implementing our designed experiment and gathering relevant information on stimuli factors that induce scytonemin production in *N. flagelliforme*.

Author contributions

Y.M. came up with the original idea and began introductory research. Y.M. contributed to the writing and proofreading of the paper.

Acknowledgements

Our gratitude goes to Western Reserve Academy, Ms. Jackie Thompson, and Biobuilder for their support of our program, including resources and time to pursue our invention. We are also thankful for the foundation laid by the previous members of this team. A special acknowledgement is given to our teacher, Dr. Beth Pethel, who we appreciate for introducing us to synthetic biology and cultivating our love for science.

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