Meso-biliverdinin IXα Production by Conversion of Cyanobacteria-Derived Phycocyanobilin

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Background

Biliverdins, such as meso-biliverdinin IXα (MBV), have been shown to exhibit strong antioxidant, anti-inflammatory, and anti-mutagenic properties that can be used in a variety of biomedical applications. Logan Lagoons Cyanobacteria 2 (LLC2), a non-toxic cyanobacterium isolated from the Logan Lagoons wastewater treatment plant, can be cultured in produced water derived from oil extraction operations. LLC2 contains phycocyanin (PC), a pigment-protein complex that is covalently linked to its cofactor phycocyanobilin (PCB) through thioether linkages. PCB can be readily cleaved away from PC and subsequently converted to MBV as recently described (Takemoto et al., 2016).

We aim to carry out the above bioprocess in its entirety, from LLC2 culturing using industry wastewater to MBV production through PC-PCB protein extraction, cleavage, and purification. Because cyanobacteria are known to produce toxic metabolites, our group also aims to knock-out toxin cassettes using CRISPR technology.

Cyanotech Corporation, a microalgae-producing company, has expressed interest in this project.

Constraints

Cyanobacteria growing in wastewater have a tendency uptake heavy metals such as lead. Since our product is meant for medical uses, preventing metal uptake is a top priority. Inductively Coupled Plasma-Mass Spectroscopy can be used to measure the lead levels.

Cyanobacteria also have the potential to produce toxins, such as BMAA. The amounts of these toxins will be determined via mass spectroscopy.

Figure 1. Reaction scheme for the conversion of phycocyanobilin (PCB) to meso-biliverdinin IXα (MBV).

Process

LLC2 is first cultured in BG-11 media on a platform shaker: A 1 L Rotating Algal Biofilm Reactor (RABR) is then inoculated with the liquid culture. The resulting LLC2 biofilm is then subsequently harvested and frozen at ~80 °C for later use in the extraction process.

PC is extracted from LLC2 using a two-step ammonium sulfate precipitation process. Each step is followed by centrifugation.

The PC-containing pellet is then blended in methanol and connected to a water column condenser and boiled for 16 hours with reflux to cleave the PC-PCB linkages.

The methanol-pellet solution is centrifuged to yield PCB-containing supernatant. Methanol is evaporated from the supernatant using a rotary evaporator. PCB is washed from the flask using 0.5 M HCl and centrifuged to yield a final pellet of PCB product.

Purified PCB is then added to a mixture of water, methanol, potassium carbonate, and sodium bicarbonate and boiled with constant stirring. 0.5 N HCl is added dropwise and the MBV precipitate is collected.

Figure 2. Apparatus used to cleave the phycocyanin-phycocyanobilin complex (left). Rotary evaporator used to evaporate methanol from PCB-containing solution. Images courtesy of the Takemoto Lab.

Benefits

Benefits of biliverdins have been well-studied, and some notable biomedical applications include (Takemoto et al., 2019):

- Organ and tissue allograft transplantation procedures
- Viral infections and wound-healing
- Alzheimer’s disease and diabetes

This production process of Mesobiliverdin will also provide a valuable use for produced wastewater. In turn, this will decrease the industry’s current reliance on potable water for MBV production, which will help decrease water scarcity.

References