Efficient Production of Extracellular Pigments from *Talaromyces purpurogenus* and Their Applications

August 2019

Sharad BHATNAGAR

Efficient Production of Extracellular Pigments from *Talaromyces purpurogenus* and Their Applications

A Dissertation Submitted to
the Graduate School of Life and Environmental Sciences,
the University of Tsukuba
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy in Agricultural Science
(Doctoral Program in Life Sciences and Bioengineering)

Sharad BHATNAGAR

[Abstract of Thesis]

Fungal pigment production offers an interesting alternative to the currently available chemical-based pigment syntheses owing to their natural and environmentally friendly production methods. But frequently, this process suffers from two major drawbacks. First, the production is usually intracellular and hence extraction is an issue, and second, since the pigments are generally water insolvent, it limits the use of pigment in some potential applications. Development of submerged fermentation processes for production of extracellular pigment would be preferable for their ease of extraction and scalability. Ascomycota *Talaromyces purpurogenus,* previously known as *Penicillium purpurogenum* has been touted as a potential producer of various *Monascus* like pigments under submerged conditions. *Monascus* like pigments are a group of azaphilone compounds consisting of yellow, orange and red colored pigments. In our research, we have attempted to realize this potential.

We have developed an initial system for efficient production of extracellular pigments which are highly water soluble. After initial screening for potential of pigment production, various factors influencing the pigment production such as carbon sources, nitrogen sources, minerals were studied. The media was optimized for pigment production, in terms of concentration of carbon and nitrogen source. Response surface methodology was used to statistically optimize the media composition. The concentration of produced pigment was quantified in terms of total absorption (U.O.D.) at 400 nm, 460 nm and 500 nm, which refers to the wavelengths for analysis of yellow, orange and red pigments. Using the optimized media, the yields of extracellular pigments were augmented to 93.9, 108.4 and 162.8 U.O.D. compared to 30.9, 30.8 and 43.7 U.O.D. achieved in unoptimized medium, for yellow, orange and red pigment respectively. Intracellular pigment extraction was studied to quantify the total pigment production by the organism. The intracellular pigment yield was found to be much lower

compared to the extracellular one. Using optimum medium, the total pigment yield was observed to be 105.8, 119.8 and 180.2 U.O.D. for yellow, orange and red pigments, respectively. Solid state fermentation was tried to test the pigment production potential of the organism on solid substrates.

The separation of pigment using thin layer chromatography revealed that the composite pigment is indeed a mixture of several different pigments. Colorimetric analysis of the pigment properties was undertaken to recognize the dyeing potential of the pigment. A standard curve of lyophilized pigment concentration versus absorbance at 400 nm, 460 nm and 500 nm was plotted to directly correlate the U.O.D. values with composite pigment concentration. The pigment produced was found to be thermally, chemically and photoprotective in nature. The pigment was found to have a protective effect and resistance against UV-A as well as UV-C. The UV-C resistance ability was found to be dependent on pigment concentration. The pigment also exhibits some antioxidant properties and antimicrobial capacity against gram positive organisms. Development of potential applications by harnessing these properties such as UV-protective agent and generation of metallic nanoparticles has been attempted.

Silver nanoparticles (AgNPs) were bio-fabricated by harnessing the extracellular pigment produced by *Talaromyces purpurogenus* as a potential reducing source. Along with the pigment, sodium hydroxide (NaOH) was found to be an important factor in synthesis. Pigment solutions at various pH were used to understand the role of NaOH in the reduction mechanism, by studying surface plasmon resonance of generated AgNPs. Production process was optimized for precursor concentration and time required for reduction. The AgNPs produced at optimum conditions were characterized using transmission electron microscopy, dynamic light scattering, fourier transform infrared spectroscopy, electron probe microanalyzer and zeta potential. The biogenic AgNPs were found to be spherical in nature with size distribution ranging from 5-40 nm. These AgNPs were tested for antibacterial and anticancer activity.

Minimum inhibitory concentration and minimum bactericidal concentration values for *E. coli* were found to be 32 μg/mL and 64 μg/mL, whereas for *S. epidermidis*, these values were found to be 4 μg/mL and 32 μg/mL. The biosynthesized AgNPs were also tested against HepG2, HeLa and HEK-293 cell lines. The effect was found to be concentration dependent in nature. The results also showed that AgNPs exhibited some selectivity in their action on cancer cell lines and normal cell lines. HepG2 cell line was most affected and an IC_{50} value of 11.1μg/mL was determined, suggesting a potent activity.