Natural “Green” Dyes for the Textile Industry

An Interim Report

to **the Toxic Use Reduction Institute, UMass Lowell**

by

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Introduction

The goal of this project is to develop biotechnology based processes to produce dyes from natural plant and microbial systems for use in the textile industry. The work performed till May 2001 was presented at the UMass Lowell meeting in May 2001. Based on some of the feed backs, further work was planned for the summer and its continuation for the second year after funding was awarded. Although the funding has not been realized as yet, the work has continued in anticipation. This work has mostly involved the development of a cranberry cell suspension culture system for effective growth and environmental manipulations. Part of the progress was reported to Pamela Eliason during the Green Chemistry Conference at UMass Amherst on October 29-30, 2001.

Cranberry Cell Suspension Culture Study

Background

Plant tissue and cell culture system may circumvent seasonal and geographic restriction of the plants, and as sources of useful secondary products. Plant cell cultures also provide effective systems for exploring plant physiology and plant biochemistry. The value of the technique of plant tissue and cell culture is that cell and tissue systems can be subjected to direct experimental control.

Cranberry plants (Vaccinium macrocarpon, Ericaceae) growing in outside bogs receive different external environmental stresses. The environmental stresses are divided into biotic stresses (attacked by insects, mites and fungi) and abiotic stresses (physical stresses: light, temperature, and wounding; chemical stresses: nutrient elements, water, and chemicals). At cellular level, there is no report on responses of the cranberry plants to these environmental stresses.

Flavonoids (anthocyanins and flavonols) are secondary products of plants; and have been found to have important therapeutic values including antioxidant, anticancer, and antibacterial activities. The accumulation of anthocyanins and flavonols in plants is largely influenced by various environmental stresses.

Previous results

We have successfully established cranberry cell culture system from cranberry (*Vaccinium macrocarpon*, Ericaceae) stems, leaves and leafstalks by using Gamborg¡¦ s B5 medium containing 5 mM 1-naphthaleneacetic acid (NAA), 5 μM 2,4-di-chlorophe-noxy acetic acid (2,4-D), and 2.5 mM kinetin at 25 ^{o}C in the dark.

Production of flavonoids in cranberry callus varies under different stresses such as light irradiation (red light of 660 nm and far-red light of 730 nm), temperature-changing (from 25 ^{o}C to 4 ^{o}C, or 37 ^{o}C), and wounding.

Cranberry callus produces anthocayanins only on exposure to light. Production of anthocyanins in cranberry callus was induced under continuous light irradiation. However, because anthocyanin is red color, it was observed that only top layer of the callus, which received light produces anthocayanins.

Rationale

Although callus provide more accessible uniform cells than the intact plant, the callus tissue is not uniform since only the base of the callus is exposed to the medium and the callus mass may contain cells at various stages of development. The alternative approach is to use cell suspensions. Cell suspensions show a faster growth rate, and all cells are exposed uniformly to the medium, and environment such as light. Cell suspensions are preferred for large scale and commercial production of secondary metabolic products.

Interim Results

We initiated cranberry cell suspension culture by transferring callus to liquid media of the same composition as the callus medium and gently agitating the suspension on a horizontal shaker at 150 rpm and 20 ^{o}C (Figures 1 and 2). We have found out that the growth of biomass of cranberry cell suspension culture in WP and MS liquid media were greater than B5 liquid medium (Figure 3). Interestingly, anthocyanin content of cranberry cell suspension culture in MS liquid medium was higher than in WP liquid medium (Figure 4). However, the flavonol content of cranberry cell suspension culture in WP liquid medium was higher than in MS liquid medium.

Current and Future plans

Now we are optimizing the culture conditions in order to obtain higher anthocyanin-producing cranberry cell culture system.

Fig. 1

Initiation of Suspension Cell Culture

Suspension cell cultures were initiated by transferring fresh mass callus to 15 ml of WP liquid medium and incubated on a rotary shaker at 150 rpm, 20 °C.

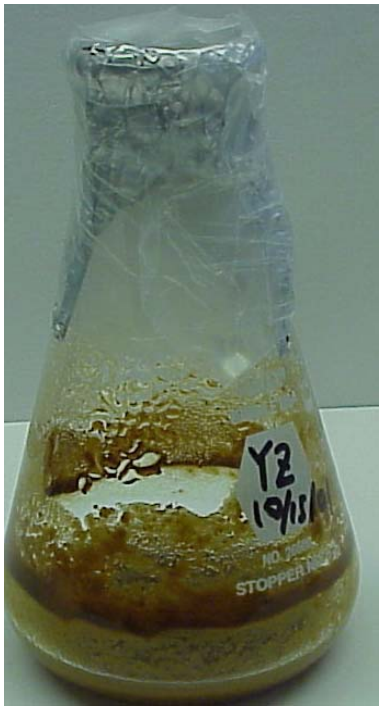

Fig. 2

Examination of Different Media (WP, B5, and MS)

Suspension cell cultures were under a continuous photosynthetic photon flux of 25μM $m^{-2}s^{-1}$ provided by cool white fluorescent lamps (F40CW-RS, General Electric Company, USA) plus continuous red light at a photon fluence rate of 12 μM $m^{-2}s^{-1}$, was obtained from six 40-w bulbs (F48T12/R-660/HO, Red, General Electric Company, USA) filtered through a red plastic sheet (Roscolux color filter # 27, ROSCO Laboratories, Port Chester, NY)

Fig. 3

Growth of Cranberry Suspension Cells in Different Media (WP, B5, and MS)

Growth was evaluated quantitatively in term of biomass, Fr.Wt. (g/flask) under different media.

Cells were separated from cell suspension medium by vacuum filtration through 3 mm Whatman filter paper, just until the pointwhen free liquid was no longer expressed, and Fr.Wt. was immediately recorded.

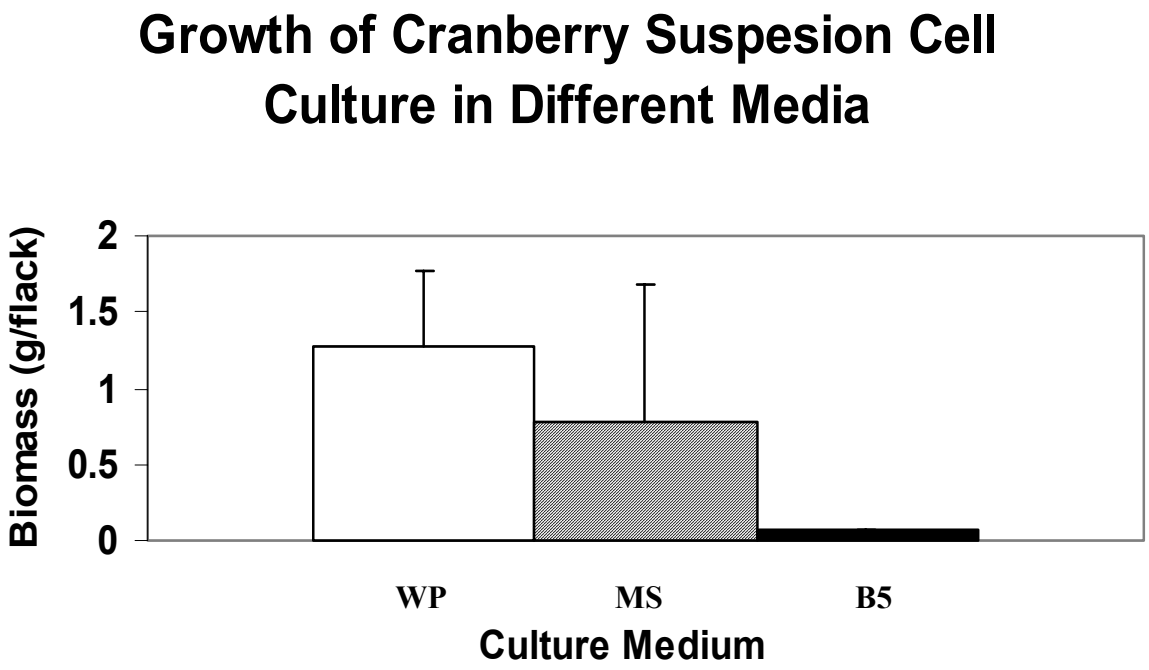

Fig. 4

Anthocyanin and Flavonol Contents in Cranberry Suspension Cell Culture

Comparison of Different Liquid Media

20.29	25.27
444	261.7