Biomass and carotenoid accumulation by green microalga PY202 under mixotrophic condition

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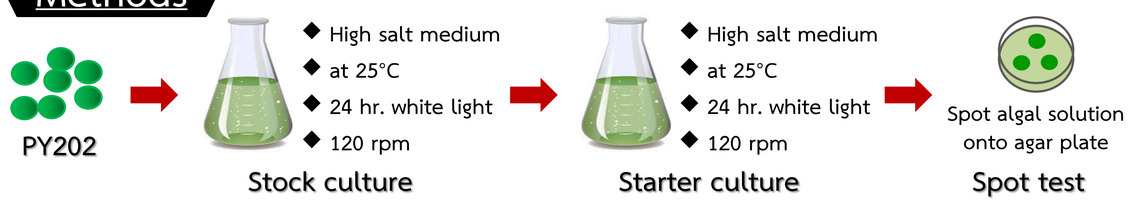
Introduction

Green microalgae already serve as a major natural source of highly valuable carotenoids. Additionally, ketocarotenoids such as astaxanthin and canthaxanthin are xanthophylls which contain a keto group. These compounds have attracted much attention, due to their strong antioxidative ability in quenching radicals, roles in cancer prevention and enhancement of immune response. They are used as additives and colorants in the food industry and aquaculture, in cosmetic, and as active ingredients in pharmaceutical products. In recent years, the potential production of ketocarotenoids from microorganisms has been a subject of intensive investigation.

Objectives

To investigate the growth pattern and the effect of glucose on biomass production and carotenoid accumulation in green microalga PY202.

Methods



1. Study of growth pattern in PY202

Autotrophic growth — HSM/Light

Heterotrophic growth — HSM/Dark/Glucose

Mixotrophic growth — HSM/Light/Glucose

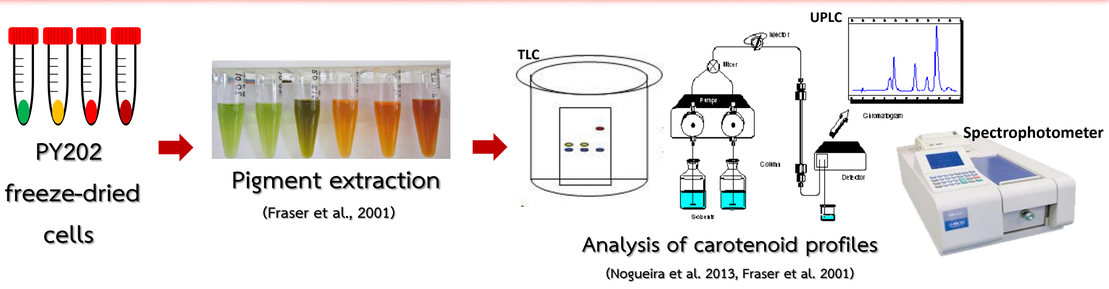
2. Effect of glucose concentration on biomass production

HSM (control) | HSM 20 g/L glucose | HSM 50 g/L glucose | HSM 100 g/L glucose

→ 24 hr. white light/25°C/35 days

PY202 cells → Lyophilisation → Biomass production via cell dry weight

3. Analysis of pigment profile



Analysis of carotenoid profiles

(Nogueira et al. 2013, Fraser et al. 2001)

Results & Discussion

Green microalga PY202

Fig.1 PY202 under light microscope

Morphologies: coccoid, solitary cells with single chloroplast and a prominent pyrenoid within (Fig.1).

Partial 18s rRNA gene sequence: ~1100 nucleotides showed 95% similarity to *Hylodesmus singaporensis*.

Morphological characters and molecular information possibly identified PY202 as *Hylodesmus* sp. However, further study on algal identification will be investigated.

1. Growth of green microalga PY202

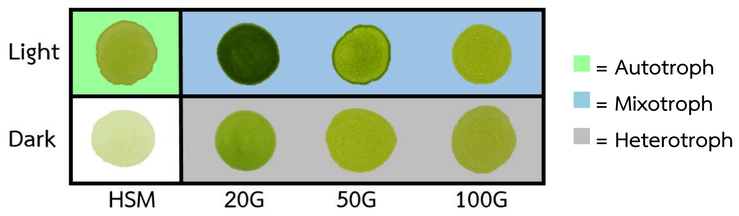


Fig.2 Growth pattern of green microalga PY202 by spot test technique at 7 days

PY202 can grow in all conditions: autotrophic (HSM/light), mixotrophic (HSM/light/glucose) and heterotrophic (HSM/dark/glucose). The best growth by spot test technique was observed under mixotrophic condition.

The best growth of PY202 was observed on HSM supplying 20 g/L glucose. The darker the green colour of algal colonies compared to control (autotrophic condition), indicates a higher cell density.

Results & Discussion (cont.)

2. Effect of glucose concentration on biomass

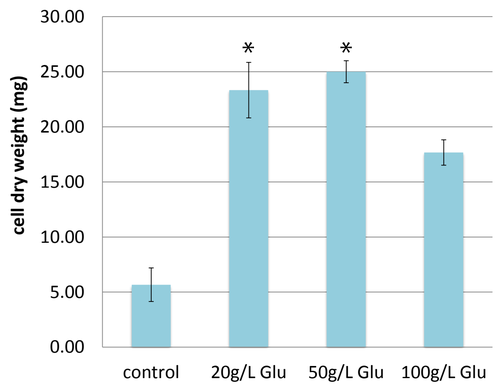


Fig.3 Glucose effect on PY202 biomass at 35 days

Supplying glucose was found to stimulate biomass production in PY202. Glucose concentration at 20 g/L and 50 g/L provided maximum cell dry weight of 23.3 ±2.5 mg and 25.0 ±1.0 mg respectively (Fig.3).

Supplementation of glucose to a mixotrophic algae led to a significant improvement in biomass production. The higher biomass of PY202 may be due to the extra carbon in its metabolic pathway from glucose. (Sun et al., 2008)

3. Effect of glucose concentration on PY202 pigment profile

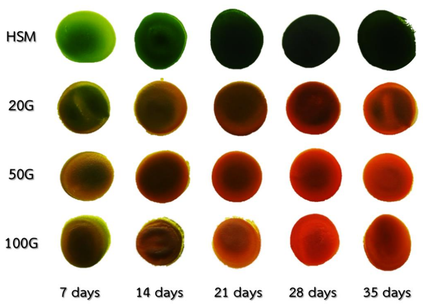


Fig.4 Glucose effect on PY202 pigment accumulation

By supplying glucose as an exogenous carbon source under white light, PY202 noticeably transformed from a green colony into a deep red colony (Fig.4). The red cells of PY202 accumulated several ketocarotenoids i.e. canthaxanthin adonixanthin 3-OH-echinenone and astaxanthin whereas canthaxanthin was predominantly detected as a major under mixotrophic culture at 35 days (Fig.5).

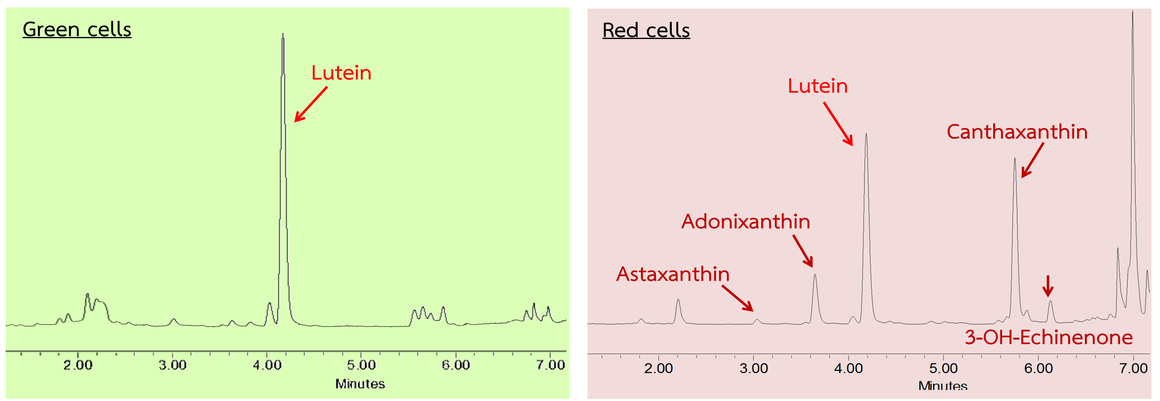


Fig.5 Ultrahigh Performance Liquid Chromatography (UPLC) chromatograms of PY202 extracts

Glucose concentration at 20 g/L and 100 g/L significantly improved total carotenoid content in PY202 (Fig.6) while canthaxanthin content enhanced 6-11.5 fold. The highest total carotenoid was discovered in 100 g/L glucose culture whereas the highest canthaxanthin production was achieved from the 20 g/L glucose culture.

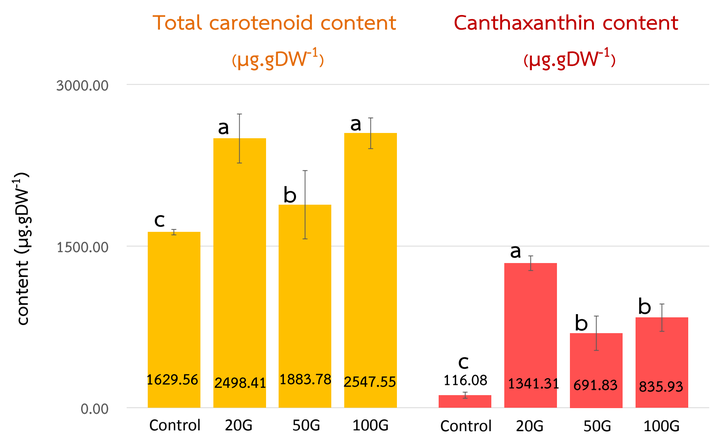


Fig.6 Glucose effect on total carotenoid and canthaxanthin content of PY202 at 35 days

Glucose concentration at 20 g/L benefit cell growth as well as canthaxanthin production. It has been proposed that the relatively high C/N ratio might stimulates ketocarotenoid biosynthesis pathway. (Sun et al., 2008)

Conclusions

- Green microalga PY202 was possibly identified as *Hylodesmus* sp. however, further identification will be sought.
- The highest cell dry weight of PY202 was obtained from glucose concentration at 20 g/L and 50 g/L glucose cultures.
- Canthaxanthin is the major ketocarotenoid found under mixotrophic condition of PY202.
- HSM supplying with light at 20 g/L glucose exhibited the highest content and production of both total carotenoid and canthaxanthin.
- The present results represent an initial step for further studies regarding the effect of different carbon sources on ketocarotenoid biosynthesis and accumulation in PY202.
- The pilot results suggested PY202 as a new potential source for the development of natural antioxidant and colourant production.

Acknowledgements

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- Department of Biology, Faculty of Science, Silpakorn University.

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