CHAPTER V

DISCUSSION

5.1 PCR fingerprints of 5 isolated strains of *Chlorella* spp. and *Scenedesmus* spp.

CRL-7 PCR fingerprints, 27f-PCR fingerprints and 1492r-PCR fingerprints of five isolates of *Chlorella* spp. and *Scenedesmus* spp. indicate that the green micro-algal isolates belong to different strains and that the strains are different from the *Chlorella* spp. and *Scenedesmus* spp. isolated by Jamkangwan (2004). All the five isolated microalgae were deposited with MIRCEN Microbiological Resources Center with the MIRCEN codes as follows: *Chlorella* sp SS1 (TISTR 8877); *Chlorella* sp. SS8 (TISTR 8878); *Scenedesmus* sp. SS4 (TISTR 8879); *Scenedesmus* sp. SS5 (TISTR 8880) and *Scenedesmus* sp. SS9 (TISTR 8881).

Since the cultures have been deposited at a culture collection center, they are available for use by researchers interested in various aspects of *Chlorella* and *Scenedesmus* biology.

5.2 β-carotene and Quercetin contents in 5 isolated strains of *Chlorella* spp. and *Scenedesmus* spp.

β-carotene contents in 5 strains of *Chlorella* spp. and *Scenedesmus* spp. as shown in Figures 4.16 and 4.17 and Table 4.2 did not distinguish between contents of 9-*cis* β-carotene and all *trans* β-carotene isomers. A C_{30} column and gradient elution are usually employed in order to determine contents of each isomer. In addition, standard β-carotene has to be subjected to photo-isomerization before being used to obtain peaks of isomerized standards by reversed-phase HPLC (Inbaraj et al., 2006). The aim of this study was to obtain β-carotene contents for use in future research to test a hypothesis that high levels of phytoene desaturase gene expression would lead to higher production and hence high β-carotene contents in *Chlorella* spp. and *Scenedesmus* spp. Therefore, it was not necessary in this work to distinguish between 9 *cis* and all trans β-carotene contents.

β-carotene contents in *Chlorella* spp.

Reversed-phase HPLC determinations showed the recovery of β-carotene contents was approximately 50% (Figure 4.14). Figures 4.16 and 4.17 showed that reversed - phase HPLC determinations yielded relatively lower contents than those obtained with spectrophotometric method. The results were obtained as such because spectrophotometric determination was not specific for β-carotene. There were more carotenoids other than β-carotene in the pigment extracts. Representative HPLC chromatograms of pigment extracts from the 5 *Chlorella* spp. and *Scenedesmus* spp. strains showed polar carotenoids (Figure D.2). Possible green algal polar carotenoids in decreasing order of polarity are auroxanthin, violaxanthin, neochrome, *cis*-neoxanthin, *cis*-lutein, 13- or 13'-*cis*-lutein, all-*tran*-lutein, zeaxanthin, and 9- or 9'-*cis*-lutein (Inbaraj et al., 2006). β-carotene contents in *Chlorella* spp. found in this study were in the range of 2,750-4,590 $\mu g.g^{-1}$ cell dry weight with continuous light intensity of approximately 3,000 lux. In 2006 Inbaraj et al. used reversed-phase HPLC with a C_{30} column to determine β-carotene contents in *Chlorella pyrenoidosa* to 4,314.3 $\mu g.g^{-1}$ cell dry weight. β-carotene contents in *Chlorella* spp. strains SS1 and SS8 were thus comparable to that reported by Inbaraj et al (2006). It is interesting to note that a lot more research has been conducted on the strain selection, production, and accumulation of β-carotene in marine green alga *Dunaliella bardawil* (BenAmortz et al., 1989; Borowitzka et al.,1993; Lers et al., 1990; Araneda et al.,1992; Markovits et al.,1993; Hejazi and Wijffels, 2003; Garcia -Gonzales et al.,2005). Perhaps stress such as heat or salinity could induce β-carotene synthesis in freshwater algae including *Chlorella* spp. and *Scenedesmus* spp.

β-carotene contents in *Scenedesmus* spp.

β-carotene contents in *Scenedesmus* spp found in this study were in the range of 290-450 $\mu g.g^{-1}$ cell dry weight with continuous light intensity of approximately 3,000 lux. Literature survey conducted so far has yielded no reports on the β-carotene contents in mid-log phase cells of *Scenedesmus* spp. However, in 1995, Bishop et al. used chemical mutagen EMS (ethyl ester of methylsulfonic acid) to obtain developmental mutant of *Scenedesmus obliquus* (mutant C-2A') and secondary mutant of *Scenedesmus obliquus* (mutant C-2A'-34). The authors employed reversed-phase

HPLC to compare pigment contents in both types of *S. obliquus* mutants under heterotrophic and mixotropic conditions at 30 °C. β-carotene contents in mutants C-2A' and C-2A'-34 grown heterotrophically in the dark for 10 days were found to be 0.016 and 0.080 μg μl^{-1} packed cell volume respectively. After the mutants were grown heterotrophically for 10 days, they were exposed to white light (10 $W.m^{-2}$) for 12 h. β-carotene contents were found to be 0.265 and 0.893 μg μl^{-1} packed cell volume respectively. β-carotene contents in C-2A' grown heterotrophically for 72 h then illuminated for 24 h under low (4 W/m^2) or high light intensities (20 W/m^2) were 16.6 or 15.9 mol β-carotene/100 mol chlorophyll a respectively. When mutant C-2A'-34 was grown heterotrophically for 72 h then exposed to low (4 W/m^2) or high light intensities (20 W/m^2), β-carotene contents were found to be 52.4 or 42.4 mol β-carotene/100 mol chlorophyll a respectively. The results indicated more β-carotene contents in mutant C-2A'-34 under all growth conditions. Since no Loroxanthin, nor Lutein, nor α-carotene were detected in the double mutant *S. obliquus* C-2A'-34, the authors concluded that the double mutant was able to produce more β-carotene under all experimental conditions due to a blockage in the lycopene to δ-carotene pathway as illustrated in Figure 5.1

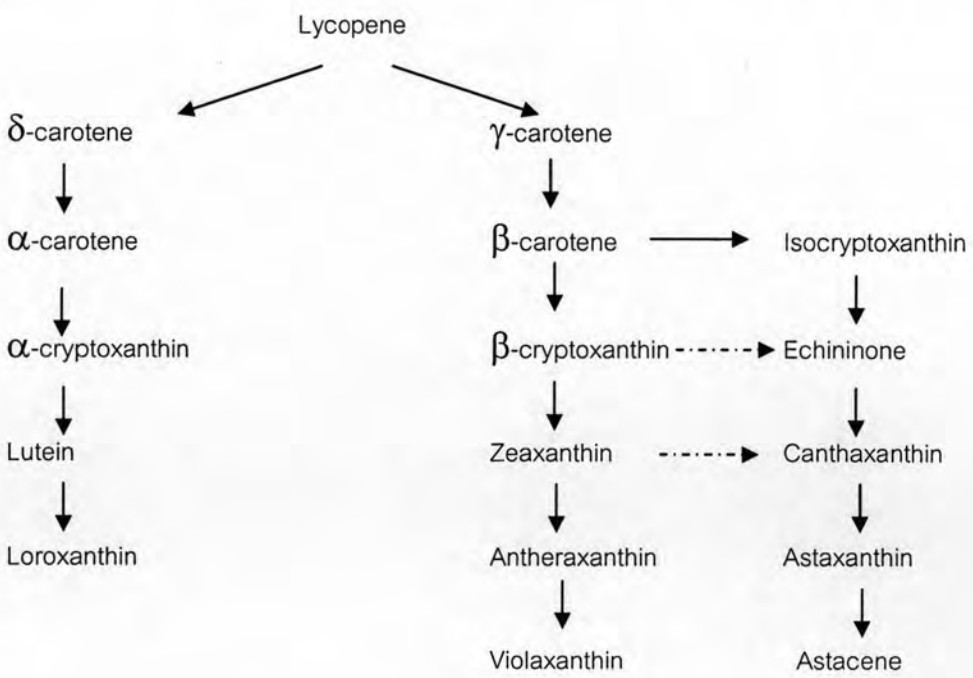

Figure 5.1 Carotenoid biosynthesis in *Scenedesmus* spp. (Bishop et al., 1995)

Since Bishop et al. (1995) reported β-carotene contents in developmental mutants of *S.obliquus* and since different units of β-carotene contents were reported by Bishop et al (1995) and in this study, it is not possible to compare β-carotene contents found in this study with those in literature. The findings point out that there is a need to conduct more research both on *Scenedesmus* taxonomy for strain identification and on β-carotene contents in mid-log phase cells. β-carotene contents should preferably be expressed in the same unit, for example, $\mu g.g^{-1}$ cell dry weight, to facilitate comparisons of β-carotene contents.

Quercetin could not be detected in *Chlorella* spp. and *Scenedesmus* spp.

Quercetin could not be detected in 200 mg – 2 g of dried algal cells. The reversed-phase HPLC system could be used to detect Quercetin as shown in the detection of Quercetin in tea leaves (Figure D.9).

More than 2 grams of algal cells may be needed to detect Quercetin (if any). Since the pathway of Quercetin production is very complicated (Figure 2.13), maybe the production of Quercetin does not contribute to growth and thus the production of Quercetin is not crucial to the survival of the green algae. In other words, the freshwater green algae may not need to spend energy to produce Quercetin. In contrast, soybeans secreted flavonoids, Genistein and Daidzein, to attract soybean rhizobia to form root nodules which lead to symbiotic nitrogen fixation. Perhaps, for *Chlorella* spp. and *Scenedesmus* spp., the production of Quercetin has no survival value. However, researchers continue to search for antioxidants in green algae including *Chlorella* spp. (Matsukawa et al., 2000). One interesting point from the findings of this research in terms of the evolution of green algae and land plants is green algae contain no lignin. However, the precursor of both lignin and flavonoids is 4-Coumaroyl-CoA. Markham and Porter (1969) could detect flavonoids in the advanced green algae, *Nitella Hookeri* Perhaps, if detection of flavonoids at the beginning of the biosynthetic pathway, for example Naringenin (Figure 2.13), is carried out, we might be able to detect flavonoids in the green algae and the findings may be interesting, evolutionary-wise.