CHAPTER I

INTRODUCTION

Green microalgae *Chlorella* spp. have been used in commercial production of single cell proteins including Sun Chlorella® and in scientific research into biochemical, genetic, and physiological aspects of oxygenic photosynthetic micro-organisms and higher plants. Another green micro-algae of interest are *Scenedesmus* spp. which have been used in research into phenotypic plasticity of their morphology, as well as in investigations on heavy metal removal and toxicity testing (Omar, 2002).

In this study, contents of β-carotene and Quercetin were determined in isolated *Chlorella* spp. and *Scenedesmus* spp. by reversed-phase HPLC. Both green algae were chosen because of the ease to obtain them in pure cultures and because of their potential uses in biotechnology. *Chlorella* spp. have been produced as health food and *Scenedesmus* spp. have been used in biochemical, genetic and physiological studies on β-carotene and anti-oxidant production. (Kranzfelder, 1992; Beremann, 1992).

It is proposed in this study that PCR fingerprints of isolated *Chlorella* spp. and *Scenedesmus* spp. be obtained for use as strain identity. Primers CRL-7 or 27f or 1492r will be used in PCR fingerprinting. CRL-7 is a GC-rich primer (Mathis and McMillan, 1996). It has been hypothesized that a large number of PCR product fragments when CRL-7 is used as the primer indicates heat-stable DNA molecules. Therefore, the organisms should be heat tolerant since it requires more energy to break the three hydrogen bonds between the nitrogenous bases G and C. Primers 27f and 1492r will also be used in PCR fingerprinting to obtain information on whether the two primers could be used in the isolation of 16S rDNA of chloroplasts of *Chlorella* spp. and *Scenedesmus* spp. Annealing of each of the latter two primers indicates that it is possible to use both primers in the isolation of 16S rDNA. If no annealing takes place which results in no PCR product fragment(s), it may be inferred that one or both primers could not be used to isolate 16S rDNA of the green micro-algae. If the isolated algal strains are used in the production of commercial products, their fingerprints can be used as baseline data for the detection of mutations that might have occurred in the

micro-organisms because changes in DNA molecules are reflected in changes of PCR fingerprint patterns. Identification of micro-algae is usually carried out by observing their morphology under the microscope. However, in some cases micro-algae isolated from different water bodies have similar morphology with differences only in sizes. These micro-organisms may or may not be the same species. The observations have given rise to the concept of "cryptic species" which refers to various genotypic strains within the same phenotypic "species" (Cassamatta et al., 2003). It has been suggested that both morphology and molecular characterization should be taken into account when identifying micro-algae and that there might be "local strains" rather than "global strains". Several molecular techniques have been used to characterize *Chlorella* spp. but not *Scenedesmus* spp. (Burja et al., 2001; Wu et al., 2001).