CHAPTER 1

INTRODUCTION

1.1 *Chlorella* (Chlorophyta)

Biologists have traditionally divided all living things into two kingdoms of plants and animals (Campbell *et al*., 2004). The plant kingdom included plants, most algae, fungi, and prokaryotes. This two-kingdom system prevailed in biology for over 200 years, but it faced problems. Then, in the late 1960's, the five kingdom system replaced the previous taxonomic system (Campbell *et al*., 2004). This new system comprised of Monera (Prokaryotes), Protista (a diverse kingdom consisting mainly unicellular organisms), Plantae, Fungi and Animalia. The prokaryotes were set apart from all eukaryotes by placing them in their own kingdom the Monera (Campbell *et al*., 2008). The trend during the 1970s and 1980s was to expand the boundaries of the kingdom Protista to include some groups of multicellular organisms, such as seaweeds (algae), classified in earlier versions of the five-kingdom system as either plants (in the case of seaweeds) or fungi. Nevertheless, the five kingdom system revealed some problems in differentiating some prokaryotes from the eukaryotes. Such difficulties led biologists to adopt the three domain system which comprise Bacteria, Archaea and Eukarya. In this system, the green algae, red algae, brown algae, golden algae, diatoms, and euglena were placed in the Domain Eukarya where land plants are present too. The blue green algae (Cyanophyta) is placed under Bacteria (Campbell *et al*., 2008).

Chlorella is a common unicellular green microalga (Chlorophyta) that belongs to the order of Chlorococcales and family Chlorellacea (Graham and Wilcox, 2000). *Chlorella* is

one of the first microalgae to be isolated as a pure culture by Beijerink in 1980's and is one of the few species that has been studied in some detail with respect to their metabolism and chemical composition (Phang and Chu, 2004). Since, *Chlorella* can be grown in simple culture medium, under limited light conditions, it can be cultured inexpensively on a large scale (Kim *et al*., 2002). *Chlorella* sp. has been used as an energy-producing and health food in Asia for centuries. It also contains high vitamin contents including Vitamin B complex such as B1, B2, B3, folic acid and high amount of lipid (EPA and DHA) and amino acids (Satin, 2004; Balch and Balch, 1990).

Knowledge on the physiological control of secondary metabolite formation and biosynthetic pathways in algae is still limited. Such studies are important in order to fully explore the potential of the microalgae, which then can allow the use of genetic engineering and recombinant DNA technology to increase the production of bioactive compounds by either developing overproducing strains, or strains in which the compound of interest is synthesized throughout the cell cycle (Lee *et al*., 1995). According to Balch and Balch (1990), microalgae have a largely untapped reservoir of novel and valuable bioactive compounds. Algal biotechnology continues to explore high value fine chemicals such as caretenoids, phycobilins, fatty acids and pharmaceuticals (Borowitzka, 1992). In the field of aquaculture, microalgae are not only used as important food sources but as feed additives in the commercial rearing of many aquatic animals, especially the larvae and spat of bivalve mollusks, penaeid prawn larvae and live food organisms such as rotifers which, in turn, are used to rear the larvae of marine finfish and crustaceans (Borowitzka, 1997).

1.2 Genetic Transformation and Microalgae

There is much interest in the use of microalgae for biotechnological applications and as plant model systems (Leon-Banares *et al*., 2004). It has been shown that genetic transformation may not be limited by taxonomic differences; DNA from an organism may be transferred into an algae to the nucleus, mitochondrion, and chloroplast using appropriate methodologies (Galun and Breiman, 1998). The basis of traditional methods used to transform microalgae was to cause, by various means, temporal permeabilization of the cell membrane, enabling DNA molecules to enter the cell while preserving viability. Permeabilization methods like particle bombardment, electroporation, *Agrobacterium*-mediated transformation, glass beads and polyethylene glycol (PEG), silica carbide (SiC) whisker and artificial transposons were commonly used in microalgae transformation (Leon-Banares *et al*., 2004). According to El-Sheekh (2005), the following DNA transfer techniques have been proven successful in algal transformation: i) the particle bombardment or biolistics process which involves high-velocity bombardment of cells or tissue with DNA-coated microparticles, ii) microinjection, iii) electroporation of cells or protoplast suspensions, iv) agitation of cell wall-deficient cells with DNA and glass beads, and v) agitation of walled cells with DNA and silicon carbide whiskers (Sanford *et al*., 1993; El-Sheekh, 1999; 2000; 2005). In the first three methods, DNA is actively transferred across the cell membrane barrier. In the last two, DNA diffusion into the cells is thought to occur through transient holes created in the membrane as a result of the abrasive action of beads or whiskers (El-Sheekh, 2005). Although biotechnological processes based on transgenic microalgae are still in their infancy, researchers and companies are considering the potential of microalgae as green cell bio-factories to produce value-added metabolites and heterologous proteins for pharmaceutical application. However, new molecular biology tools are needed to optimize genetic modification methods of microalgae (Leon-Banares *et al*., 2004).

1.3 Objective

The objective of this research was to investigate a transformation system for the tropical green microalga, *Chlorella vulgaris* UMACC 001. This task was accomplished by:

i) Genetically transforming *Chlorella vulgaris* UMACC 001 with the hepatitis B surface antigen (HBSAg) gene via particle bombardment.

ii) Verifying the presence and analyzing the integration of the HBSAg gene in the host genome by Polymerase Chain Reaction (PCR) and Southern Blot Analysis.

iii) Determining the expression of the HBSAg gene by performing Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) and Western Blot Analysis.

1.4 Hypothesis

The hypothesis of this research was;

H_o: A foreign gene could be introduced into *Chlorella vulgaris* UMACC 001 via particle bombardment.

H_1: A foreign gene could not be introduced into *Chlorella vulgaris* UMACC 001 via particle bombardment.