CHAPTER I

INTRODUCTION

Gaucher disease is an inherited metabolic disorder caused by mutations in the gene encoding acid-β-glucosidase (or glucocerebrosidase), the enzymes that catalyzes the breakdown of glycolipid glucosylceramide. These mutations result in glycolipid glucosylceramide accumulation mainly in macrophages but also in other tissues. Approximately 5000 people worldwide were affected by this disease, with a particularly high incidence in the Ashkenazi Jewish population.

Among available treatments for Gaucher's disease enzyme replacement therapy using Cerezyme® which was approved by FDA was the only treatment widely accepted. Details of cerezyme are provided in Table 1.2

Table 1.1 Recombinant glucocerebrosidase details

Product	Recombinant glucocerebrosidase
Trade name	Cerezyme
Principal uses	Treatment for Gaucher's disease Type I
Manufacturer	Genzyme Corporation, USA
Donor organism	Fetal human lung fibroblasts
Host organism	Chinese hamster ovary
Advantages	Highly clinically effective for treatment of Gaucher's disease Safe and unlimited supply Near absence of any side effects

Basically, Cerezyme® was produced via recombinant DNA technology. At present, most recombinant proteins are based on transgenic bacteria, yeast and mammalian cells with famous example on insulin had been well addressed (John, 1983). Several recombinant DNA products had been accepted for clinical used. These included one of the approaches

using recombinant proteins for enzyme replacement therapy (ERT) (Ernest, 1987), among more cases use in medical applications.

Although bacteria, yeast and mammalian cells are used as models to produce recombinant proteins, they are high invasive, unstable and contaminable by undesirable molecules or virus in mammalian-cell case (Hefferon and Fan, 2004). Thus microalgae and plants become more feasible protein-produced system. Comparing with plant, microalgae is lower in productive cost but more efficient in growth rate (Leon-Banarez, 2004). Using microalgae as recombinant-protein productive system could be a better alternative.

Unicellular green microalgae are of interest in biological research as simple plant models for higher plants and from a biotechnological perspective as a natural source of high value compounds for health and medical applications (Avron and Ben-Amotz, 1992) (Table 1.2).

Table 1.2 Microalgae – based products and their related businesses

Microalgae – based product	Company
Food additives (carotinoids, food dyes and dietary supplements)	Cyanotech (http://www.cyanotech.com) Mera Pharmaceuticals (http://www.aquasearch.com) Nikken Sohonsha Corporation NBT (http://www.clostanin.co.jp) Cognis (http://www.cognis.com/cognis.html)
Polyunsaturated fatty acids	Subitech (http://www.suitech.com) Far East Microalgae Ind. Co. (http://www.allproducts.com.tw/manufacture11/fareast/supplier.html)
Polysaccharides	Nikken Sohonsha Corporation
Fluorescent pidments (phycobiliproteins)	Cyanotech
Bioactive compounds (anti-tumer and vaccines)	Phycotransgenic (http://www.phycotransgenics.com)

Biomass (animal feed, aquaculture, nutritional and healthy products)	Cyanotech Earthrise Farms (http://www.earthrise.com/ERFarms.html)
Eukaryotic expression systems for mammalian proteins	Entelechon (http://www.entelechon.com) Subitech

Several reports had been focused on the experiment on transgenic green microalgae. Most of them were *Chlamydomonas* sp. and *Chlorella* sp. However, *Dunaliella* sp. was considered an interesting model for transgenic green microalgae because it had properties closer to plant protoplast than others.

Dunaliella sp., a unicellualar geen microalgae, had been used to produce carotenoids such as β-carotene (Avron and Ben-Amotz, 1992), glycerol (Barclay *et al.*, 1994), astaxantin and canthaxanthin, used as pigments in food products and cosmetics, vitamin A, antioxidant supplements, used in health food products and feed additives for poultry, livestock, fish and crustaceans (Walker, 2005). Over 80% of the world's supply of natural β-carotene came from this halophilic green alga (Walker, 2005). Since it was photoautotrophic, its characteristics obviated the need for exogenous carbon source of energy and making its large-scale culture comparatively cheap.

D. salina was also an ideal organism for study of salt-resistant mechanisms because it can grow in salt media ranging from less than 0.5 mol/L to saturated salt solutions (Ben-Amotz *et al.*, 1982). Moreover, it was a good candidate for producing recombinant protein because of its uniqe characteristics. First, a halotolerant eukaryote that can grow in media with high salt concentrations. In that condition, there was less chance that *D. salina* would be contaminated by other less-halotolerant organisms (De-Gui *et al.*, 2004). Second, it was a naturally protoplast-like because it lacked rigid polysachharide cell wall (De-Gui *et al.*, 2004). This allowed transgenic vecters to be easily transferred into cells and again the expressed recombinant protein was also be easily harvested and purified. Third, it was able to culture rapidly in an inexpensive medium containing only simple salts making cost of protein-

production stood at very low (De-Gui *et al.*, 2004). All these advantages made *D. salina* a good model species for producing recombinant proteins.

This experiment, was divided into 4 major parts; 1) production of *D. salina* using simple media for the substitution of original medium used fine chemicals, 2) the study on method for gene transformation in *D. salina* using PEG-mediated method, 3) the construction of transforming vector containing a target GBA gene, and 4) the selection and detection of target gene expression in recombinant algae. The objective of this thesis is employing *D. salina* as a model system to produce recombinant protein using bialaphos resistant gene and glucocerebrosidase gene as target model.