CHAPTER 6

CONCLUSION

The main objective of this research was to establish a transformation system for the tropical microalga, *Chlorella vulgaris* UMACC 001. Hence, particle bombardment was used as a method to transfer the pCAT-HBSAg vector into *C. vulgaris. C. vulgaris* was proven to be a good candidate for transformation as it grows fast in simple medium like BBM and also shared similar characteristics to higher plants and other eukaryotes.

The CAT gene was considered to be a very reliable and good selectable marker. In our research, we found that 200 µg mL^{-1} of chloroamphenicol was sufficient to inhibit *Chlorella vulgaris* growth. The implication that can be made when the CAT gene was expressed in transformed *C. vulgaris* was that the, SV40 promoter which was located at the upstream of the CAT genes, was a good promoter to be used in microalgal transformation.

The presence of the HBSAg gene in transformed *Chlorella vulgaris* was successfully detected via PCR. Sequencing results and PCR-Southern Analysis further confirmed the presence of the HBSAg gene in *C. vulgaris*. The ANOVA two way test showed that there were no differences between the all the four parameters used for transformation , therefore suggesting that rupture disc pressures of 900 psi and 1100

psi at the distance of 6 cm and 9 cm were good parameters for transforming *C. vulgaris* cells.

Southern blot analysis that gave genomic bans from transformed samples showed that there is a possible integration of the foreign gene (HBSAg gene) into the genomic of the host. A 271 bp band that was obtained after RT-PCR further indicated that the HBSAg gene was successfully transcribed into mRNA. However, in western blot analysis, a 47kDa band was obtained instead of expected 24 kDa. The larger size protein (47 kDa) may be a fusion product of the expression of both the HBSAg and CAT genes.

The presence of the HBSAg gene was detected from the 22^{nd} (day 44), 32^{nd} (day 64), 42^{nd} (day 84), 53^{rd} (day 106), 63^{rd} (day 126), 69^{th} (day 138), 72^{nd} (day 144), 82^{nd} (day 164) and until the 92^{nd} (day 184) cell generation via PCR. The HBSAg gene could be considered to be stably integrated into the microalga genome because southern blot analysis on the 82^{nd} cell generation (day 164) showed the integration of the HBSAg gene. The RT-PCR results were obtained during the 10^{th} cell generation (day 20), and western blot protein bands were obtained in 42^{nd} (day 84) and 50^{th} (day 100) cell generation. There was no HBSAg protein band detected when crude protein samples of transformed *Chlorella vulgaris* after the 50^{th} cell generation (day 100) was tested, which led us to believe that transgene silencing could have occurred.

As a conclusion, particle bombardment was a good method for transforming *Chlorella vulgaris* as it was easy and efficient. The SV 40 worked well in *C. vulgaris* and could be used to express other genes besides the HBSAg and CAT gene in other

tropical microalgae. This research has proved that the HBSAg gene was successfully introduced, integrated, transcribed and translated in bombarded *C. vulgaris*.