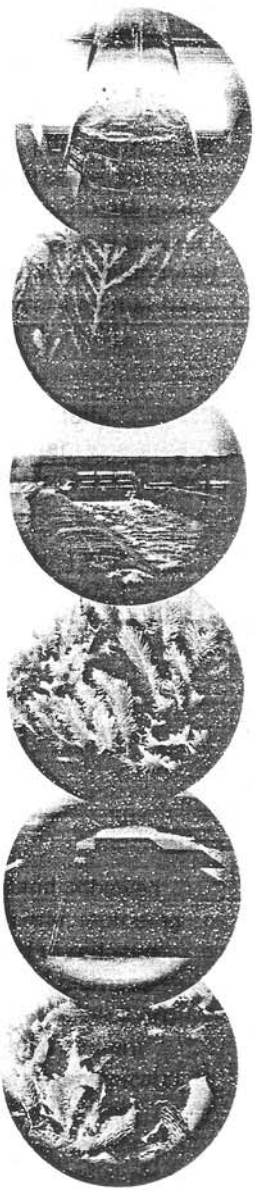
APCAB & ICCB 2012

ALGAE FOR THE FUTURE

8th Asia-Pacific Conference on Algal Biotechnology
and **1st** International Conference on Coastal Biotechnology



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APCAB Poster 8

Expression of a novel fluorescent protein (VFP) in the *Chlamydomonas reinhardtii* chloroplast: a new marker for recombinant protein production in microalgae

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Unicellular algae such as *Chlamydomonas reinhardtii* are attracting interest as platforms for synthesis of high-value heterologous proteins, due to their various benefits in terms of manipulation and protein expression, in comparison to other biological systems. Insertion of foreign genes into the *C. reinhardtii* chloroplast genome has proved to be a reliable strategy since genes can be precisely targeted to a specific locus and high levels of expression obtained without gene silencing issues.

Fluorescent proteins such as green fluorescent protein (GFP) provide a simple tool for rapid monitoring of recombinant protein expression *in vivo*. We wish to use such a reporter protein to optimise culture conditions (temperature, stirring and lighting), and therefore achieve increased cell growth rate and recombinant protein production. The ultimate goal is to develop genetic cassettes encoding recombinant proteins for various applications fused to the fluorescent protein, and thus evaluate its expression. However, low levels of GFP fluorescence in the *C. reinhardtii* chloroplast have hindered such studies. Recently, a newly discovered protein called Verde Fluorescent Protein (VFP) has been described that has superior fluorescence properties when compared to GFP and its variants (Ilagan *et al.* 2010, FEBS J. 277: 1967). We have therefore investigated VFP as an alternative reporter.

A codon-optimised version of the VFP gene fused to a HA epitope was synthesised. The gene was successfully introduced into the chloroplast genome and expressed under the control of the endogenous *atpA* promoter. Western blotting using α-HA antibodies revealed a high level of VFP in transformant lines, and fluorescence was readily observed by confocal microscopy.

Ongoing work aims at testing whether recombinant protein levels can be markedly improved by introducing VFP into the chloroplast genome such that it is translationally fused to a highly expressed endogenous gene such as *rbcL*, encoding the large subunit of ribulose bisphosphate carboxylase.

APCAB Poster 9

Optimization of Chlorococcum humicola for biodiesel production

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Chlorococcum humicola, a wild-type strain newly isolated from Bueng Boraphet, Nakhorn Sawan, Thailand achieved the highest lipid yield in 3N Bold's Basal Medium (3NBBM) culture. The eight major ingredients of 3NBBM, including $NaNO_3$, $CaCl_2$, $MgSO_4$, K_2HPO_4, KH_2PO_4, NaCl, trace element solution and vitamin addition were adjusted to reduced concentrations. The results showed that the reductions in nutrient concentration usually led to lower final biomass and lipid yields but the effects on the maximum specific growth rates were variable. Combined medium compositions were adjusted to lower production cost in batch and semi-continuous systems. The final biomass and lipid content were still the highest with the standard 3NBBM. The variation of biochemical content (protein, lipid and carbohydrate) in each treatment of combined nutrients was not statistically different from standard medium. When effects of pH on *C. humicola* in 3NBBM were examined, there was no statistically significant difference on the maximum specific growth rate within the range of pH 5 to 10. However, the biomass and lipid productivity appeared to be optimal at pH 5. The effects of initial pH at 5 and 7 and the light intensity at 40 and 100 μmol/m^2/s to *C. humicola* cultivated in optimal combined nutrient were examined in the raceway ponds (RWP) and flat panel photobioreactors (PBR). The highest specific growth rate and biomass were obtained under high light intensity in both systems. The variation of biochemical compositions and fatty acid profiles were significantly different at initial pH and light intensity. These results suggested that it is feasible to scale up *C. humicola* culture to the commercial scale production.