

Piyanast Sornchai 2010: Certain Factors Affecting Transformation of *Spirulina platensis* by Electroporation. Master of Science (Agricultural Biotechnology),
Major Field: Agricultural Biotechnology, Interdisciplinary Graduate Program.
Thesis Advisor: Assistant Professor Sermsiri Chanprame, Ph.D. 82 pages.

Transformation of *Spirulina platensis* strain TISTR 8217 by electroporation was done. The recombinant plasmids, pBAD-TOPO[®]/*gus* contained *gus* reporter gene and *Amp* as the selectable marker gene. *S. platensis* in the exponential phase of growth was used for spheroplast isolation by enzymatic treatment using lysozyme at the concentration of 0.035% (w/v) with 0.08 % (w/v) ethylenediaminetetraacetic acid (EDTA). The reaction was incubated at 37 °C for 3 hours. The percentage of spheroplast isolation was verified by microscopic examination. The results indicated that 55% of initial cells were produced spheroplast. The isolated spheroplasts were then cultured in ½ x Zarrouk medium for regeneration. The highest percentage of regenerated spheroplasts of 33% was obtained after 20 days in culture. For electroporation field strength of 4 kv/cm was used. Selection of transformed cell was done in 300 ug/ml ampicillin containing ½ x Zarrouk medium. The putative transformed *S. platensis* was determined for transient expression by Gus histochemical assay and the existence of *gus* gene and pBAD-TOPO[®] by PCR technique. The result showed 1,800 base pair of *gus* and 731 base pair of a part of pBAD-TOPO[®]. The existence of *gus* gene was confirmed by dot blot hybridization and Southern PCR hybridization technique, which also found the 1,800 bp of *gus* gene. But 30 day after transformation *gus* gene and pBAD-TOPO[®] were not found using PCR technique. It was indicated that transformation in *S. platensis* is not stable.

Student's signature

Thesis Advisor's signature