

Improved protocol for the preparation of *tetraselmis suecica* axenic culture and adaptation to heterotrophic cultivation.

ABSTRACT

The effectiveness of various physical and chemical methods for the removal of contaminants from the microalgae, *Tetraselmis suecica*, culture was investigated. The information obtained was used as the basis for the development of improved protocol for the preparation of axenic culture to be adapted to heterotrophic cultivation. Repeated centrifugation and rinsing effectively removed the free bacterial contaminants from the microalgae culture while sonication helped to loosen up the tightly attached bacterial contaminants on the microalgae cells. Removal of bacterial spores was accomplished using a mixture of two antibiotics, 5 mg/mL vancomycin and 10 mg/mL neomycin. Walne medium formulation with natural seawater was preferred for the enhancement of growth of *T. suecica*. Adaptation of growth from photoautotrophic to heterotrophic conditions was achieved by the repeated cultivation of photoautotrophic culture with sequential reduction in illumination time, and finally the culture was inoculated into the medium containing 10 g/L glucose, incubated in total darkness to obtain heterotrophic cells. Changes in the morphology and composition of *T. suecica* cells during the adaptation from photoautotrophic to heterotrophic condition, as examined under Transmission Electron Microscope, were also reported.

Keyword: Axenic microalgae culture; *Tetraselmis suecica*; Adaptation; Heterotrophic; Phototrophic; Microalgae cell composition.