RECOVERY OF HIGHLY CONCENTRATED MICROALGAE CELLS AFTER ENZYMATIC AND MECHANICAL TREATMENT

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ABSTRACT

Although selection techniques based on spontaneous mutation are conventionally used for different strains improvement, genetic manipulations like DNA transformation and protoplast fusion are supposed to be more effective. The above procedures, when applied to microorganisms that present a rigid cell-wall, require the efficient isolation and regeneration of protoplasts, which facilitate the applications of classical molecular and genetic studies. Protoplast electroporation is a particular method that allows the introduction of foreign DNA or nanoparticles into a great variety of cells by means of electric pulses. Transformed cells can further exploited depending on the effectiveness of the regeneration protocol. *Scenedasmus almeriensis* and *Nannochloropsis oceanica* are two well-known green microalgae that are widely used as a health food and feed supplement, as well as in the pharmaceutical and cosmetics industries. The objective of this study was to develop an efficient protocol for recovery highly concentrated protoplastic cells from *Scenedasmus almeriensis* and *Nannochloropsis oceanica.* The final goal is to propose a protocol that can find applications for genetic studies and manipulations of several algal species.

The first step in protoplast preparation is the removal of cell wall through enzymatic digestion. Cell wall complexity is the major factor that influences the digestion efficiency, so the selection of the appropriate degrading enzymes is the key factor for isolating protoplasts. The algal cell wall is comprised of algal 'wall protein' and 'wall polysaccharides'. Our results showed that an enzymatic solution containing 2% cellulose was more effective than other kind of enzyme. The effects of the mechanical treatment are also presented.

It is worth emphasizing the vital role of different culture medium for the recovery of protoplasts, since the photosynthetic activity is impaired after cell-wall removal. In general, microalgae membranes are constituted by glycoproteins and phospholipids and also single amino acids. 0.1% w/v casamino acids were necessary due to the fact that it can be directly used as the building blocks of the membranes. Also, glucose and fructose are the major components for glycolysis, that can start from glucose degradation or by using fructose and lead to production of glycerol (glyceraldehyde-3-phosphate) and then to triglycerides and phospholipid production. In both strains, 2% of glucose in the culture medium helped to increase the glycosylation as well as the glycoprotein levels and that served for the reconstruction of the cell-wall and membranes. As a constituent, glucose and fructose play a double role for phospholipid and glycoprotein production supporting the membrane synthesis. In conclusion, this work provides for the first time a very efficient protocol for the preparation of protoplasts from *Scenedasmus almeriensis* and *Nannochloropsis oceanica*, and the recovery of highly concentrated microalgae cells, which will find useful applications in both classical molecular and genetic studies.

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