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Poster Abstract – I.07

GENETIC MANIPULATION OF FOUR UNICELLULAR GREEN ALGAL SPECIES USEFUL FOR WASTEWATERS REMEDIATION

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Unicellular green algae have been a model system for elucidating biological process important for plant and animals and, in the recent years, there have been efforts to improve our understanding algal molecular biology and biotechnology. The importance of transgenic techniques applied to algae is now being developed for various biotechnological applications including the synthesis of recombinant antibodies and vaccines and bioremediation of soil and water contaminated with heavy metals and organic pollutants. However, only few nuclear algal genomes can be routinely manipulated.

In this presentation we report results on nuclear transformation mediated by particle bombardment or *Agrobacterium tumefaciens* of four unicellular green algae species, previously tested for their ability to degrade phenols pollutants or steroids: *Ankistrodesmus braunii* CCAP202.7a, *Scenedesmus vacuolatus* SAG211-8b, *Scenedesmus obliquus* SAG276-1, and *Selenastrum capricornutum* UTEX1648 (Pinto *et al.*, Biotechnology Letters *24*: 2047-2051, 2002; Pollio *et al*., Phytochemistry *42*: 685-688, 1996).

Preliminary *in vitro* resistance experiments have been made to find the minimum concentrations of the antibiotic kanamycin (kan) or glufosinate-ammonium-based erbicide Basta useful for selection of putative transformed algal cells. The above-mentioned algal species were thus cultured in liquid or solid medium added with the selectable compound. It resulted that the concentration of kanamycin useful to discriminate putative transgenic algal cells was equal to 80 mg/l, whilst it was equal to 80 mg/l for Basta. Experiments of nuclear genetic transformation were carried out using microprojectile bombardment or co-culture with *A. tumefaciens*; two vectors were assayed, p35S-GUS-INT (*nos-kan*) and pG0229 (*nos-bar*).

Molecular analyses (PCR and RT-PCR) performed on putative transformed algal clones revealed the stable nuclear integration and expression of the transgenes in three species out four. *Ankistrodesmus braunii, Scenedesmus vacuolatus* and *Scenedesmus obliquus* expressed the *kan* gene while only *Scenedesmus vacuolatus* species expressed the *bar* gene. However, *Selenastrum capricornutum* did not reveal any sign of transgenes stable insertion. Frequencies of kanamycin and Basta resistant colonies were monitored and ranked between 17.2 to 19.3 x 10^{-6} for co-culture experiments and between 0.3 to 3.3 x 10^{-6} for microprojectile bombardment experiments. Further experiments are now in course to improve the efficiency of genes delivery and expression.