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Poster Abstract – C.19

WASTEWATER PHYTOREMEDIATION: GENOMIC ANALYSIS AND SCREENING OF GREEN MICROALGAE SPECIES FOR EXTRACELLULAR LACCASE ACTIVITY

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Phytoremediation deals with the use of plants, or other green photosynthetic organisms, to reduce organic or inorganic pollutant in the environment, mainly waters and soils (Pivetz. B.E. 2001. EPA
/540/S-01/500). Our research group is involved in studies regarding the use of plants for soil phytoremediation (Galante *et al.*, 2005. Proc. SIGA Congress, L04) and, more recently, of unicellular green algae species (green microalgae) for wastewater treatments. Some microalgae species have been recently tested to degrade an array of pollutants such as phenols, polyphenolic aromatic compounds (PAH) and even hormones (Pollio *et.al*., 1994. Phytochemistry, *37*:1269-1272;. Pinto *et.al*., 2003. Biotechnol Lett., *25*:1657-1659). It is worthy to note that about 2,500 species belong from Chlorophyceae, seldom living in contrasting habitat under severe environmental conditions. So far, a little has been done to exploit this genetic biodiversity bonanza; thus, few reports have been published on enzymes implicated in their degradative action (Semple *et.al*., 1996. Appl. Envr. Micr, *62*:1265-1273).

Since a wide collection of green microalgae species are available at the University of Naples, Department of Biological Science, recently we have started a research aimed to (a) find algae species with extracellular phenoloxidase enzymatic activity; (b) identify extracellular enzymes able to degrade xenobiotic like synthetic dyes and other PAHs; (c) clone and overexpress genes producing phenoloxidases in homologous and in heterologous systems, in order to use these enzymes primarily for phytoremediation of milling oil wastewaters. Among phenoloxidaes, we focused our interest on laccases (EC 1.10.3.2) that are phenol-oxidoreductases able to catalyze the oxidation of various aromatic compounds (particularly phenols) with the concomitant reduction of oxygen to water.

Selected algae strains were grown in liquid culture at 22°C under continuous light conditions, starting with an inoculum of 0.1 OD. After ten days, the algal growth was measured as optical density at 600 nm. A screening was performed by detecting the laccase activity in the broth medium culture, deprived of algae cells, in the presence of 2,2-azino-bis 3-ethybenz-thiazoline-6-sulfonic acid (ABTS) at 420 nm. The laccase activity was referred to the polyphenol oxidase activity of *Trametes versicolor*; thus, each positive strain was assayed on industrial azo-dye Remazol Brilliant Blue R (RBBR) and on the natural phenol compound syringaldazine by kinetic analysis. Preliminary results, obtained comparing different species, showed a wide variation both within the same substrate and among the different microalgae.

Microalgae strains able to produce and secrete laccase enzymes were further chosen for more detailed genetic studies. To clone phenoloxidase genes from those species, we have started a bio-informatics approach, on the basis of highly conserved coding sequences of laccases already isolated and sequenced from several higher plants. Primers drawn on the alignment of those sequences have been used to amplify genomic DNA.