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Directed evolution, biotechnology and in silico analysis of reaction centre proteins for microorganisms and biomimetic-based biosensors in environmental toxicity monitoring

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Tons of chemical compounds derived from human and industrial activities are incessantly threatening our environment. Current approaches for monitoring of pollutants include precise and accurate assessment of individual compounds by chemical analyses, which are however unable to provide information about bioavailability, effect on living organisms, and synergistic or antagonistic behaviour in mixtures, thus requiring combination with biomarker assays and ecosystem monitoring. These methodology strategy is time and labour intensive, demands ex-situ collection at individual locations and extensive sample preparation, and has elevated costs depending on the complexity.

To overcome these challenges, biosensor and bioassay technology can furnish advanced devices for water monitoring with greater efficiency. Indeed, integrated, cost-effective, easy to use, and fast biosensors can be projected to characterize the extent of pollution at relevant spatio-temporal scales and in terms of ecological effects. Despite this great potential, most of the published works focused on analyses of fresh water, mainly because of the highly demanding working environment that seawater constitutes. To face the challenges posed by real environments, biosensors need to be fully automated, very robust (resistant to physical impacts, high corrosion, and biofouling), drift-free or with accurate calibration, with minimal power consumption, user-friendly, and enough sensitive to measure pollutants at very low concentrations. Several examples of biosensor development for marine measurements of eutrophication, pesticides, anti-biofouling agents, polycyclic aromatic hydrocarbons, endocrine disruptors, trace metals, organism detection and algal toxins have been described in literature.

Algal biosensors react very broadly to toxicity and their detection mechanism frequently relies on measurement of the photosynthetic activity caused by 33% of pesticides actually in the market. Biosensing applications of photosynthetic organisms are based on the inhibition of the electron transfer occurring after a few minutes exposure of photosystem II (PSII) to certain pollutants, or to adverse physicochemical conditions changing the local chemical equilibrium. Indeed, when pollutants such as photosynthetic pesticides are present and encounter the photosystem, they can bind the reaction centre D1 protein and directly or indirectly inhibit the transport of electrons from the primary acceptor, plastoquinone A (QA), to the secondary quinone (QB) along the photosynthetic chain. This inhibition results in a variation of PSII fluorescence emission in a pollutant concentration-dependent manner that can be monitored by optical transduction. Based on this approach, several microalgal biosensors have been designed for pesticide and heavy metal detection in fresh water. However, hyper-saline conditions present in marine environment and stress conditions during environmental monitoring may affect the photosynthetic process resulting in significant changes in the bioassay performance. Herein we present the development of an optical bioassay for detection of photosynthetic pesticides from different chemical classes in real water samples by exploiting various green microalgae strains. Therefore, the main objectives were to select the most appropriate microalgae strains to achieve stability and adaptability into real matrices, and to develop a bioassay integrated with portable fluorescence instrumentation allowing pesticide detection at relevant environmental concentrations. Several microalgae species from Chlorophyceae, Trebouxiophyceae, Dinoflagellates, Diatoms and Eustigmatophyceae groups with different marine and non-marine origins, including fresh water and soil, were analysed. Lipid content of selected microalgae suggested that *C. mirabilis* and symbiotic associations between *C.vulgaris* and protozoa were the microorganisms with higher potential to acclimate to high salinity environments being mainly constituted by unsaturated lipids involved in responses to several environmental stresses.

Among the wide range of microalgae species, which have been employed to develop biosensor technology, *Chlamydomonas reinhardtii* was especially studied since it possess a number of features that suite perfectly the requirements of an early warning environmental biosensor. It is a grass organism, easily cultivable having 8 hours doubling time and it can grow with or without carbon source, besides, it is easily transformable and all 3 genomes are sequenced. Recent our efforts have focused on increasing the stability and selectivity of PSII from microalgae for the detection of different subclasses of pollutants. These goals were achieved by using the alga *C. reinhardtii* mutated at the D1 protein herbicide-binding site by site-directed mutagenesis. *C. reinhardtii* was also modified introducing in the chloroplast antioxidant peptides, known in food able to reduce the content of free radicals, thus lessening the photooxidative membrane damage. Measurements of *in-vivo* antioxidant activity showed that mutant strains have improved their survival rate in the presence of singlet oxygen precursors, which highly exceeds the survival rate of control algae, showing increased stability and sensitivity for biosensor applications.

Beyond these scientific achievements, nowadays the market needs highly specific and precise *in situ* measurement devices able to collect and send the data in real-time for periods of months without maintenance under multi-stressors. These devices demand more robust algal biomediators. Thus, the challenge is the preservation of the algal photosynthetic functionality when integrated with electronic components or operated under fluctuating environmental conditions. To this end *C. reinhardtii* mutants able to quench $1O_2$ and other ROS, were integrated into a newly developed miniature and portable electrochemical/optical device, to measure and collect PSII data in real-time for long periods. Several photosynthetic pollutants spiked in real samples were detected within 10 min in concentrations between ng/L-µg/L and the different algae species tested showed diverse pesticide sensitivities.

Always towards to increase the biomediator performance, biomimetic peptides of the photosynthetic D1 binding niche of the microalgae C. reinhardtii were developed, both by chemical and biological synthesis, as suggested by in silico analysis. Standing out among the others, the biomimetic mutant peptide, D1pepS268C, bound to specific quantum dots, showed high ability to mimic the microalgae in binding pesticides. Replacement of whole microalgae cells or their photosynthetic apparatus by mimetic peptide improved the system in terms of stability.

This approach allowed also the integration of the biomediators with quantum dots and innovative stretchable printed electrodes-based electrochemical biosensor as a wearable point-of-use screening tool for toxicity environmental analyses.

Biography

Maria Teresa Giardi has worked as the manager of research at the National Council of Research (IC-CNR) in Rome till 2015. She is now associated to CNR, working at the company Biosensor srl as research director and CEO. Her background is in industrial chemistry with extensive experience in biochemistry and molecular biology; her main interest is on photosynthetic protein stabilization and utilization in biosensors for real toxicity environmental monitoring. She is a supervisor-coordinator of several national and international projects in the field of biosensors based on plants and microorganisms and of European Space Agency's projects involving space flights of engineered microorganisms to low orbit and to International Space

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