EVALUATION OF PESTICIDES TOXICITY USING BIOSENSORS OF THE MARINE PHOTOSYNTHETIC PICOEUKARYOTE *OSTREOCOCCUS TAURI*

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Abstract
Biosensors expressing the firely luciferase driven by specific genes were developed in the marine photosynthetic picoeukaryote *Ostreococcus tauri* and used to measure the toxicity of different pesticides (diuron and its metabolites DCPU and DCPMU, irgarol 1051, glyphosate, chlorpyrifos). Results were compared to the conventional assay of growth inhibition. Our results demonstrate that the use of luminescent biosensors can constitute a sensitive, high-throughput and non-invasive approach to assess the toxicity of pesticides. The gene encoding the cycling-dependent-kinase A (*CDKA*) implicated in cell cycle appeared to be the most sensitive to the pesticides tested.
Keywords: Ecotoxicology, Phytoplankton, Pesticides, Biotechnologies, Physiology

Introduction - Coastal ecosystems are exposed to various pollutants including pesticides. These pesticides can come from agricultural activities or from the leaching of biocides used as marine antifouling agents. These different pesticides may affect non-target organism including phytoplankton. There is a great interest to develop new approaches to detect the toxicity of theses pollutants for marine organisms and to perform ecotoxicological tests considering the interactions between different pollutants or the interactions of pollutants with environmental parameters. To resolve this complexity a high-throughput and sensitive assay is required. We propose here the use of the marine photosynthetic picoeukaryote *Ostreococcus tauri* for which biosensors expressing the luciferase under specific gene control have been developed [3]. *O. tauri* belongs to the *Prasinophyceae* and is reported as a globally abundant in Mediterranean coastal lagoons and in the oceans [1,2]. The most striking feature of *O. tauri* is it minimal cellular organization (a naked, 1-micron cell, lacking flagella, with a single chloroplast and mitochondrion), and a small genome (12.56 Mb) completely sequenced [4]. This is the first eukaryotic algal model where stably transformed luciferase lines accurately report gene expression [3].

Material and methods - For the growth inhibition assays, *O. tauri* strain 0TTH0595 was grown in 96-wells microplate at 25°C with Keller media under constant illumination at 56 µmole.quanta. $cm^{-2.}$ sec^{-1}. Cells were introduced at 5 x 10^6 cell/ml and the pesticides were added 24 h after inoculation. Growth of cultures was measured by flow cytometry (Cell Lab Quanta^{TM} MPL, Beckman Coulter) over 72 h. For the luminescence assays, four different transformed luciferase lines were used in this study (Table 1). The different modified genetic lines were grown in 96-wells white microplate at 20°C with Keller media and luciferin (10 µM) under constant illumination at 13 µmole.quanta. $cm^{-2.}$ sec^{-1}. Cells were introduced at 10 x 10^6 cell/ml and the pesticides were added 24 h after inoculation. Luminescence was acquired every 2 h (Berthold LB Centro automated luminometer) and over 72 h to follow *in vivo* expression of genes of interest.

Results and discussion - Based on the growth inhibition assays it was possible to classify the toxicity of the pesticides as follow: irgarol > diuron > DCPU > DCPMU > glyphosate > chlorpyrifos. The use of luminescent biosensors gave the same order of toxicity but the effective concentration of each pesticide leading to a decrease of 50% of luminescence was most of the time lower than the concentration leading to 50% of growth inhibition, suggesting a more sensitive assay. Moreover, the automated, non-invasive and highly reproducible measurements of luminescence give additional advantages to the use of the biosensors compared to the growth inhibitions assays, promoting this new assay as a method of choice to monitor pesticide toxicity in the marine environment. When the different biosensors were compared we observed that pesticides tested had a stronger inhibitory effect on the expression of cell cycle-regulated genes (*CyclineA* and *CDKA*) compared to the photosynthesis (*CAB*) and the circadian clock (*TOC1*) regulated genes. The *CDKA:Luc* reporter gene was the most promosing biosensor since it had high basal level of luminescence and yielded highly reproducible results. The results are very promising for the design of a fast and automated ecotoxicological test based on *O. tauri* luminescent biosensors, which could constitute a useful tool for studying i) the impact of environmental parameters influencing the sensitivity of microorganisms to toxic compounds and ii) the interactive effects of different pollutants.

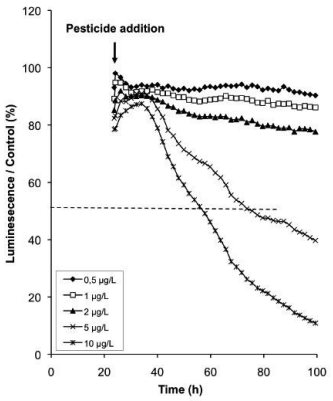

Fig. 1. Effect of different concentrations of diuron on the gene expression of *CDKA* in *O. tauri* as measured with the luminescent biosensor. The luminescence data are expressed in percentages relative to control cultures and are the mean of three independent wells. The dashed line indicate 50% of inhibition of *CDKA* gene expression

Tab. 1. The different luminescent biosensors used

Gene	Function	Luciferase gene reporter system
Chlorophyll a Binding protein *(CAB)*	Photosynthesis	Transcriptional fusion
Cycline Dependant Kinase A *(CDKA)*	Cell cycle	Translational fusion
CyclineA	Cell cycle	Translational fusion
Timing Of *CAB* expression *(TOC1)*	Circadian clock	Translational fusion

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