Marine Pollution Bulletin 129 (2018) 212–221

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Fast pesticide pre-screening in marine environment using a green microalgae-based optical bioassay

Laura Moro[a,*], Gianni Pezzotti[a], Mehmet Turemis[a], Josep Sanchís[b], Marinella Farré[b], Renata Denaro[c], Maria Grazia Giacobbe[c], Francesca Crisafi[c], Maria Teresa Giardi[a,d]

[a] Biosensor Srl, Via degli Olmetti 44, 00060 Formello, Rome, Italy
[b] Water and Soil Quality Research Group, Institute of Environmental Assessment and Water Research (IDAEA-CSIC), c/ Jordi Girona 18-26, 08034 Barcelona, Catalonia, Spain
[c] Institute for Coastal Marine Environment (IAMC-CNR), Spianata S. Raineri 86, 98122 Messina, Italy
[d] Crystallography Institute, CNR Area della Ricerca di Roma, Via Salaria km 29,300, 00015 Monterotondo, Rome, Italy

ARTICLE INFO

Keywords:
Bioassay
Pesticide
Photosynthesis
Microalga
Chlorella mirabilis

ABSTRACT

The present study evaluates an optical bioassay based on green photosynthetic microalgae as a promising alternative for monitoring of relevant seawater pollutants. Photosystem II fluorescence parameters from several microalgae species were examined in the presence of three common marine pesticides that act as photosynthesis inhibitors. The three pollutants were detected within 10 min in concentrations between ng/L-μg/L. The different algae species showed slightly diverse pesticide sensitivities, being *Chlorella mirabilis* the most sensitive one. Potential interferences due to oil-spill pollutants were discarded. The lipid content was characterized to identify microorganisms with suitable mechanisms that could facilitate stress acclimatization. *C. mirabilis* presented elevated content of unsaturated lipids, showing a promising potential for biosensing in saline stress conditions.

The optimized microalgae-based bioassay was preliminarily incorporated into a marine buoy for autonomous pre-screening of pesticides in coastal areas, demonstrating its suitability for real-time monitoring of marine water and quantitative evaluation of total biotoxicity.

1. Introduction

Tons of chemical compounds derived from human and industrial activities are incessantly threatening marine environments through direct routes, riverine contributions, and atmospheric deposition (Sun et al., 2016; Talvitie et al., 2015). The so-called "contaminants of emerging concern" of industrial, pharmaceutical, personal care and agriculture provenance represent an important pollution source for marine and coastal environments (Martínez Bueno et al., 2009). Pesticides of agricultural use are water soluble and constitute a concerning source of pollution entering marine ecosystems (Mercurio et al., 2014; Polidoro et al., 2017). For instance, herbicide use for weed control in agriculture has been largely restricted in the last decades by the European Union (EU) to minimize the impact on environment and human health. Atrazine, a pesticide from the triazine family, was banned in 2004 in the EU but it remains extensively applied in around sixty other countries worldwide (Sass and Colangelo, 2006). Similarly, the use of the triazine simazine is not approved in the EU, and application of diuron, an algicide and herbicide of the phenylurea class, has been extensively limited. In spite of the regulations, they are still found in soils and waters in many parts of Europe, finally reaching marine ecosystems, due to their long persistence and accumulation. Analyses in water samples from careening areas of several ports showed concentrations of atrazine up to 0.82 μg/L, while diuron presence reached up to 0.21 μg/L, in both cases being the Mediterranean the most polluted sea (Munaron et al., 2012; Nödler et al., 2014).

Moreover, ocean-going ships release different hazardous chemicals including petroleum hydrocarbons, organic compounds, toxins and heavy metals. Among others, antifouling agents such as tributyltin, copper-based pigments, zinc oxide, irgarol, and diuron, are largely exploited to prevent the growth of organisms on boats although they present detrimental environmental effects in semi-enclosed marine systems and highly affect populated coastal areas (Karlsson et al., 2010; Turner, 2010). For example, irgarol has been found above detection limits in 63% of seawater and freshwater samples from Denmark (Vorkamp et al., 2014). Previous studies from monitored sites in ports,

* Corresponding author.
E-mail addresses: l.mororesearch@gmail.com (L. Moro), g.pezzotti@biosensor.it (G. Pezzotti), m.turemis@biosensor.it (M. Turemis), josep.sanchis@idaea.csic.es (J. Sanchís), marinella.farre@idaea.csic.es (M. Farré), renata.denaro@iamc.cnr.it (R. Denaro), mariagrazia.giacobbe@iamc.cnr.it (M.G. Giacobbe), francesca.crisafi@iamc.cnr.it (F. Crisafi), mt.giardi@biosensor.it (M.T. Giardi).

https://doi.org/10.1016/j.marpolbul.2018.02.036
Received 4 October 2017; Received in revised form 15 February 2018; Accepted 20 February 2018

Table 1
Description of microalgae species from different taxonomic groups used along this work. The fluorescence parameter F_v/F_m was determined at the maximum growth under optimal conditions for each species. Mean F_v/F_m values ± standard deviations from three measurements are shown.

	Culture collection & strain number	Origin	F_v/F_m
Chlorophyceae			
Chlamydomonas reinhardtii (intronless)[a]	SAG 11-32b[b]	Soil, Massachusetts, USA	0.869 ± 0.003
Chromochloris zofingiensis	CAUP H6006[c]	Soil, Báb, Slovakia	0.851 ± 0.003
Trebouxiophyceae			
Chlorella mirabilis	CAUP H1988[c]	Unknown	0.799 ± 0.004
Chlorella sorokiniana	CAUP H1957[c]	Warm local surface waters, Austin, Texas, USA	0.777 ± 0.002
Chlorella vulgaris	CAUP H1987[c]	Sweden	0.804 ± 0.005
Chloroidium saccharophilum	CAUP H1912[c]	Sap from wounded *Populus alba*, Germany	0.853 ± 0.005
Choricystis parasitica	CAUP H1983[c]	Endosymbiont of *Spongilla lacustris*, Manumet Beach, Massachusetts, USA	0.799 ± 0.003
Dinoflagellate			
Alexandrium minutum	CNR AMI-D6[d]	Marine	Nd
Alexandrium tamarense	CNR ATA-C2[d]	Marine	Nd
Alexandrium taylori	CNR ATAY-sard[d]	Marine	Nd
Coolia monotis	CNR CM-A6[d]	Marine	0.428 ± 0.007
Scrippsiella spp.	CNR SCRIPPS[d]	Marine	0.655 ± 0.006
Diatom			
Chaetoceros sp.	CBA 5[f]	Marine	0.611 ± 0.006
Skeletonema marinoi	CBA 22[f]	Marine	Nd
Cylindrotheca fusiformis	CBA 115[f]	Marine	Nd
Eustigmatophyceae			
Nannochloropsis sp.	CCAP 211/46[e]	Marine	0.338 ± 0.009

Nd, not detectable.
[a] *Chlamydomonas reinhardtii* mutant with an intronless *psbA* gene (Johanningmeier and Heiss, 1993).
[b] SAG; Culture Collection of Algae at Göttingen University (Germany).
[c] CAUP, Culture Collection of Algae of Charles University of Prague (Czech Republic).
[d] CNR, National Research Council, Culture collection of IAMC, Messina (Italy).
[e] CCAP, The Culture Collection of Algae and Protozoa (UK).
[f] CBA, Centre of Environmental Biology, University of Urbino (Italy).

marinas and estuarine areas outside of Europe reported irgarol concentrations between tens and thousands of ng/L (Eguchi et al., 2010; Knutson et al., 2012; Sapozhnikova et al., 2013).

Furthermore, modifications in environmental conditions due to climate change are expected to cause alterations in the bioavailability and toxicity of chemicals and their spread in the marine ecosystem (Lehtonen et al., 2017). Therefore, holistic solutions need to be applied for reducing waste and pollutants entering marine ecosystems while providing environmental restoration of waterways, coastlines and oceans.

Current approaches for monitoring of marine 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 (Brayner et al., 2011), thus requiring combination with biomarker assays and ecosystem monitoring (Galloway, 2006; Hamza-Chaffai, 2014). This 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 marine water monitoring with greater efficiency (Verma and Bhardwaj, 2015). Indeed, integrated, cost-effective, easy to use, and fast biosensors can be projected to characterize the extent of marine 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 and wastewater, mainly because of the highly demanding working environment that seawater constitutes (Kröger et al., 2002; Kröger and Law, 2005). In order to face the challenges posed by marine 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 (PAHs), endocrine disruptors, trace metals, organism detection and algal toxins have been described in literature (Kröger and Law, 2005). Biosensor strategies for pesticide detection in marine ecosystems are mainly based on the use of enzymes (Sturm et al., 1999), antibodies (Belkhamssa et al., 2016), or microorganisms, such as bacteria (Ranjan et al., 2012).

Algal biosensors react very broadly to toxicity and their detection mechanism frequently relies on measurement of the photosynthetic activity (D'ors et al., 2010; Podola et al., 2004; Tahirbegi et al., 2017). 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 (Q_A), to the secondary quinine (Q_B) along the photosynthetic chain (Rea et al., 2009). 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 (Brayner et al., 2011; Ferro et al., 2012; Pardos et al., 1998; Védrine et al., 2003). However, high salinity conditions present in marine environment 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 marine water samples by exploiting various green microalgae strains. Microalgae species were selected among different taxonomic groups available from algae collections (Table 1), in order to evaluate their potential use as biocomponents for toxicity measurement in seawater.