American Journal of Plant Sciences, 2015, 6, 2502-2509
Published Online September 2015 in SciRes. http://www.scirp.org/journal/ajps
http://dx.doi.org/10.4236/ajps.2015.615252

Assessing Ecotoxicity in Marine Environment Using Luminescent Microalgae: Where Are We At?

Sophie Sanchez-Ferandin[1,2]

[1]Sorbonne Universités, UPMC Univ Paris 06, UMR7232 Biologie Intégrative des Organismes Marins (BIOM), Observatoire Océanologique, Banyuls-sur-Mer, France
[2]CNRS, UMR7232 Biologie Intégrative des Organismes Marins (BIOM), Observatoire Océanologique, Banyuls-sur-Mer, France
Email: sophie.sanchez-ferandin@obs-banyuls.fr

Received 27 July 2015; accepted 27 September 2015; published 30 September 2015

Abstract

Nowadays, microalgae are particularly used to assess the environmental impact of contaminants in aquatic systems. Naturally present in some algal species, bioluminescence is highly used in application fields related to environmental monitoring. Bioluminescent dinoflagellates have played a pivotal role in this domain. When exposed to heavy metals or toxic organic compounds, bioluminescent dinoflagellates have the capacity to decrease light emission. In addition, new molecular tools allow the possibility to produce genetically modified microorganisms which are able to perform luminescence. Combined with the luciferase reporter gene, two main genetic constructions can be employed. Activation of a specific inducible promoter induces the luminescence gene transcription and this signal increases over time. Constitutive promoters result in a high basal expression level of the reporter gene. During exposure to a potential toxic pollutant, the basal expression level will decrease due to the toxic effect. Toxicity bioassays based on engineered luminescent Chlorophyta microalgae are among the most sensitive tests and are an invaluable complement to classical toxicity assays.

Keywords

Ecotoxicity, Microalgae, Bioluminescence, Luciferase, Gene Reporter System

1. Introduction

Persistent organic and inorganic environmental pollutants can be toxic and accumulate in numbers of aquatic

How to cite this paper: Sanchez-Ferandin, S. (2015) Assessing Ecotoxicity in Marine Environment Using Luminescent Microalgae: Where Are We At? *American Journal of Plant Sciences*, **6**, 2502-2509.
http://dx.doi.org/10.4236/ajps.2015.615252

species. To predict the effect of environmental stressors on populations, communities and ecosystems, ecotoxicological tests using microorganisms are particularly efficient. Marine microalgae are a major constituent of the aquatic food chain and play an important role in coastal ecosystems [1]. These unicellular cells tend to be more sensitive than animal species to a wide variety of both organic and inorganic pollutants [2]. They are particularly used for the development of new toxicity bioassays and to assess the environmental impact of contaminants in aquatic systems [3]. The use of flow cytometry is an important step in the development of growth inhibition tests. It offers the possibility to perform multiparameter analysis on a wide range of cell properties (algal cell densities, cell size and shape or granularity) [4]. In addition, compared to traditional tests, a high growth rate and good level of biomass are reached in a short time. Microalgae are widely used in ecotoxicological tests to evaluate the toxicity effects of diverse pollutants: dichloromethane and dichloroethane on *Chlorella vulgaris* [5], nanoparticles on freshwater (*Chlorococcum* sp. and *Scenedesmus rubescens*) and marine species (*Dunaliella tertiolecta* and *Tetraselmis suesica*) [6]; pentachlorophenol on co-cultures of freshwater phytoplankton species (the cyanobacterium *Microcystis aeruginosa* and the microalgae *Chlorella vulgaris*) [7]. Nowadays, new microalgae models continue to emerge like *Phaeocystis antarctica*, a species isolated from Antarctic coastal marine environments, which will be suitable to bring information on the sensitivity of Antarctic microalgae to metal contamination [8].

Bioluminescent microalgae are often responsible for large-scale phenomena known as the “shining seas” in marine waters. Basically, the reaction of bioluminescence requires an enzyme, the luciferase, to catalyze an oxidation reaction. The natural capacity to produce bioluminescence by different species of microalgae appears as an interesting asset. Dinoflagellate and bacteria are the main bioluminescent microorganisms in marine environment (for a review see [9]) and ecological aspects and molecular mechanisms that regulate bioluminescence phenomenon are well known in these microorganisms. Nowadays, naturally produced luminescence can be used to monitor environmental quality: some bioluminescent dinoflagellates have a unique capacity of decreasing light emission when are exposed to heavy metals or toxic organic compounds. In addition, new molecular tools allow the possibility to produce genetically modified microorganisms which are able to perform luminescence. Although an exhaustive description of all applications of bioluminescence is outside the scope of this review, we will present here how the bioluminescence has inspired scientists in the field of ecotoxicological tests.

2. Bioluminescent Microalgae in Ecotoxicity

2.1. Bioluminescent Genes Used as a Detection Tool

Detection of harmful and innocuous bioluminescent microorganisms in environmental sea water can be done using various molecular techniques. Direct search in global environmental databases is sometimes difficult because the majority of libraries are collections of 18S rDNA sequences. To overcome this problem, the development of species-specific probes (targeting the LuxA gene of the lux operon in bacteria) has helped to identify luminescent bacteria in both laboratory cultures [10] and seawater samples [11]. In the same way, molecular methods helped to characterize bioluminescent microalgae species [12]. A particular attention was given to the dinoflagellates luciferase gene. “Universal” primers were designed for the development of PCR-oriented approaches to specifically detect bioluminescent dinoflagellates in environmental waters samples [13]-[15]. The development of PCR primers targeting a longer gene sequence helped to assess bioluminescent potential in multiple strains of one selected species [15]. The authors found that out of 34 tested dinoflagellate strains, 23 possessed the luciferase gene while only 18 were able to produce light in laboratory culture conditions [15]. Several transcriptomics’ studies also suggested other sequences that could serve as indicators of bioluminescent species possessing luciferin binding protein as this protein is highly expressed in *Lingulodinium polyedrum* [16] [17] and in *Alexandrium tamarense* [18], and is the most highly expressed protein in *Alexandrium catenella* [19] [20]. Highly localized bioluminescence in marine environmement is sometimes considered as an early indicator of algal bloom development [21]. A large number of harmful dinoflagellate taxa luminesce [22] and in the future, bioluminescence detection will be probably a useful tool to localize them and prevent vulnerability of human populations.

2.2. Bioluminescent Dinoflagellates in Ecotoxicological Tests

Bioluminescent microorganisms can be used as natural whole-cell biosensors to sense environmental signals

because of their natural capacity of decreasing light emission when exposed to toxic conditions ("natural luminescence", **Figure 1**). Historically, biossays using bioluminescent bacteria were firstly developed. The Microtox bioassay [23] is available since 1990. It proposes the detection of over 2000 toxic chemicals thanks to the reduction of light emission from the bioluminescent marine bacteria *Vibrio fischeri* as a means for measuring toxicity. For this purpose, the toxicity of different chemicals is compared by classifying them according to the half maximal effective concentration (EC_{50}) parameter. This EC_{50} parameter represents the concentration of a compound which causes 50% of its maximal effect (for example, a 50% decrease of luminescence). In other words, for a given compound, the lowest is the concentration, the most toxic is the compound. The Microtox bioassay has become the commonly accepted method in ecotoxicity assessments [24] and is currently used in a wide range of applications. This 15-min test allows the detection of products as diverse as cadmium chloride ($CdCI_2$) (EC_{50} = 56.8 mg·L^{-1} ± 8.46 mg·L^{-1}) or mercury chloride ($HgCl_2$) (EC_{50} = 0.060 or 0.093 mg·L^{-1} depending on studies) [25] [26]. Another bioluminescent bacteria, *Photobacterium phosphoreum*, was proposed for detecting and signalling the presence of toxicants in water systems [27]. Microalgae are also attractive systems for the development of new toxicity bioassays. Some bioluminescent dinoflagellates (*Lingulodinium polyedrum*, *Pyrocystis lunula*...) show reduced light emission when exposed to heavy metals or toxic organic compounds ("natural bioluminescence", **Figure 1**). When exposed to lead (EC_{50} = 321 ppb) and copper (EC_{50} = 23 ppb), *Lingulodinium polyedrum* bioluminescence was affected by the metals in a dose-dependent manner [28] and yielded accurate and more sensitive results when compared to traditional test like the crustacean *Mysidopsis* survival test (LC_{50} = 3130 ppb and LC_{50} = 120 - 140 ppb respectively) [28] [29]. In the Lumitox bioassay that uses a *Pyrocystis lunula* monospecific culture, recovery of luminescence is sometimes achieved by decreasing pH depending on the toxicants used [30]. The ASTM-approved QwikLite bioassay, developed with *Pyrocystis lunula* and *Lingulodinium polyedrum*, utilizes only 300 cells per experimental condition. Four other microalgae species (*Ceratocorys horrida*, *Pyrocystis noctiluca*, *P. fusiformis* and *Pyrophacus steinii*) can be used in these bioassays. Cells are separately maintained in a Laboratory Plankton Test Chamber (LPTC) [29] and evaluated following a 24 h exposure period. Whatever the test used, assays generally result in similar toxicity rankings for the metal tested. However, comparison between QwikLite and Microtox tests revealed that QwikLite assay was generally one to two orders of magnitude more sensitive than the Microtox test. For example, after exposure to copper, *L. polydreum* EC_{50} was only 0.090 ± 0.012 mg·L^{-1} while *V. fischeri* EC_{50} was 0.397 ± 0.030 mg·L^{-1} and after exposure to lead, *L. polydreum* EC_{50} was only 0.747 ± 0.088 mg·L^{-1} while *V. fischeri* EC_{50} reached 34.6 ± 11.7 mg·L^{-1}

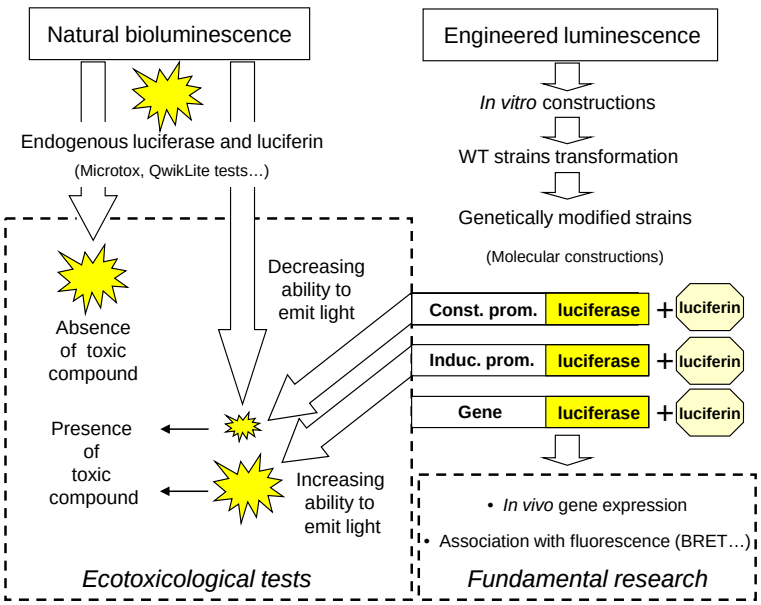

Figure 1. Natural and engineered luminescence used for environmental and fundamental research themes. "WT": Wild-Type; "Induc. prom.": Inducible promoter; "Const. prom.": Constitutive promoter; "BRET": Bioluminescence Resonance Energy Transfer.

[26]. In the same way, QwikLite is much more sensitive to total ammonia (10 ± 1.1 $mg \cdot L^{-1}$) than Microtox, in which the 15 min EC_{50} for inhibition of bioluminescence is reported to be higher than 900 $mg \cdot L^{-1}$ [31] [32]. In contrast, although QwikLite generally shows better precision than classical algal growth rate bioassays, it can be insensitive to other compounds like diuron [32].

3. Engineered Luminescent Microalgae in Ecotoxicity

3.1. Genetic Engineering with Non-Naturally Luminescent Organisms

Genes responsible for bioluminescence can be transferred and expressed in several microorganisms. Luminescent reporter genes are popular tools for the real-time monitoring of gene expression in living cells. Utilization of luminescence has become an important component of biomedical research [33] [34]. Biological processes can be monitored *in vivo*. Pathogens can be tracked over time. Our understanding of the effect of infections and host immune responses was substantially improved thanks to the production of recombinant virus and bacteria [35]-[39]. More recently, in food industry (for a review see [40]), luminescence tools permit to follow the survival of pathogenic microorganisms and the expression of bacterial toxins in foods. Interestingly, specific bacteriophages genetically transformed with the Lux genes allow the direct detection of food pathogens [41]. Genetically modified whole-cell bioreporters using luminescence detection are practical tools for the detection and monitoring of contaminants in environmental context. *In vitro* toxicity assays using recombinant *Escherichia coli* (encoding a luciferase from *Photorhabdus luminescens*) was used as an alternative to the *Vibrio fischeri* Microtox test [42].

3.2. Genetic Transformation with a Luciferase Gene Reporter System

Toxicity bioassays sometimes require utilization of genetic engineering with non luminescent organisms [43]. Different expression systems exist. Gene reporter systems using inducible or constitutive promoters are two ways currently used for the evaluation of sample toxicity. An inducible expression system is based on a specific inducible promoter. Inducible promoter activation depends on a substance of interest which induces promoter activity, expression of the reporter gene, and transcription of an adjacent resistance gene. Luciferase gene reporter gives the opportunity to follow the detection of the substance of interest by luminescent signal. This signal increases over time as a result of inducible promoter activity ("Induc. prom.", **Figure 1**). Using this approach, 20 genetically modified *E. coli* strains were designed and responded differently in the presence of diverse chemicals [44]. In the same way, whole-cell biosensor assays for arsenic detection have been proposed. Various indicator tests were developed for the detection of this compound in the environment [45]-[47]. On the opposite way, a constitutive expression system generally uses a gene promoter that is highly expressed under normal conditions. It results in a high basal expression level of the reporter gene (see the following section). During exposure to a pollutant, the basal expression level and the emission of luminescence decrease due to the toxic effect ("Const. prom.", **Figure 1**). This substrate-dependent reporter system sometimes requires luciferin addition when only luciferase is present in the genetic construction (**Figure 1**). The use of these technologies produces fast and economical high-throughput biosensor systems for detecting environmental pollution.

3.3. Towards New Models

As for bacteria, the integration of genetic material by injection or transformation can be performed in eukaryotic organisms. Recently, new genetically modified microorganisms have emerged. Discovered in a Mediterranean lagoon, the unicellular alga *Ostreococcus tauri* (Chlorophyta, Mamiellophyceae) is the smallest free-living eukaryotic cell known to date [48] [49] and its compact genome is entirely sequenced and published [50] [51]. Although this photosynthetic green alga does not have the intrinsic ability to produce bioluminescence naturally, it can easily be cultivated and transformed in the laboratory thus allowing functional genomics approaches. *In vitro* molecular constructions using luciferase gene reporter system can be integrated in the genome of *O. tauri*, enabling this eukaryote to emit light ("Const. prom.", **Figure 1**). Expression of genes of interest can then be studied *in vivo* in the context of fundamental research. Cell division mechanisms [52] and circadian clock pathways [53] were thereby analysed. Very recently, recombinant *O. tauri* cells expressing firefly luciferase were used to monitor the impact of herbicides on this marine alga and may thus be valuable biosensors for environmental monitoring [54]. More precisely, a luminescence assay using CDKA-luc biosensor (integration in *O. tauri* genome of CDKA gene fused with luciferase coding sequence) gave reasonable range of EC_{50} recorded for

two biocides, diuron (EC_{50} = 5.65 ± 0.44 µg·L^{-1}) and Irgarol 1051 (EC_{50} = 0.76 ± 0.10 µg·L^{-1}) after 48 hours [54] compared to standard growth inhibition assays using other phytoplankton strains. This assay, using CDKA-luc lines, detected lesser diuron concentrations to those using a QwikLite test using *Pyrocystis lunula* (19 ± 13 mg·L^{-1}) [32]. These *O. tauri* CDKA-luc lines detected similar Irgarol 1051 concentrations compared to the growth inhibition assay using the marine chlorophyte *Dunaliella tertiolecta* [55] but was about ten-fold more sensitive than using growth inhibition with the freshwater species *Raphidocelis subcapitata* [56]. Moreover, for each of the two biocides, lower EC_{50} were obtained from the luminescent marker approach when compared to wild-type *O. tauri* growth experiments with the same 48 hours exposure time (five-fold lower EC_{50}) [54], underlining the improved sensitivity of this biosensor. To our knowledge, the *O. tauri* luminescence assay is the most sensitive test of all whole cell luminescence biosensors for detecting diuron and Irgarol 1051. It was particularly sensitive to both biocides compared to the frequently used *Vibrio fischeri* Microtox luminescent test, with a difference of three to four orders of magnitude (Microtox gave EC_{50} = 58 and 51 mg·L^{-1} for diuron and Irgarol 1051 respectively) [54] [57] [58].

4. Conclusions and Future Perspectives

The use of microorganisms provides easiness of use, rapid response and cost effectiveness. They can be used in diverse applied fields notably in environmental monitoring. Knowledge acquired from natural bioluminescence allows the development of sophisticated molecular tools. Luminescent genetically modified microorganisms are easy to manipulate and allow rapid and reproducible experiments. They fulfil the major requirements of whole cell miniaturized reporters and constitute a new generation of biosensors. They are among the most sensitive tests and will probably be intensively used in emerging environmental issues. Recently, the transfer of metal oxide nanoparticles from microalgae (*Cricosphaera elongata*) to sea urchin larvae (*Paracentrotus lividus*) was shown [59]. Investigating the potential toxicity of such particles on aquatic organisms and their entry into the food chain has become an important issue. Microalgae like *Ostreococcus tauri* are thus good candidates to help to answer to these questions.

Acknowledgements

Thanks to the three anonymous referees who evaluated this paper, and to the partial support from the “Oceanomics” project (ANR-11-BTBR-0008).

References

[1] Behrenfeld, M.J., O’Malley, R.T., Siegel, D.A., McClain, C.R., Sarmiento, J.L., Feldman, G.C., Milligan, A.J., Falkowski, P.G., Letelier, R.M. and Boss, E.S. (2006) Climate-Driven Trends in Contemporary Ocean Productivity. *Nature*, **444**, 752-755. http://dx.doi.org/10.1038/nature05317

[2] Klaine, S.J. and Lewis, M.A. (1995) Algal and Plant Toxicity Testing. In: Hoffman, D.J., Rattner, B.A., Burton Jr., G.A. and Cairns Jr., J., Eds., *Handbook of Ecotoxicology*, Lewis Publishers, Boca Raton, 163-184.

[3] Stauber, J.L. and Davies, C.M. (2000) Use and Limitations of Microbial Bioassays for Assessing Copper Bioavailability in the Aquatic Environment. *Environmental Reviews*, **8**, 255-301. http://dx.doi.org/10.1139/a00-010

[4] Stauber, J.L., Franklin, N.M. and Adams, M.S. (2002) Applications of Flow Cytometry to Ecotoxicity Testing Using Microalgae. *Trends in Biotechnology*, **20**, 141-143. http://dx.doi.org/10.1016/S0167-7799(01)01924-2

[5] Wu, S., Zhang, H., Yu, X. and Qiu, L. (2014) Toxicological Responses of *Chlorella vulgaris* to Dichloromethane and Dichloroethane. *Environmental Engineering Science*, **31**, 9-17. http://dx.doi.org/10.1089/ees.2013.0038

[6] Aravantinou, A.F., Tsarpali, V., Dailianis, S. and Manariotis, I.D. (2015) Effect of Cultivation Media on the Toxicity of ZnO Nanoparticles to Freshwater and Marine Microalgae. *Ecotoxicology and Environmental Safety*, **114**, 109-116. http://dx.doi.org/10.1016/j.ecoenv.2015.01.016

[7] de Morais, P., Stoichev, T., Basto, M.C.P., Ramos, V., Vasconcelos, V.M. and Vasconcelos, M.T.S.D. (2014) Pentachlorophenol Toxicity to a Mixture of *Microcystis aeruginosa* and *Chlorella vulgaris* Cultures. *Aquatic Toxicology*, **150**, 159-164. http://dx.doi.org/10.1016/j.aquatox.2014.03.008

[8] Gissi, F., Adams, M.S., King, C.K. and Jolley, D.F. (2015) A Robust Bioassay to Assess the Toxicity of Metals to the Antarctic Marine Microalga *Phaeocystis antarctica*. *Environmental Toxicology and Chemistry*, **34**, 1578-1587. http://dx.doi.org/10.1002/etc.2949

[9] Haddock, S.H.D., Moline, M.A. and Case, J.F. (2010) Bioluminescence in the Sea. *Annual Review of Marine Science*,

2, 443-493. http://dx.doi.org/10.1146/annurev-marine-120308-081028

[10] Wimpee, C.F., Nadeau, T.-L. and Nealson, K.H. (1991) Development of Species-Specific Hybridization Probes for Marine Luminous Bacteria by Using *in Vitro* DNA Amplification. *Applied and Environmental Microbiology*, **57**, 1319-1324.

[11] Lee, K.-H. and Ruby, E.G. (1992) Detection of the Light Organ Symbiont, *Vibrio fischeri*, in Hawaiian Seawater by Using Lux Gene Probes. *Applied and Environmental Microbiology*, **58**, 942-947.

[12] Frommlet, J.C. and Iglesias-Rodriguez, M.D. (2008) Microsatellite Genotyping of Single Cells of the Dinoflagellate Species *Lingulodinium polyedrum* (Dinophyceae): A Novel Approach for Marine Microbial Population Genetic Studies. *Journal of Phycology*, **44**, 1116-1125. http://dx.doi.org/10.1111/j.1529-8817.2008.00566.x

[13] Baker, A., Robbins, I., Moline, M.A. and Iglesias-Rodriguez, M.D. (2008) Oligonucleotide Primers for the Detection of Bioluminescent Dinoflagellates Reveal Novel Luciferase Sequences and Information on the Molecular Evolution of This Gene. *Journal of Phycology*, **44**, 419-428. http://dx.doi.org/10.1111/j.1529-8817.2008.00474.x

[14] Valiadi, M., Painter, S.C., Allen, J.T., Balch, W.M. and Iglesias-Rodriguez, M.D. (2014) Molecular Detection of Bioluminescent Dinoflagellates in Surface Waters of the Patagonian Shelf during Early Austral Summer 2008. *PLoS ONE*, **9**, e98849. http://dx.doi.org/10.1371/journal.pone.0098849

[15] Valiadi, M., Iglesias-Rodriguez, M.D. and Amorim, A. (2012) Distribution and Genetic Diversity of the Luciferase Gene within Marine Dinoflagellates. *Journal of Phycology*, **48**, 826-836. http://dx.doi.org/10.1111/j.1529-8817.2012.01144.x

[16] Machabee, S., Wall, L. and Morse, D. (1994) Expression and Genomic Organization of a Dinoflagellate Gene Family. *Plant Molecular Biology*, **25**, 23-31. http://dx.doi.org/10.1007/BF00024195

[17] Tanikawa, N., Akimoto, H., Ogoh, K., Chun, W. and Ohmiya, Y. (2004) Expressed Sequence Tag Analysis of the Dinoflagellate *Lingulodinium polyedrum* during Dark Phase. *Photochemistry and Photobiology*, **80**, 31-35. http://dx.doi.org/10.1562/2004-03-12-RA-110.1

[18] Hackett, J.D., Scheetz, T.E., Yoon, H.S., Soares, M.B., Bonaldo, M.F., Casavant, T.L. and Bhattacharya, D. (2005) Insight into a Dinoflagellate Genome through Expressed Sequence Tag Analysis. *BMC Genomics*, **6**, 80. http://dx.doi.org/10.1186/1471-2164-6-80

[19] Uribe, P., Fuentes, D., Valdes, J., Shmaryahu, A., Zuniga, A., Holmes, D. and Valenzuela, P.D. (2008) Preparation and Analysis of an Expressed Sequence Tag Library from the Toxic Dinoflagellate *Alexandrium catenella*. *Marine Biotechnology*, **10**, 692-700. http://dx.doi.org/10.1007/s10126-008-9107-8

[20] Toulza, E., Shin, M., Blanc, G., Audic, S., Laabir, M., Collos, Y., Claverie, J. and Grzebyk, D. (2013) Gene Expression in Proliferating Cells of the Dinoflagellate *Alexandrium catenella* (Dinophyceae). *Applied and Environmental Microbiology*, **76**, 4521-4529. http://dx.doi.org/10.1128/AEM.02345-09

[21] Le Tortorec, A.H., Hakanen, P., Kremp, A., Olsson, J., Suikkanen, S. and Simis, S.G.H. (2014) Stimulated Bioluminescence as an Early Indicator of Bloom Development of the Toxic Dinoflagellate *Alexandrium ostenfeldii*. *Journal of Plankton Research*, **36**, 412-423. http://dx.doi.org/10.1093/plankt/fbt116

[22] Van den Hoek, C., Mann, D.G. and Jahns, H.M. (1995) Algae: An Introduction to Phycology. Cambridge University Press, Cambridge.

[23] Bulich, A. (1979) Use of Luminescent Bacteria for Determining Toxicity in Aquatic Environments. *ASTM International*, **667**, 98-106. http://dx.doi.org/10.1520/stp34880s

[24] Johnson, B.T. (2005) Small-Scale Freshwater Toxicity Investigations—Microtox® Acute Toxicity Test. In: Blaise, C. and Férard, J.-F., Eds., Springer, Netherlands, 69-115.

[25] McFeters, G.A., Bond, P.J., Olson, S.B. and Tchan, Y.T. (1983) A Comparison of Microbial Bioassays for the Detection of Aquatic Toxicants. *Water Research*, **17**, 1757-1762. http://dx.doi.org/10.1016/0043-1354(83)90197-5

[26] Rosen, G., Osorio-Robayo, A., Rivera-Duarte, I. and Lapota, D. (2008) Comparison of Bioluminescent Dinoflagellate (QwikLite) and Bacterial (Microtox) Rapid Bioassays for the Detection of Metal and Ammonia Toxicity. *Archives of Environmental Contamination and Toxicology*, **54**, 606-611. http://dx.doi.org/10.1007/s00244-007-9068-3

[27] Vetrova, E., Esimbekova, E., Remmel, N., Kotova, S., Beloskov, N., Kratasyuk, V. and Gitelson, I. (2007) A Bioluminescent Signal System: Detection of Chemical Toxicants in Water. *Luminescence*, **22**, 206-214. http://dx.doi.org/10.1002/bio.951

[28] Okamoto, O.K., Shao, L., Hastings, J.W. and Colepicolo, P. (1999) Acute and Chronic Effects of Toxic Metals on Viability, Encystment and Bioluminescence in the Dinoflagellate *Gonyaulax polyedra*. *Comparative Biochemistry and Physiology Part C*, **123**, 75-83.

[29] Lapota, D., Robayo Osorio, A., Liao, C. and Bjorndal, B. (2007) The Use of Bioluminescent Dinoflagellates as an Environmental Risk Assessment Tool. *Marine Pollution Bulletin*, **54**, 1857-1867.

http://dx.doi.org/10.1016/j.marpolbul.2007.08.008

[30] Craig, J.M., Klerks, P.L., Heimann, K. and Waits, J.L. (2003) Effects of Salinity, pH and Temperature on the Re-Establishment of Bioluminescence and Copper or SDS Toxicity in the Marine Dinoflagellate *Pyrocystis lunula* Using Bioluminescence as an Endpoint. *Environmental Pollution*, **125**, 267-275. http://dx.doi.org/10.1016/S0269-7491(03)00059-9

[31] Adams, M.S., Stauber, J.L., Binet, M.T., Molloy, R. and Gregory, D. (2008) Toxicity of a Secondary-Treated Sewage Effluent to Marine Biota in Bass Strait, Australia: Development of Action Trigger Values for a Toxicity Monitoring Program. *Marine Pollution Bulletin*, **57**, 587-598. http://dx.doi.org/10.1016/j.marpolbul.2007.12.012

[32] Stauber, J.L., Binet, M.T., Bao, V.W.W., Boge, J., Zhang, A.Q., Leung, K.M.Y. and Adams, M.S. (2008) Comparison of the QwikLite™ Algal Bioluminescence Test with Marine Algal Growth Rate Inhibition Bioassays. *Environmental Toxicology*, **23**, 617-625. http://dx.doi.org/10.1002/tox.20400

[33] Luker, K.E. and Luker, G.D. (2010) Bioluminescence Imaging of Reporter Mice for Studies of Infection and Inflammation. *Antiviral Research*, **86**, 93-100. http://dx.doi.org/10.1016/j.antiviral.2010.02.002

[34] Zinn, K.R., Chaudhuri, T.R., Szafran, A.A., O'Quinn, D., Weaver, C., Dugger, K., Lamar, D., Kesterson, R.A., Wang, X. and Frank, S.J. (2008) Noninvasive Bioluminescence Imaging in Small Animals. *Institute of Laboratory Animal Research Journal*, **49**, 103-115. http://dx.doi.org/10.1093/ilar.49.1.103

[35] Luker, G., Bardill, J., Prior, J., Pica, C., Piwnica-Worms, and Leib, D.A. (2002) Noninvasive Bioluminescence Imaging of Herpes Simplex Virus Type 1 Infection and Therapy in Living Mice. *Journal of Virology*, **76**, 12149-12161. http://dx.doi.org/10.1128/JVI.76.23.12149-12161.2002

[36] Cook, S. and Griffin, D. (2003) Luciferase Imaging of a Neurotropic Viral Infection in Intact Animals. *Journal of Virology*, **77**, 5333-5338. http://dx.doi.org/10.1128/jvi.77.9.5333-5338.2003

[37] Hwang, S., Wu, T.-T., Tong, L., Kim, K., Martinez-Guzman, D., Colantonio, A., Uittenbogaart, C. and Sun, R. (2008) Persistent Gammaherpesvirus Replication and Dynamic Interaction with the Host *in Vivo*. *Journal of Virology*, **82**, 12498-12509. http://dx.doi.org/10.1128/JVI.01152-08

[38] Zaitseva, M., Kapnick, S., Scott, J., King, L., Manischewitz, J., Sirota, L., Kodihalli, S. and Golding, H. (2009) Application of Bioluminescence Imaging to the Prediction of Lethality in Vaccinia Virus-Infected Mice. *Journal of Virology*, **83**, 10437-10447. http://dx.doi.org/10.1128/JVI.01296-09

[39] Andreu, N., Zelmer, A., Fletcher, T., Elkington, P.T., Ward, T.H., Ripoll, J., Parish, T., Bancroft, G.J., Schaible, U., Robertson, B.D. and Wiles, S. (2010) Optimisation of Bioluminescent Reporters for Use with Mycobacteria. *PLoS ONE*, **5**, e10777. http://dx.doi.org/10.1371/journal.pone.0010777

[40] Morrissey, R., Hill, C. and Begley, M. (2013) Shining Light on Food Microbiology; Applications of Lux-Tagged Microorganisms in the Food Industry. *Trends in Food Science and Technology*, **32**, 4-15. http://dx.doi.org/10.1016/j.tifs.2013.05.001

[41] Griffiths, M.W. (2000) How Novel Methods Can Help Discover More Information about Foodborne Pathogens. *Canadian Journal of Infectious Diseases*, **11**, 142-153.

[42] Kurvet, I., Ivask, A., Bondarenko, O., Sihtmäe, M. and Kahru, A. (2011) LuxCDABE—Transformed Constitutively Bioluminescent *Escherichia coli* for Toxicity Screening: Comparison with Naturally Luminous *Vibrio fischeri*. *Sensors*, **11**, 7865-7878. http://dx.doi.org/10.3390/s110807865

[43] Xu, T.T., Close, D.M., Sayler, G.S. and Ripp, S. (2013) Genetically Modified Whole-Cell Bioreporters for Environmental Assessment. *Ecological Indicators*, **28**, 125-141. http://dx.doi.org/10.1016/j.ecolind.2012.01.020

[44] Lee, J.H., Mitchell, R.J., Kim, B.C., Cullen, D.C. and Gu, M.B. (2005) A Cell Array Biosensor for Environmental Toxicity Analysis. *Biosensors and Bioelectronics*, **21**, 500-507. http://dx.doi.org/10.1016/j.bios.2004.12.015

[45] Tauriainen, S., Karp, M., Chang, W. and Virta, M. (1997) Recombinant Luminescent Bacteria for Measuring Bioavailable Arsenite and Antimonite. *Applied and Environmental Microbiology*, **63**, 4456-4461.

[46] Stocker, J., Balluch, D., Gsell, M., Harms, H., Feliciano, J., Daunert, S., Malik, K.A. and van der Meer, J.R. (2003) Development of a Set of Simple Bacterial Biosensors for Quantitative and Rapid Measurements of Arsenite and Arsenate in Potable Water. *Environmental Science and Technology*, **37**, 4743-4750. http://dx.doi.org/10.1021/es034258b

[47] Sharma, P., Asad, S. and Ali, A. (2013) Bioluminescent Bioreporter for Assessment of Arsenic Contamination in Water Samples of India. *Journal of Biosciences*, **38**, 251-258. http://dx.doi.org/10.1007/s12038-013-9305-z

[48] Courties, C., Vaquer, A. and Troussellier, M. (1994) Smallest Eukaryotic Organism. *Nature*, **370**, 255. http://dx.doi.org/10.1038/370255a0

[49] Chrétiennot-Dinet, M.-J., Courties, C., Vaquer, A., Neveux, J., Claustre, H., Lautier, J. and Machado, M.-C. (1995) A New Marine Picoeucaryote: *Ostreococcus tauri* Gen. et sp. nov. (Chlorophyta, Prasinophyceæ). *Phycologia*, **34**, 285-292. http://dx.doi.org/10.2216/i0031-8884-34-4-285.1

[50] Derelle, E., Ferraz, C., Rombauts, S., Rouze, P., Worden, A.Z., Robbens, S., Partensky, F., Degroeve, S., Echeynie, S., Cooke, R., Saeys, Y., Wuyts, J., Jabbari, K., Bowler, C., Panaud, O., Piegu, B., Ball, S.G., Ral, J.-P., Bouget, F.-Y., Piganeau, G., De Baets, B., Picard, A., Delseny, M., Demaille, J., Van de Peer, Y. and Moreau, H. (2006) Genome Analysis of the Smallest Free-Living Eukaryote *Ostreococcus tauri* Unveils Many Unique Features. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 11647-11652. http://dx.doi.org/10.1073/pnas.0604795103

[51] Palenik, B., Grimwood, J., Aerts, A., Rouze, P., Salamov, A., Putnam, N., Dupont, C., Jorgensen, R., Derelle, E., Rombauts, S., Zhou, K., Otillar, R., Merchant, S.S., Podell, S., Gaasterland, T., Napoli, C., Gendler, K., Manuell, A., Tai, V., Vallon, O., Piganeau, G., Jancek, S., Heijde, M., Jabbari, K., Bowler, C., Lohr, M., Robbens, S., Werner, G., Dubchak, I., Pazour, G.J., Ren, Q., Paulsen, I., Delwiche, C., Schmutz, J., Rokhsar, D., Van de Peer, Y., Moreau, H. and Grigoriev, I.V. (2007) The Tiny Eukaryote *Ostreococcus* Provides Genomic Insights into the Paradox of Plankton Speciation. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 7705-7710. http://dx.doi.org/10.1073/pnas.0611046104

[52] Moulager, M., Corellou, F., Vergé, V., Escande, M.-L. and Bouget, F.-Y. (2010) Integration of Light Signals by the Retinoblastoma Pathway in the Control of S Phase Entry in the Picophytoplanktonic Cell *Ostreococcus*. *PLoS Genetics*, **6**, e1000957. http://dx.doi.org/10.1371/journal.pgen.1000957

[53] Djouani-Tahri, E., Christie, J.M., Sanchez-Ferandin, S., Sanchez, F., Bouget, F.-Y. and Corellou, F. (2011) A Eukaryotic LOV-Histidine Kinase Regulates Circadian Clock Function in the Picoalga *Ostreococcus*. *The Plant Journal*, **65**, 578-588. http://dx.doi.org/10.1111/j.1365-313X.2010.04444.x

[54] Sanchez-Ferandin, S., Leroy, F., Bouget, F.-Y. and Joux, F. (2013) A New, Sensitive Marine Microalgal Recombinant Biosensor Using Luminescence Monitoring for the Toxicity Testing of Antifouling Biocides. *Applied and Environmental Microbiology*, **79**, 631. http://dx.doi.org/10.1128/AEM.02688-12

[55] Gatidou, G. and Thomaidis, N. (2007) Evaluation of Single and Joint Toxic Effects of Two Antifouling Biocides, Their Main Metabolites and Copper Using Phytoplankton Bioassays. *Aquatic Toxicology*, **85**, 184-191. http://dx.doi.org/10.1016/j.aquatox.2007.09.002

[56] Devilla, R.A., Brown, M.T., Donkin, M. and Tarran, G.A. (2005) Impact of Antifouling Booster Biocides on Single Microalgal Species and on a Natural Marine Phytoplankton Community. *Marine Ecology Progress Series*, **286**, 1-12. http://dx.doi.org/10.3354/meps286001

[57] Bonnet, J.-L., Bonnemoy, F., Dusser, M. and Bohatier, J. (2007) Assessment of the Potential Toxicity of Herbicides and Their Degradation Products to Nontarget Cells Using Two Microorganisms, the Bacteria *Vibrio fischeri* and the Ciliate *Tetrahymena pyriformis*. *Environmental Toxicology*, **22**, 78-91. http://dx.doi.org/10.1002/tox.20237

[58] Fernández-Alba, A., Hernando, M., Piedra, L. and Chisti, Y. (2002) Toxicity Evaluation of Single and Mixed Antifouling Biocides Measured with Acute Toxicity Bioassays. *Analytica Chimica Acta*, **456**, 303-312. http://dx.doi.org/10.1016/S0003-2670(02)00037-5

[59] Gambardella, C., Gallus, L., Gatti, A.M., Faimali, M., Carbone, S., Antisari, L.V., Falugi, C. and Ferrando, S. (2014) Toxicity and Transfer of Metal Oxide Nanoparticles from Microalgae to Sea Urchin Larvae. *Chemistry and Ecology*, **30**, 308-316. http://dx.doi.org/10.1080/02757540.2013.873031