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Diatoms for Omics Applications in Water Monitoring

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Abstract

Diatoms are driving forces in marine and freshwater ecosystems by sustaining aquatic food webs and by having leading roles in the fixation of carbon and silica. The morphological diversity of the silicified cell walls from different diatom species has for long been exploited for the biotic characterization of environmental samples. Diatomic indices based on microscopic approaches have been developed and are broadly accepted, with some being able to correlate with the eutrophication level or pollution conditions. Moreover, molecular-based diatomic indices are expected to replace existing indices because of their increased sensitivity. Recent developments in omics technologies, coupled with bioinformatic tools for complex omics data analysis is opening the door for the use of diatoms as target organisms in the assessment of water quality using molecular biomarkers. Accordingly, fully sequenced diatom species are currently being applied to toxicological studies aiming to unveil the mode of action of pollutants. It is envisioned that molecular studies in diatoms will become major tools in the ecological assessment of environmental samples, matching the key role of these organisms in the ecosystem.

Introduction

Diatoms are eukaryotic photosynthetic organisms with a worldwide distribution in freshwater and marine environments and they play a dominant role in the global carbon cycle [1]. It is estimated that over 200,000 species of diatoms exist, differing in their size and cell wall shape and pattern [2]. Diatoms play a dominant role in the biogeochemical cycle of silicon by biomineralizing silicon to produce their unique cell walls [3], in contrast to other algae that have cell walls made of organic matter or calcium. Diatoms uptake silicon through silicon transporter (SIT) protein channels at the plasma membrane and accumulate it in silica deposition vesicles until biomineralizing the silica to form new valves during cell division [4]. Silica polymerization into intricate meso- and micro-scale patterns is under genetic control and is accomplished by silaffin proteins, silacidins and long chain polyamines [4] in close association with the cytoskeleton [5]. The success of diatoms as dominant marine primary producers has been related to the genetic diversity accumulated during a complex evolutionary series of endosymbiotic events. These included an ancient acquisition of a significant number of diatom nuclear genes from green algae, plastids from red algae, and by successive gains or losses of genetic material [6,7]. These features have likely helped diatoms to adapt to new ecological niches during evolution and are expected to determine the success of some species in the current changing environmental conditions [7]. The unique chimera of genes and metabolic pathways in diatoms makes them evolutionary distinct from plant and animal lineages [8,9]. However, diatoms share specific pathways to both, and therefore information gathered on the effects of pollutants in diatoms could be translated into other species even from different trophic levels. They became particularly relevant in this field ever since the first full genome sequence of a diatom was first described in 2004 [9]. Since then, there has been a steadily increase in research on diatoms, which is boosting their applications as model organisms in systems ecotoxicology.

Diatomic indices for environmental quality assessment

Diatoms are sensitive to their environment and are considered good sentinel species to assess environment quality. Diatom biotic indices, which are based on the estimation of diversity and relative abundance of diatom species in water bodies, have been developed and widely used for decades as water quality indicators [10-13]. Moreover, indices such as the Specific Pollution Sensitivity Index (IPS) or Generic Diatom Index (GDI) exploit the known sensitivity of some diatom species to environmental contamination [14]. These indices are able to correlate the relative abundance of specific diatom species with the presence of pollutants or high eutrophication levels providing a biological measure of water quality [14]. The use of diatom biological indices has usually implied lengthy microscopic analysis for the identification and enumeration of the diatom species based on key morphological features. This analysis is laborious and requires highly trained technicians that are able to distinguish the different valve shapes and, consequently it is also operator-dependent. To overcome these limitations, diatom indices based on molecular tools are currently being investigated [15-18] (Figure 1). These methods will likely be more expedite, reliable and user-friendly than the traditional approaches. Recent efforts in this field have focused on the development of DNA barcoding systems that use sequence comparisons of a short region of DNA to identify diatom species [16,17,19]. Several gene regions are being considered for this type of analysis based on the universality of the sequence and ability to distinguish closely related species [16,20,21].

Alternative molecular approaches to detect and quantify diatoms in environmental samples, based on quantitative real-time polymerase chain reaction (qRT-PCR), are also being explored. This tool has already been employed to monitor algal blooms and determine their toxicity potential. This is because molecular based approaches are able to discriminate between toxic and non-toxic *Pseudo-nitzschia*

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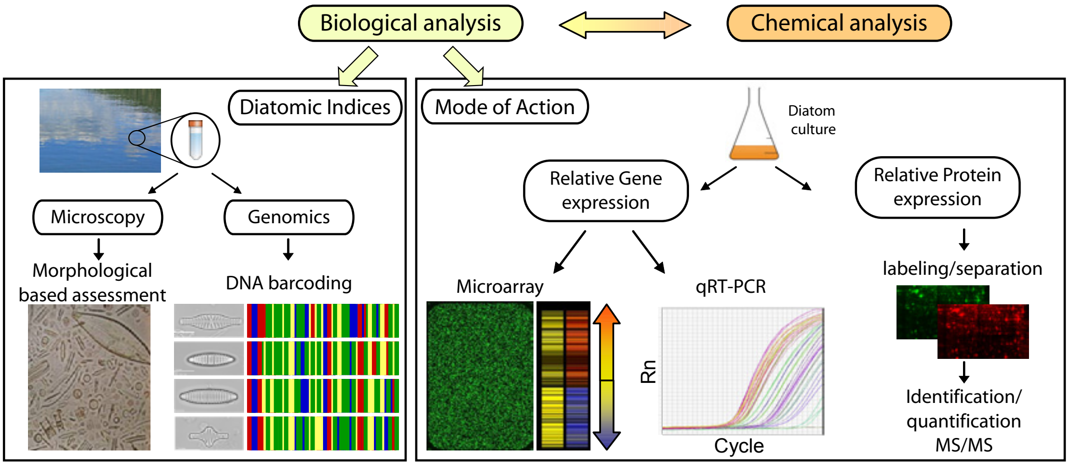

Figure 1: Application of molecular tools to diatoms for the biological-based analysis in environmental risk assessment (Diatom images from ADIAC project; CEC contract MAS3-CT97-0122).

diatom species while the optical microscopy approach can only discern taxonomy to the genus level within this taxon [22].

Thus, molecular techniques could be helpful to determine microorganism diversity and relative abundance of different species in an ecosystem. The analysis of the diatom diversity present in a water sample can be accomplished both by DNA barcoding and metagenomics (the sequencing of the entire genetic material from environmental samples), while qRT-PCR is suitable to target specific diatom species. Additionally, as the microscopic-based indices, molecular-based diatom indices should be able to correlate any observed changes in the ecosystem with the presence of a contamination, by focusing on known sensitive diatom species.

Diatoms as model organisms in systems ecotoxicology

In addition to the application of diatomic indices, diatoms are also relevant organisms in systems ecotoxicology. In this respect, the use of diatoms has great advantages to study the effects of chemical contaminants when compared to other organisms. First, diatoms have high growth rates and are easy to cultivate. Second, diatoms are able to absorb pollutants in aquatic environments and since they are at the base of the food web, they may facilitate the accumulation of xenobiotics in higher organisms and increase the toxicity. Third, diatoms are unicellular organisms, helping to overcome the usual difficulties encountered in higher eukaryotes regarding the molecular variability of different cells and tissues or the different bioaccumulation rates of contaminants in different organs [23]. Finally, several of the molecular biomarkers associated with the mechanisms of toxicity of pollutants identified in diatoms are likely conserved in higher organisms.

The full genome sequence of two diatom species has become available in recent years, the centric diatom *Thalassiosira pseudonana* [9] and the pennate diatom *Phaeodactylum tricornutum* [24]. The diatom genome repository is being further expanded by the additional genomes of two pennate diatoms, the polar specie *Fragilariopsis cylindrus* and the toxigenic coastal specie *Pseudo-nitzschia multiseries* (DOE Joint Genome Institute; http://www.jgi.doe.gov/genome-projects/).

Studies on the diatom genome or transcriptome have unveiled the molecular elements in metabolic pathways important for the success of diatoms as major players in marine phytoplankton-driven carbon cycle. These include genes responsible for silicon and iron uptake, silicon metabolism, carbon fixation and possibly a C4-like photosynthesis [9,25,26]. At the moment, functional studies on particular diatom genes are scarce and the annotation of the different genes has been derived from sequence homology to other species. However, a high proportion of genes do not have sufficient sequence similarity to characterized genes in other species to warrant functional assignments. To reduce the complexity of looking at the entire transcriptome, recent proteomics studies focused on the snapshot of proteins and pathways being used by the cell at a given time point or condition of interest [27,28]. Targeted studies have also allowed the identification of sub-sets of proteins belonging to either specific pathways or subcellular compartments. These include the silicon bioprocessing pathway [26], proteins involved in programmed cell death [29] or the cell wall proteome [30].

The diatom *T. pseudonana* has already been the target of various omics studies to identify cellular changes at the molecular level upon conditions of stress. Analyzed stress conditions included nutrient limitation, temperature and pH [26], exposures to copper and hydrogen peroxide [31], and to the polycyclic aromatic hydrocarbon benzo(a)pyrene [27,32]. Interestingly, a comparative analysis of the transcriptome response in *T. pseudonana* caused by the different stress conditions in these studies revealed several common regulated molecules (Carvalho and Lettieri, personal communication). These genes may become good biomarker tools to detect general hazards caused by contaminated water samples. Alternatively, the molecular responses of toxicity and information on the mode of action (MoA) specific to each stress condition may provide candidate biomarkers to recognize the specific contaminant in environmental samples. Recently, the effect of a sub-lethal concentration of benzo(a)pyrene (BaP) has been studied in the diatom *T. pseudonana* both at the transcriptome and proteome levels. Highly regulated pathways identified by DNA microarray included lipid metabolism, silicon metabolism, cell cycle and stress-induced molecules either involved in protein folding or in the detoxification of reactive oxygen metabolites (Carvalho, Bopp and Lettieri, personal communication). Similar pathways were observed when performing a quantitative proteomics study of *T. pseudonana* under the same exposure conditions [27].

Moreover, using some of the identified gene biomarkers of exposure to BaP, a similar regulation was confirmed when diatom cultures were treated with marine surface sediments contaminated with polycyclic aromatic hydrocarbons [33]. This work suggests that molecular fingerprints could be used to screen environmental samples by working as warning signals whenever a contamination is present and perhaps even identify classes of chemicals. To discriminate between pollutants in complex mixtures, however, additional information will be required on the mechanism of toxicity of the different compounds or classes of compounds, to allow the selection of specific biomarkers.

Omics data and a step forward to the environmental monitoring

The traditional biological studies in ecotoxicology have mainly dealt with effects on physiological acute endpoints like growth, survival, reproductive success, development or behavior [34,35]. Currently, the use of molecular tools focused on the mechanism of toxicity of contaminants is gaining increasing support for their promise to identify hazards even at low concentrations, where no effect on the commonly used endpoints is visible [36]. This is because the effects of a chemical pollutant will be first visible at the molecular level, including the interaction with target molecules, the metabolism of the substance or a specific toxic disruption of the cellular homeostasis, followed by adaptive and detoxification mechanisms. Ultimately, the challenge is in linking these molecular effects with changes at the organism physiology and population levels to attain a complete description of the MoA of the substance. The use of MoA to describe the toxicity potential of a compound is not a new idea, nor is it restricted to the environmental field, and important progresses are being made in the toxic assessment of substances to human health [37].

The current limitations in systems ecotoxicology is still the reduced amount of ecologically relevant organisms with known sequence information and restricted classes of compounds for which the MoA is studied. The first fully sequenced organisms have been mainly used as models in biomedical research to study chemical toxicity, drug discovery and human disease. Very few model organisms with a full genome sequence available have applications to aquatic toxicology, with zebrafish (*Danio rerio*), the nematode *Caenorhabditis elegans* and the waterflea (*Daphnea pulex*) being the most commonly used for toxicity testing. However, the ongoing genome projects for several ecologically relevant organisms will likely increase the commercially available microarrays in the near future. The increasing knowledge on the diatom *T. pseudonana*, including recent omics studies on MoA of chemical pollutants in these organism is likely to strengthen its use as a model organism in ecotoxicology. In addition, ecologically relevant non-model organisms, for which there is not a high-quality genome sequence available, can still be used to link molecular and physiological responses to chemical exposure, as shown by Williams et al. [38] for the European flounder (*Platichthys flesus*). They could reconstruct a global network linking transcriptional and metabolic responses to physiology in this fish, representative of the chemical contaminant exposure in the sampling site. The developed model also revealed a remarkable similarity between network modules predictive of chemical exposure in the environment and pathways involved in relevant aspects of human pathophysiology [38].

Although omics approaches still require standardization and validation for use in regulatory toxicology [39], it is a feasible way forward for chemical environmental monitoring which so far in Europe is mainly based on chemical measurements [40]. A strategic approach for including omics technologies in environmental assessment will require further studies with special focus on i) gathering omics data for a wider number of substances; ii) a measurement of the reliability of this kind of approaches, e.g. inter-laboratory exercises; iii) the applicability of biomarkers across relevant trophic levels; iv) the choice of relevant species to be used as model organisms, including primary producers like diatoms; v) the need to communicate and train legislators and environmental agencies on omics approaches and vi) the social-economic impact of including molecular methods in the environmental monitoring workflow.

The foreseen shift in monitoring approaches

The good ecological status of a water body is assessed by considering both the anthropogenic pressures on an ecosystem, and the quality of the environment (biological and physico-chemical parameters). Quality standards for hazardous substances are usually defined to limit maximum annual average concentrations to prevent the effects of chronic exposures, as well as maximum allowable concentrations to prevent the effects of acute exposures [40]. To check compliance with the water quality standards for substances of priority concern, periodic monitoring of the different waters bodies is required. Important progresses have been made in recent years within chemical monitoring activities with the establishment of chemical analytical methods for the detection and quantification of most hazardous contaminants in water with acceptable uncertainty [40]. In addition, there has been an effort to link the chemistry information with ecological data by assessing the various human impacts on the ecosystem e.g. physical properties of water bodies and biodiversity [41,42]. However, there are still many challenges in the water quality assessment by chemical analytical methods including the need for new methodologies for the identification of emerging pollutants, toxicological assessment of mixtures of pollutants, as well as the bioavailability of compounds. In this respect, effect-based monitoring methods, particularly omics approaches would complement chemical-based monitoring (Figure 1).

Omics technologies combined with outstanding advances in bioinformatics provide enormous amount of information on the effect of single compounds, allowing to identify molecular or pathway signatures that are characteristic of certain toxic effects. The integration of toxicological information for the most relevant hazardous priority substances in the environment and from a set of relevant model organisms (when available), will allow the identification of compound-specific biomarkers as well as the most relevant biomarkers of a general stress-induced cellular response. Thus, the use of substance-specific biomarkers could provide a way to assess the effect of complex mixtures present in a water sample. The evaluation of mixtures of chemicals as a whole has great advantages when compared to the assessment of single substances or sample fractionation approaches that mostly ignore possible synergistic or antagonistic effects. This is evident from the recent study by Garcia-Reyero et al. [43] showing different modes of action when using binary mixtures of estrogenic and anti-estrogenic compounds in the fish fathead minnow [43]. Additional studies have used un-fractionated environmental samples to link molecular changes to the complex mixture exposure. Thus, after sampling European flounder from different field sites, the hepatic transcriptomic comparison of stress response genes could correlate with the contamination levels from the sampling sites [44]. Moreover, different mixtures of chemicals were tested in *Daphnia magna*. The transcriptomic analysis of the mixtures based on 15 selected genes showed a non-additive response when compared to the single chemicals [45].

In the coming years omics approaches will have a considerable impact on environmental monitoring strategies integrating the biological counterpart with chemical monitoring.

Due to the growing amount of available information on diatoms and their relevance in any ecosystem, diatom species should be selected as one of the model organisms of choice in this integrated approach. Ongoing sequencing projects of more diatom species, especially those from freshwater ecosystems, would provide even better tools for the characterization of all water bodies. We envisage that diatoms will be the key model in the environmental field as yeast has been for years in the biomedical field.

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