UNIVERSITY OF
GOTHENBURG

FACULTY OF SCIENCE

DEPARTMENT OF PLANT AND ENVIRONMENTAL SCIENCES

2008

Impact of Antifouling Compounds on Photosynthesis, Community Tolerance and *psbA* Genes in Marine Periphyton

Doctoral thesis in Environmental Sciences

K. MARTIN ERIKSSON

Akademisk doktorsavhandling för filosofie doktorsexamen i Miljövetenskap med inriktning mot fysiologisk botanik, som enligt beslut i lärarförslagsnämnden i biologi kommer att offentligen försvaras fredagen den 23:e januari 2009, kl 10:15 i föreläsningssalen, Institutionen för växt- och miljövetenskaper, Carl Skottsbergs gata 22B, Göteborg.

Examinator: Prof. Anna-Stina Sandelius

Fakultetsopponent: Dr. Jean-Francois Humbert, Research Director, Pasteur Institute, National Centre for Scientific Research, Unit for Cyanobacteria, Paris, France.

ISBN 978-91-85529-24-7

Eriksson, K. Martin, 2008, Impact of Antifouling Compounds on Photosynthesis, Community Tolerance and *psbA* Genes in Marine Periphyton, Department of Plant and Environmental Sciences, University of Gothenburg, Box 461, 40530 Göteborg, Sweden. ISBN 978-91-85529-24-7

ABSTRACT

Toxicants can act as selective pressures in the environment, eliminating sensitive genotypes or species and favouring tolerant ones. Such toxicant-induced selection can be detected in natural communities using the Pollution-Induced Community Tolerance (PICT) concept. Mechanisms of tolerance to toxicants in communities can potentially be studied using metagenomic approaches in which the pool of genes or genomes of all community members are analysed. Such approaches have the potential to unravel how toxicants interact with molecular targets, and combined with phylogeny, they can also unravel what tolerance mechanisms different organisms are compelled to use. Combining PICT and traditional measures of community function and structure with metagenomic and phylogenetic approaches, can potentially enable integrated studies of how toxicants interact with biological entities from the molecular to the community level, including important ecological interactions.

Antifouling compounds are toxicants which by their toxicity prevents attachment and growth of organisms on ship hulls and underwater installations. The major part of this thesis (Paper II-IV), concerns selection pressure from the antifouling compound irgarol on periphyton communities in Swedish coastal waters. It is shown that community tolerance to irgarol developed slowly over the years from 1994 to 2004, and that PICT was dependent on the contamination pattern over the boating season. Although not statistically significant in our studies, a small tolerance increase was observed at all sites investigated, indicating that irgarol might affect organisms adversely over larger areas in Swedish coastal waters.

PICT to irgarol was verified in flow-through microcosm experiments. Clone libraries of *psbA*, the gene coding for the target protein of irgarol - D1 - was made from communities highly and moderately tolerant to irgarol. Irgarol caused a clear shift in *psbA* haplotypes, D1 protein types and morphologically distinct species. None of the previously known mutations, conferring tolerance to compound with the same mechanism of action as irgarol, was found in any of the libraries. However, another region of D1, corresponding to the so-called PEST region, was identified as important for irgarol tolerance. Since the PEST region is suggested to regulate the degradation of the protein, a mechanism of increased degradation and turnover of the target protein is proposed. Tolerant communities were less diverse at the gene, protein and species levels, and the dominance of diatoms and cyanobacteria increased. Phylogenetic analysis enabled the determination of diatoms as the taxonomic group in which the proposed tolerance mechanism is important, whereas the cyanobacteria were identified as a group that probably use other tolerance mechanisms.

Irgarol seems to exert a specific selection pressure in the Swedish coastal marine environment, with the potential to restructure the distributions of genes, proteins and morphologically distinct species and thereby induce community tolerance.

In addition, this thesis evaluates the capacity of short-term photosynthetic endpoints in detecting toxicity of five additional antifouling compounds. The use of such endpoints when testing compounds with mechanisms of action not directed towards photosynthesis might underestimate toxicity. Since short-term toxicity tests are crucial for PICT detection it was tested whether prolonging the exposure time, thereby allowing for toxic effects to be propagated to photosynthesis, increased the performance of the photosynthetic endpoints.

Keywords: PICT, antifouling, periphyton, photosynthesis, D1, *psbA*, tolerance, resistance