BIOSENSE: EVALUATING MOLECULAR PROBE BASED TAXONOMY OF PHYTOPLANKTON WITH A VIEW TO INCORPORATING SPECIES IDENTIFICATION IN AUTOMATED MARINE OBSERVATION SYSTEMS

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Introduction

Knowledge of phytoplankton species composition is important for understanding ecosystem processes, as they form the base of

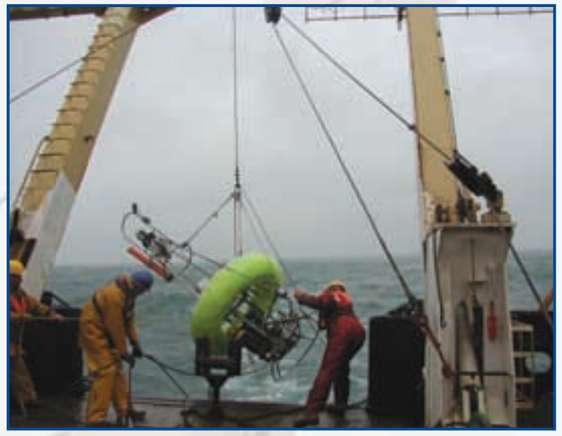

Figure 1: Recovery of SmartBuoy with payload.

the marine food web. The lack of field observations at critical time scales has led to the development of a data buoy (SmartBuoy), capable of collection of high frequency (1h-1day) data sets and relaying the results in real-time to shore using telemetry. For example by quantifying concentrations of oxygen and nitrate alongside a range of environmental parameters, such as temperature, conductivity, turbidity, and downwelling photosynthetically active radiation as well as chlorophyll fluorescence, correlation and trends can be identified. These high-resolution data sets are essential for relating changes in phytoplankton, sensitive indicators of change, to environmental conditions.

Sandwich and wholecell assays

While the quantification of chlorophyll fluorescence is useful as an indicator of algal bloom formation and decay, it gives little information about the species succession within the bloom and any potential danger from harmful algal species present. Research is therefore in progress on the application of molecular probe based techniques, such as the **sandwich- *and* wholecell-hybridisation assays** (1), to phytoplankton monitoring programmes. Initially, discrete samples are being screened for the presence of *Alexandrium tamarense* using the Saigene sandwich assay kits in microtiter plate format and automated processor with probes specifically designed to differentiate between North American, Temperate Asian and Western European lineages. Samples from Weymouth inner harbour, where *Alexandrium tamarense* has bloomed repeatedly have been tested and gave positive signals with the Western European probe. This is consistent with sequence information obtained and the absence of toxicity typical for strains of European origin (2). No false positives were obtained with mixed field samples and non-target organisms such as *Prorocentrum lima* or *Alexandrium minutum*.

Figure 2: Saigene processor with assay plate for Sandwich hybridisation.

Analysing preserved material

The applicability of the nucleic acid probe based methods to cultures treated with **Lugol's iodine** was evaluated, as routine monitoring samples usually are preserved in this fashion. Positive identification using the sandwich hybridisation method was still possible even after prolonged periods (maximum storage time to date 6 month), though some decrease in signal was apparent, which will affect the detection limit. Fluorescence labelling of preserved whole cells also resulted in positive signals, with Figure 3 showing an example of a culture treated with Lugol's iodine 6 days prior to conducting the hybridisation experiment.

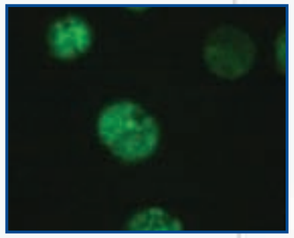

Figure 3: Alexandrium tamarense CCAP 1119/6 preserved with Lugol's iodine and treated with fluorescin labelled whole cell format probes.

Electrochemical sensor development

While the optical detection methods presented have proven very useful for laboratory tests, integration of molecular biological techniques with rapid *in-situ* measurements is the ultimate aim of the work. **Electrochemical signal generation** was therefore evaluated as an alternative means of visualising the hybridisation event, as it is considered easy to integrate with data acquisition, logging and transmission.

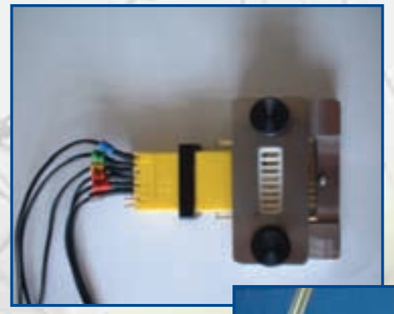

Figure 4a: Array of gold electrodes evaporated onto glass substrate clamped into small volume incubation chamber.

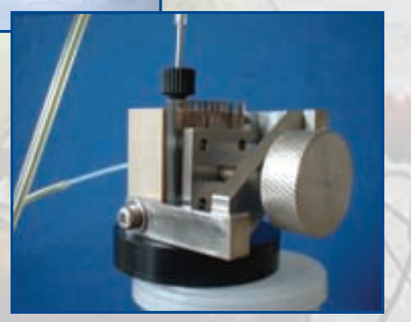

Figure 4b: Thin-layer flow cell with exchangeable electrode array.

Two different electrochemical detection systems were tested: impedance spectroscopy and amperometry. Gold was chosen as working electrode surface material as it is chemically relatively inert and provides an excellent immobilisation surface for self-assembled monolayers (SAMs). Using terminal thiol-groups and spaces groups to minimise steric hindrance, oligo-nucleotides were attached to the electrode surfaces. Alternatively, to generate a generic immobilisation surface, thiol-biotin can act as the primary SAM, subsequently functionalised with different probes via streptavidin as a linker and nucleotide biotinylation.

Impedance spectroscopy

Impedance measured at an electrode surface is the vector of capacitance and resistance and therefore directly related to surface processes such as the immobilisation of a molecular probe and the subsequent hybridisation of target nucleic acid. The advantage of this technique is the direct nature of the signal generation, which eliminates the need for secondary probes and enzymatic labels. While the subsequent reduction in assay steps and reagents involved is very positive, the additional safeguard against non-specific signals afforded by the assembly of a two-probe sandwich is lost. Experiments were conducted using ferro-ferricyanide as redox probes to quantify surface coverage and monitor changes following exposure to complementary nucleic acids and cell lysates. Variations in results obtained demonstrated that very stringent control both over environmental conditions and surface processes such as molecular realignment is required to interpret the observed changes in electron transport resistance with any confidence.

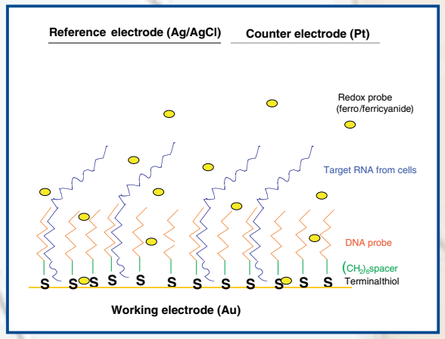

Figure 5a: Schematic representation of impedance measurement.

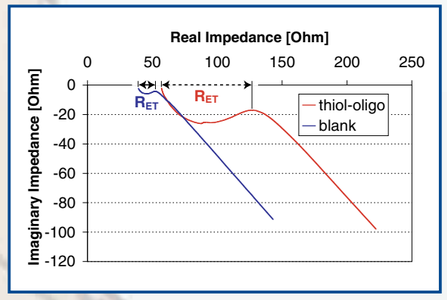

Figure 5b: Nyquist plot of impedance, in which the diameter of the semi-circle indicates the surface electron transfer resistance (R_{ET}). The modification of the gold surface with the thiolated oligo clearly increased R_{ET}.

Amperometry

This detection method is based on enzymatic production of a redox-active substance that results in a current signal. The format chosen was equivalent to the sandwich assay, using peroxidase (HRP) as label enzyme and hydrogen peroxide plus tetramethylbenzidine (TMB) as substrate. By modifying gold wire electrodes with thiolated oligonucleotide probes, and replacing the signal solution with a modified reagent it was possible to run the assay either using the Saigene processor or in batch incubations. Amperometric detection of HRP activity is know to be very sensitive, with 8.5×10^{-14} mol l^{-1} enzyme detectable (3), and instrumentation required to measure the resulting current is related to circuitry used for example in oxygen electrodes. Good correlation between both methods was obtained and transfer of the protocol into flow-injection mode is in progress.

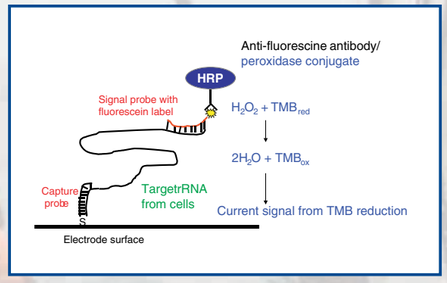

Figure 6a: Schematic representation of amperometric measurements.

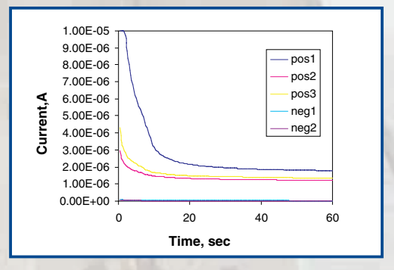

Figure 6b: Example of current signal obtained with assay sandwich formed on gold electrode (pos) compared to blank (neg).

Advantages of this detection method over optical detection include the possibility of reducing the probe and sample volume, insensitivity to discoloration, the availability of miniaturised electrode arrays to increase confidence through multiplication of measurement points, the robustness of the measurement electronics and its convenient interface with data logging devices. In summary, the vision is to obtain an instrument capable of detecting the presence of individual phytoplankton species thus enhancing our capability for conducting ecological studies at microbial level. While the primary focus was on harmful algae, the concept extends to other classes of microorganisms, which are currently under-sampled but play a major role in ecosystem processes.

References:

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3. Volpe, G., Compagnone, D., Draisci, R., and Palleschi, G. 1998: 3,3',5,5'-Tetramethylbenzidine as electrochemical substrate for horseradish peroxidase based enzyme immunoassays. A comparative study. *Analyst* 123:1303-1307.