Detection of Marine Microorganisms using Immuno-based Methods

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Introduction: Detection of a harmful alga (Brown Tide) and other marine microorganisms

Marine Microorganisms

Harmful Algal Blooms

- Blooms of harmful algae that are toxic to marine life and dangerous to human health are increasing nationally and globally
- Many bloom-forming algae are small in size and patchy in distribution, making detection problematic
- The conditions under which blooms occur are still poorly understood and require massive sampling efforts on both temporal and spatial scales.

Aureococcus anophagefferens

Brown Tides of the Mid-Atlantic eastern US

- Recurrent discoloration of water off Mid-Atlantic coasts caused by massive blooms of *A. anophagefferens* (>10^6 cells/ml)
- Harmful to commercial shellfisheries, specifically scallops and hard clams
- Small (2-3 µm), non-descript morphology makes traditional counting techniques (e.g. light and epifluorescence microscopy) difficult

Problem Description: Devise new technology and application schemes needed for detection of microorganisms

ELISA and Flow Cytometry

- **Enzyme-Linked ImmunoSorbent Assay**
  - Utilizes highly specific monoclonal antibody against *A. anophagefferens* cell surface antigens
  - Allows for analysis of 24 samples in multiwell format in 4-5 hours
  - Capable of detecting concentrations of 5000 cells/ml.
- **Flow cytometry**
  - Utilizes fluor-labeled monoclonal antibody
  - Individual samples run in minutes following 20 minute incubation period.
  - Capable of detecting concentrations of <1000 cells/ml

Atomic force microscopy

- **Force-distance analysis of antibody-antigen interactions**
  - Utilizes AFM tip functionalized with monoclonal antibody
  - Allows detection of single *A. anophagefferens* cell
  - Biotin-avidin model system provides better understanding of typical ligand-receptor interactions
- **Resonance frequency shifts**

  AFM tip is oscillated and changes in resonant frequency are detected with the addition of mass (e.g. cells) onto the antibody-coated tip or cantilever

Proposed Solution: Spatially significant sampling coupled with rapid detection

Experimental setup

- **Experimental testbed**
  - Glass column, grow light and water chiller simulate water column with cold water (4°C) at bottom and warm water (28°C) at the surface, forming a sharp thermocline (Figure 1)
  - Column is filled with media formulated specifically for *A. anophagefferens* and then inoculated with culture.
  - Cell concentration is monitored using flow cytometry and ELISA.
  - Great deal of optimization in last six months for parameters such as salinity, light intensity, and nutrients
  - Some response in algal distribution to hydrographic barrier created by thermocline (Figure 2)
  - Utilizes a "wired" thermistor strand, although detection of thermocline is processed as if individual thermistors can only communicate with their immediate neighbors.
- **Flow cytometry**
  - Optimization of protocol for counting *A. anophagefferens* using the FITC-conjugated MAb
  - MAb and *A. anophagefferens* show low reactivity in seawater; reactions performed in PBS (phosphate buffered saline) show strong labeling after 20 minute incubation period (Figure 3, A-C)
  - The FITC-conjugated MAb shows high specificity for *A. anophagefferens* relative to non-toxic co-occurring plankton which have similar sizes and/or share similar ecological niches as *A. anophagefferens* (Figure 3, D-F)
- **AFM**
  - *A. anophagefferens* cells have been imaged by AFM on poly-L-lysine coated mica surfaces.
    - Imaging was performed in intermittent tapping mode with a silicon nitride tip (Figure 4).
  - Measurement of adhesion forces for simpler biotin-avidin system.
    - Silicon nitride tips were functionalized with a poly-ethyleneglycol moiety terminating with biotin
    - Mica surfaces were coated with avidin, and the force-distance analysis was perfomed in liquid
    - Multiple biotin-avidin interactions were observed with adhesion forces between 1-4nN (Figure 5)
      - However, smaller quantized adhesion forces of several hundred piconewton were observed in almost all of the pull-off forces. This could be attributed to the rupture of individual biotin-avidin bonds.
  - Work on biotin-avidin system will now be extended to study the interaction between *A. anophagefferens* and the monoclonal antibody.
  - The measurement of changes in resonant frequency is being explored as a possible detection technique.

Results

Experimental testbed

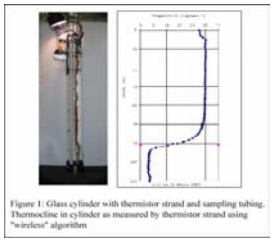

Figure 1: Glass cylinder with thermistor strand and sampling tubing. Thermocline in cylinder as measured by thermistor strand using "wireless" algorithm

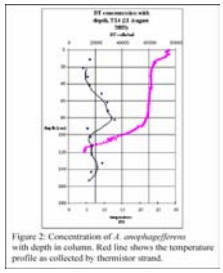

Figure 2: Concentration of *A. anophagefferens* with depth in column. Red line shows the temperature profile as collected by thermistor strand.

Flow cytometry

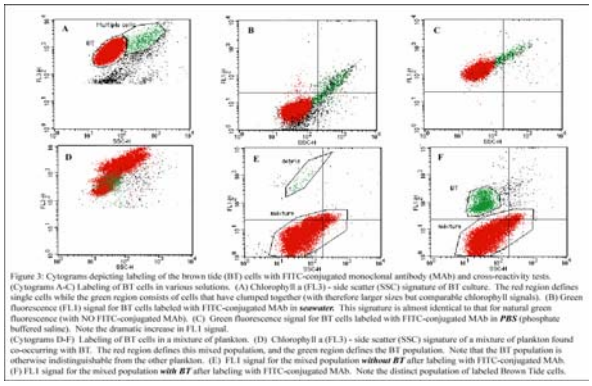

Figure 3: Cytograms depicting labeling of the brown tide (BT) cells with FITC-conjugated monoclonal antibody (MAb) and cross-reactivity tests. (Cytograms A-C) Labeling of BT cells in various solutions. (A) Chlorophyll a (FL3) - side scatter (SSC) signature of BT culture. The red region defines single cells while the green region consists of cells that have clumped together (with therefore larger sizes but comparable chlorophyll signals). (B) Green fluorescence (FL1) signal for BT cells labeled with FITC-conjugated MAb in ***seawater***. This signature is almost identical to that for natural green fluorescence (with NO FITC-conjugated MAb). (C) Green fluorescence signal for BT cells labeled with FITC-conjugated MAb in ***PBS*** (phosphate buffered saline). Note the dramatic increase in FL1 signal.
(Cytograms D-F) Labeling of BT cells in a mixture of plankton. (D) Chlorophyll a (FL3) - side scatter (SSC) signature of a mixture of plankton found co-occurring with BT. The red region defines this mixed population, and the green region defines the BT population. Note that the BT population is otherwise indistinguishable from the other plankton. (E) FL1 signal for the mixed population ***without BT*** after labeling with FITC-conjugated MAb. (F) FL1 signal for the mixed population ***with BT*** after labeling with FITC-conjugated MAb. Note the distinct population of labeled Brown Tide cells.

AFM

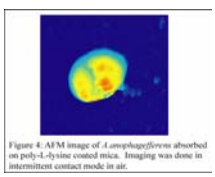

Figure 4: AFM image of *A.anophagefferens* absorbed on poly-L-lysine coated mica. Imaging was done in intermittent contact mode in air.

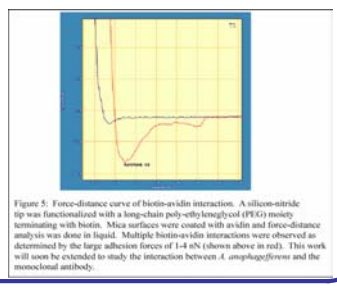

Figure 5: Force-distance curve of biotin-avidin interaction. A silicon-nitride tip was functionalized with a long-chain poly-ethyleneglycol (PEG) moiety terminating with biotin. Mica surfaces were coated with avidin and force-distance analysis was done in liquid. Multiple biotin-avidin interactions were observed as determined by the large adhesion forces of 1-4 nN (shown above in red). This work will soon be extended to study the interaction between *A. anophagefferens* and the monoclonal antibody.