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 multwell 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

Experimental Setup

• Experimental testbed
  – Glass column, grow light and water chiller simulate water column with cold (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 performed 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

Flow cytometry

AFM