Title
Detection and Identification of Marine Microorganisms

Permalink
https://escholarship.org/uc/item/1134z204

Authors
Beth Stauffer
David Caron
Mrinal Mahapatro
et al.

Publication Date
2005
Experimental Results

Flow cytometry

- Utilizes fluor-labeled monoclonal antibody specific to *A. anophagefferens*.
- Combines fluorescent and cell size information.
- Allows for analysis of single sample in <5 minutes.
- Detects concentrations of 1000 cells/ml

ELISA

- Allows for analysis of 24 samples in multiwell plate format in 4-5 hours.
- Detects concentrations of 5000 cells/ml

**Proposed Solution: Immuno-based Detection Techniques: Flow Cytometry, AFM & QCM**

**Problem Description: Application of New Technologies for Detection and Identification**

**Flow cytometry**

- Utilizes fluor-labeled monoclonal antibody specific to *A. anophagefferens*.
- Combines fluorescent and cell size information.
- Allows for analysis of single sample in <5 minutes.
- Detects concentrations of 1000 cells/ml

**ELISA**

- Allows for analysis of 24 samples in multiwell plate format in 4-5 hours.
- Detects concentrations of 5000 cells/ml

**Quartz Crystal Microbalance (QCM)**

- 5 MHz quartz crystals with gold electrodes were functionalized with MAb using a variety of schemes. Following reaction with *A. anophagefferens* in the QCM, crystals were microscopically analyzed and the number of attached cells counted.
- N-Succinimidyl 3-(2-pyridylidythio) propionate (SPDP, targetting amines in the MAb) and 3-(2-pyridylidythio) propionyl hydrazide (PDPH, targetting carbohydrates) have yielded the best results.
- Frequency curves, however, have shown only small changes in resonant frequency with the addition of BT cells (Figure 2).

**Atomic Force Microscopy (AFM)**

- BT cells have been immobilized on Si/SiO2 surfaces using polyethylene imine (PEI, Figure 3B) silicon AFM tips have been functionalized with MAB using the scheme in Figure 3A.
- Force-distance analyses for antibody-coated tips and immobilized *A. anophagefferens* were performed on cell surfaces (Figure 3C top) and bare surfaces (Figure 3C bottom). There is a distinct difference between the force-distance curves; the forces on the cell surface are much higher on average than those on bare Si/SiO2 surfaces.

**Experimental Design**

- **Column Testbed**
  - A bloom of *A. anophagefferens* was stimulated in a 3-meter high glass column of nutrient-rich seawater.
  - The growth of BT and distribution with depth was monitored over time using ELISA (Figure 1A).
  - *Pedinella*, a known grazer of *A. anophagefferens* was added and the decline of the BT population with depth was monitored.

- **Comparison of ELISA and flow cytometric enumeration techniques**
  - Cultures of *A. anophagefferens* were analyzed using both ELISA and flow cytometric techniques.
  - In the absence of grazing, the techniques yielded comparable data (not shown); however, in the presence of grazing, the flow cytometric technique yielded lower, more accurate concentrations (Figure 1B).
  - The flow cytometric technique incorporates size information, which ELISA does not, thus distinguishing between whole cells and fragments.

**Quartz Crystal Microbalance (QCM)**

- Utilizes quartz crystals functionalized with monoclonal antibody specific to *A. anophagefferens*.
- Detects adsorption of molecules to the crystal surface as changes in resonance frequency.
- Allows for theoretical detection of ~5ng/cm² (Q-Sense, Inc.)

**Atomic Force Microscopy (AFM)**

- Utilizes silicon AFM tips coated with monoclonal antibody specific to *A. anophagefferens*.
- Images immobilized cells and measures adhesion forces between the MAb-functionalized tip and a surface containing target cells.
- Allows for theoretical detection of a single target molecule.

**Flow cytometry and ELISA**

- Enzyme-Linked ImmunoSorbent Assay (ELISA)

**Problem Description: Application of New Technologies for Detection and Identification**

**Introduction:**

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

- *Aureococcus anophagefferens* (Brown Tide Alga)
  - Brown Tides of the Mid-Atlantic Eastern US
    - Recurrent discoloration of water off Mid-Atlantic coasts caused by *massive blooms* of *A. anophagefferens* (~10⁶ 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.

- 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.

- Cultures of *A. anophagefferens* were added and the number of attached cells counted.
- ELISA does not, thus distinguishing between whole cells and fragments.

**QCM and AFM**

- Utilizes quartz crystals functionalized with monoclonal antibody specific to *A. anophagefferens*.
- Detects adsorption of molecules to the crystal surface as changes in resonance frequency.
- Allows for theoretical detection of ~5ng/cm² (Q-Sense, Inc.)

- Utilizes silicon AFM tips coated with monoclonal antibody specific to *A. anophagefferens*.
- Images immobilized cells and measures adhesion forces between the MAb-functionalized tip and a surface containing target cells.
- Allows for theoretical detection of a single target molecule.

**Proposed Solution: Immuno-based Detection Techniques: Flow Cytometry, AFM & QCM**

**Experimental Results**

- **A) Growth of BT Alga in column testbed**
- **B) Enumeration of BT in culture with Pedinella grazer**

**Figure 1:** A) BT growth and decline, following addition of *Pedinella grazer* in testbed column. B) Enumeration of BT in culture with *Pedinella* using ELISA and flow cytometry

**Figure 2:** Frequency curves using crystals functionalized with SPDP (A) and PDPH (B). Yellow, green, and red curves are the 3rd, 5th, and 7th overtones, respectively.

**Figure 3:** A & B) Functionalization schemes for Si AFM tips & Si/SiO2 surface with BT cells. C) Force-distance analysis of MAb and BT cells (top: on cell surface; bottom: on bare surface).