National Aeronautics and
Space Administration

Jet Propulsion Laboratory
California Institute of Technology
Pasadena, California

JPL is able to apply its technologies, facilities, and expertise to assist our partners in product improvement and problem solving to reduce risk.

Microbial Burden Approach

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New Monitoring Approach for Measuring Microbial Burden

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Presentation Agenda

- Advantages of new approach for differentiating live cells/ spores from dead cells/spores
- Four examples of *Salmonella* outbreaks leading to costly destruction of dairy products
- List of possible collaboration activities between JPL and other industries (for future discussion)
- Limitations of traditional microbial monitoring approaches
- Introduction to new approach for rapid measurement of viable (live) bacterial cells/spores and its areas of application
- Detailed example for determining live spores using new approach (similar procedure for determining live cells)

Determination of Live Cells/Spores

- JPL has developed a patented approach for measuring amount of live and dead cells/spores
- This novel “molecular” method takes less than 5 to 7 hrs. compared to the seven days required using conventional techniques
- Conventional “molecular” techniques can not discriminate live cells/spores among dead cells/spores
- The JPL-developed novel method eliminates false positive results obtained from conventional “molecular” techniques that lead to unnecessary delay in the processing and to unnecessary destruction of food products

Examples of *Salmonella* outbreak

- Ice cream firm linked to *Salmonella* outbreak
  - http://findarticles.com/p/articles/mi_m1370/is_n5_v29/ai_17067476/
- Food and Drug Administration.
  - "FDA warns consumers to avoid drinking raw milk," FDA NEWS, December 16, 2005.
- Centers for Disease Control and Prevention.
  - "*Salmonella* Typhimurium infection associated with raw milk and cheese consumption—Pennsylvania, 2007." JAMA. 2008;299(4): 402-404.
  - http://jama.ama-assn.org/content/299/4/402.full

Ice cream firm linked to *Salmonella* outbreak

- In September and October 1994, Minnesota's health department logged a sharp rise in the state's *Salmonella* illness cases. A few weeks later, FDA and state officials determined that contaminated ice cream was the likely cause of a *Salmonella* outbreak that may have sickened more than 3,000 people in as many as 41 states.

- Schwan's Sales Enterprises of Marshall, Minn., recalled ice cream made at its Marshall plant after learning last Oct. 7 that the products were linked to the illnesses. The recall involved dozens of frozen dairy products, including ice cream, yogurt, dietary desserts, sherbets, cones, and ice cream sandwiches.

Salmonella Typhimurium infection associated with raw milk and cheese consumption—Pennsylvania, 2007

- In February 2007, the Pennsylvania Department of Health received reports, through routine electronic laboratory disease reporting, of two persons with recent laboratory-confirmed infections with *Salmonella enterica* serotype Typhimurium. Both persons had reported drinking raw (unpasteurized) milk from the same York County, Pennsylvania, dairy (dairy A).
- *S*. Typhimurium isolates from these persons had pulsed-field gel electrophoresis (PFGE) patterns that were indistinguishable by use of the *Xba*I restriction enzyme.
- The same month, the Pennsylvania Department of Agriculture (PDA) received reports of illness from raw-milk customers of dairy A. PDA obtained milk samples from the raw-milk bulk tank at dairy A, which yielded *S*. Typhimurium with a PFGE pattern that was identical to the pattern from patient isolates.
- On February 26, the Pennsylvania Department of Health and PDA launched an investigation to determine the source and scope of the outbreak.
- The investigation determined that 29 cases of diarrheal illness caused by *S*. Typhimurium were associated with consumption of raw milk or raw-milk products from dairy A.
- The findings underscore the need to inform policymakers and the public of the potential health risks associated with raw-milk consumption.

Roseburg's Umpqua Dairy *Salmonella* outbreak

- August, 2010
- Umpqua Dairy in Roseburg has suspended operations and ordered a recall of milk products and drinks in a *Salmonella* outbreak that stumped scientists in Oregon for months.
- The outbreak, which has sickened 23 people in Oregon, including many kids, prompted the company to recall its milk, half & half, cream and buttermilk sold under various labels as well as its own brand of orange juice and fruit drinks.
- The outbreak, which first appeared in October, involves Salmonella Braenderup, a relatively rare strain that sickens an average of about six people a year in Oregon. State health officials pinpointed the strain's molecular pattern as cases continued to trickle into, with a few reported each month, said Dr. Paul Cieslak, head of the communicable disease section at Oregon Public Health.
  - http://www.oregonlive.com/health/index.ssf/2010/08/roseburgs_umpqua_dairy_recalls.html

Emerging Biomolecular Technologies of Mutual Interest

Current capabilities of other industries:
Total biomass
Indicator microorganisms
Specific food borne pathogens

- JPL proposes a three phase task to develop an automated biosensor unit to measure viable cells of various kinds that are important to the food and dairy industry

Proposed Space Act Agreement between JPL and other industries

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- **Phase I:**
  - **Prove the concept by quantifying viable microbes using existing JPL validated PMA-based system (1 month; $50K)**
  - **Evaluate other industries Decontamination Systems – a survey**
    - **raw material to end product testing (3 months; $100K)**
- **Phase II:**
  - **Design and test advanced system for measuring viable cells of various kinds of importance to other industries food and dairy products (6 months; $300K)**
- **Phase III:**
  - **Develop automated biosensor for detecting:**
    - **Indicator microbes (Coliforms, *E. coli* etc.)**
    - **Specific pathogens (*Salmonella, Legionella* etc.)**
    - **Problematic cell types of other industries's interest**
    - **Live microbes and discriminate from dead cells/spores**
  - **Sensitivity is 100 gene copies**
  - **Species discrimination**
  - **Cost: $1M**

Back up slides

Traditional Microbial Monitoring

- BAM (Bacteriological Analytical Method)
  - Only cultivable counts
  - It is limiting and could cover only 1% of known microbes that are viable
  - This also cannot measure the injured microbes that could proliferate at a later stage

What is PMA

- Propidium monoazide (PMA) is a fluorescent DNA intercalating dye.
- PMA can only penetrate the membrane of dead cells and can be used to distinguish between viable and dead cells/spores.
- Once PMA is cross-linked to DNA, only the dead cells/spores will fluoresce.
- Fluorescence can also be exploited to measure total viable and dead cells/spores.

Technical Approach

- Test the feasibility for rapid viable and dead cells/spore quantification using PMA system
  - For spores, heat shock sample, add PMA, measure fluorescence
  - For cells, heat kill all of the sample, add PMA, measure fluorescence
  - Subtract final fluorescence from initial fluorescence for total viability
- Validate total fluorescence quantification with PCR for low bio-mass samples
- Test and implement PMA system in relevant environment

Significance of PMA - System

- Validation of sterility and microbial quality
  - Food, Pharmaceutical, Medical industries etc.
- Automated biosensors development
- The PMA-system can be used for validating the sterilization processes of dairy products, USPS postal products, equipment and facilities; water treatment systems, and in various public health applications.
- U.S. Department of Defense and Homeland Security (funds received – work in progress for anthrax spore detection)
- U.S. Food and Drug Administration

How does PMA – system work?

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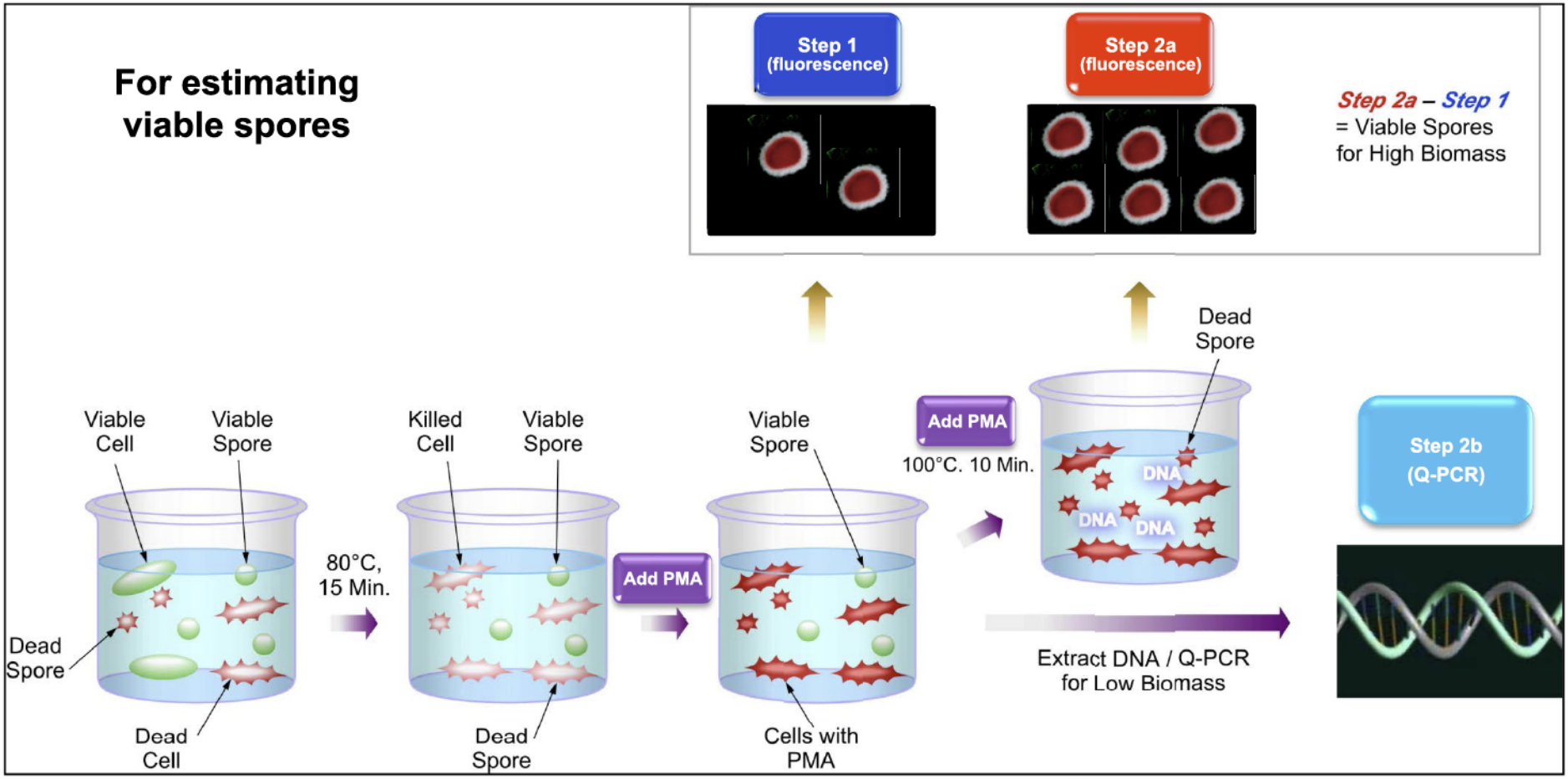

Step 2a – Step 1 = Viable cells for high biomass

Step 2b via Q-PCR = Viable cells for low biomass

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These laboratory activities are carried out at the Jet Propulsion Laboratory, California Institute of Technology, under a contract with the National Aeronautics and Space Administration. Copyright 2013 California Institute of Technology. Government sponsorship acknowledged.