USP Virtual Symposium

Emerging technologies

in probiotic, live biotherapeutic product and microbiome analysis

Oct. 6-7, 8:30-11:30am ET

Final Agenda

All times are in Eastern Daylight Time (EDT) – Washington, DC time zone

as of September 29, 2022

DAY ONE: Thursday, October 06, 2022

Moderator: Pierre Burguière, Ph.D., Probiotic Expert Panel Member, and R&D and Innovation executive leadership, Strategic Senior Advisor

8:30 a.m. **Welcome, Rules of Engagement**
Speaker: Jacqueline D. Starkes, Senior Stakeholder Affairs Coordinator | USP

8:35 a.m. **Opening Remarks, USP Overview**
Speaker: Kit Goldman, Ph.D., Senior Director, Dietary Supplements and Herbal Medicines | USP

8:40 a.m. **Challenges and Opportunities in the Analysis of Live Microorganisms**
Speaker: Jean Schoeni, Ph.D., Vice-Chair, Probiotic Expert Panel, and a Member of the Non-Botanical Dietary Supplements Expert Committee | USP

Presentation overview: A statement of challenges related to the analysis of live microorganisms (e.g., CFU, time, variability, need to link to clinical data), gaps where emerging technologies could provide a path forward, and desired outcome of the symposium.

8:55 a.m. **Quantitative Real-Time PCR for the Determination of Probiotic Potency**
Speakers: Andrzej Benkowski, B.Sc., Senior Technical Leader | Eurofins

Presentation overview: Quantitative/real-time PCR (qPCR) applications can be a useful and rapid tool to evaluate probiotic potency and viability to the strain level. Specificity and sensitivity of the method are key benefits to the technology, but some drawbacks exist. An overview and nuances of the methodology will be discussed, including case studies on ways qPCR can be used for diagnostic and quality-related purposes.

9:15 a.m. **Advanced Molecular Tools for the Analysis of Beneficial Microbes in Foods**
Speaker: Mickaël Boyer, Ph.D., Head of Discovery Lead Nutrition & Health Science | Lesaffre International

9:35 a.m. Absolute Quantification of Organisms by Droplet Digital PCR (ddPCR) – An Overview of the Technology and Method
Speaker: Michael Geimer, B.S., Lead Biologist, Manufacturing Science, and Technology | ATCC

Presentation overview: Traditional methods for quantification of probiotic organisms can be labor intensive, take a long time to obtain results, and results tend to be inconsistent. Recent advances in PCR technology allow for quicker and more accurate quantification, which has led to the development of droplet digital PCR (ddPCR). This technique improves upon the sensitivity of qPCR, allowing for the detection of rare events and absolute

quantification of a sample. This presentation will take a closer look at ddPCR technology and the method of performing ddPCR. Some advantages and limitations of the technology will be discussed in addition to a case study using the technology in the production of a virome product (MSA-2008) and a few examples of how ddPCR can be implemented in probiotics research and product development.

9:55 a.m. **Break**

10:10 a.m. **Droplet Digital PCRF (ddPCR) Case Study**
Speaker: Anthony Kiefer, Assistant Scientist | International Flavors & Fragrances

10:30 a.m. **Challenges and opportunities of DNA-based identification, characterization, and authentication of probiotic strains**
Speaker: Antonio Del Casale, CEO | Microbion SRL

Presentation Overview

- Challenges of the classic bacterial identification methods;
- Solutions to species identification and strain authentication;
- Innovative genome-based strain characterization

10:50 a.m. **NextGen Sequencing and Metagenomics**
Speaker: Scott Jackson, Leader – Complex Microbial Systems Group | National Institute of Standards and Technology

11:05 a.m. **Q&A (live or chat)**
Moderator: Pierre Burguière
Panelist:

- Andrzej Benkowski, B.Sc.
- Mickaël Boyer, Ph.D.
- Michael Geimer, B.S.
- Kit Goldman, Ph.D.
- Scott Jackson
- Anthony Kiefer
- Antonio Del Casale
- Jean Schoeni, Ph.D.

11:20 a.m. **Day One Summary "What we Heard" and Highlights for Day Two**
Speaker: Jean Schoeni. Ph.D., Vice-Chair, Probiotic Expert Panel, and a Member of the Non-Botanical Dietary Supplements Expert Committee | USP

11:30 a.m. **Adjourn**

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DAY TWO: Friday, October 7, 2022

Moderator: Binu Koshy, Ph.D., Senior Scientist II, Dietary Supplements and Herbal Medicines | USP

8:30 a.m. **Welcome, Rules of Engagement**
Speaker: Jacqueline D. Starkes, Senior Stakeholder Affairs Coordinator | USP

8:35 a.m. **Opening Remarks**
Speaker: Binu Koshy, Ph.D., Senior Scientist II, Dietary Supplements and Herbal Medicines | USP

8:40 a.m. **Intro to Flow Cytometry Antibody Identification**
Speaker: Pierre Burguière, Ph.D., Probiotic Expert Panel Member, and R&D + Innovation executive leadership, Strategic Senior Advisor | USP

9:00 a.m. **Label-free Enumeration of Bacteria by Impedance Flow Cytometry**
Speaker: Peter Lüttge Jordal, Ph.D. | SBT Instruments A/S

Presentation Overview: BactoBox is a novel, label-free instrument for enumerating bacteria in liquid samples. While a fluorescence flow cytometer exploits lasers for bacterial detection, BactoBox uses electricity to probe the lipid integrity status of bacterial membranes. This provides the user with information on both bacterial concentration and a live/dead proxy. The presentation will introduce the impedance flow cytometry principle and give examples of head-to-head comparison to other analytical techniques. Emphasis will also be put on possible use cases where BactoBox can be used close to the action.

9:20 a.m. **Assessment of Long-Term Shelf-Life (and Metabolic Viability) of a Multi-strain Synbiotic Using Standard and Innovative Enumeration Methodologies**
Speaker: Marco Pane, Ph.D., R&D Director | Probiotical Research srl

Presentation Overview: We will discuss how plate count (PC) and flow cytometry (using membrane integrity as a proxy of viability) compare during a long-term stability study of up to 12 months and four different temperatures. The study is supported by predictive microbiology models using Arrhenius equations, and the results will be compared to the product's metabolic activity.

9:40 a.m. **Break**

10:00 a.m. **Rapid Monitoring of Microbiota Composition and Targeted Culturomics of Species of Interest using Polyclonal Antibodies in Combination with Flow Cytometry**
Speaker: Vincent Thomas, Pharm.D., Ph.D., Head of Microbiome Program | Bioaster

10:20 a.m. **Thermocalometric Cell Counting**
Speaker: Marta Veses Garcia, Ph.D., Principal Scientist | Symcel

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10:40 a.m. **In-process Real-time Probiotic Strain Identity Tracking: The Use of Fourier Transform Infrared Spectroscopy in a Simulated Industrial Production Process**
Speaker: Marco Pane, Ph.D., R&D Director | Probiotical Research srl

Presentation overview: Strain identity during probiotic industrial production is a challenging task, mostly for a long time before results and costs of the main typing technologies (PFGE, MLST, genome sequencing). FT-IR spectroscopy is a phenotypic method traditionally used in chemistry to determine the molecular composition of a wide range of sample types. It is a rapid, non-destructive, simple, inexpensive, and high-throughput analytical tool based on the differential vibrational modes of distinct chemical bonds when exposed to an infrared beam. We will present a proof-of-concept for the use of FT-IR of real-time strain bio-typing during a simulated industrial process in comparison with the gold-standard PFGE methodology.

11:00 a.m. **Q&A (live or chat)**
Moderator: Binu Koshy, Ph.D.

Panelists:

- Pierre Burguière, Ph.D.
- Peter Jordal
- Marco Pane, Ph.D.
- Vincent Thomas, Ph.D.
- Marta Veses Garcia, Ph. D.

11:25 a.m. **Summary/Next Steps/Closing Remarks**
Speakers: Pierre Burguière, Ph.D., and Kit Goldman, Ph.D.

11:30 a.m. **Adjourn**