Challenges and Opportunities in the Analysis of Live Microorganisms

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Emerging Technologies

Live Microorganisms & Health Benefits

- The production of beneficial health effects are restricted to certain strains of live microorganisms
  - These abilities are not found across entire species or genera
- Beneficial strains must be administered in adequate amount to produce health benefits

Challenge

- Quality Management:

  Products have been found in the marketplace that do not meet label claim

  - Mis-identified strains
  - Labeled species or strains missing
  - Species present but not on label
  - Lower numbers than claimed

- Identification
  - Correct to the strain level
- Enumeration
  - Accurate strain counts

Area of Opportunity: Improving Identification

PROVIDE A WELL RECEIVED, COMMERCIALLY VIABLE WAY TO IDENTIFY LIVE MICROORGANISMS TO THE STRAIN LEVEL

- FAO/WHO (2001) Probiotics in food: Health and nutritional properties and guidelines for evaluation
  - Suggests identification be made to the strain level by genetic typing
- USP General Chapter <64> Probiotic Testing
  - Many regulatory filings for commercial probiotics are made at the strain level
- Dronkers et al. (2020) Global analysis of clinical trials with probiotics
  - Complete strain identification given in only 49% of registered clinical trials

Identification Methods

Phenotypic

Genomic

Hopes & Expectations

- Strain level identification
- Detect and identify minor populations
- Minimal rates
  - Mis-identification
  - Misinterpretation
  - Non-interpretable results
- Method does not need other methods to confirm results
- Little to no sample prep
- Sensitive, specific, accurate, reliable
- Rapid and cost effective

Area of Opportunity: Improving Enumeration

PROVIDE A METHOD THAT ACCURATELY DETERMINES VIABLE CELL COUNTS OF EACH STRAIN OF LIVE MICROORGANISM PRESENT

- FAO/WHO (2001), Probiotics in food: Health and nutritional properties and guidelines for evaluation
  - The label should state the viable concentration of each probiotic present at the end of shelf-life
- Hill et al. (2014), Consensus statement on scope and appropriate use of the term probiotic
  - If making health claim(s) there must be proof of efficacious dose delivery of viable strain(s) at end of shelf-life

Enumeration Methods

Phenotypic

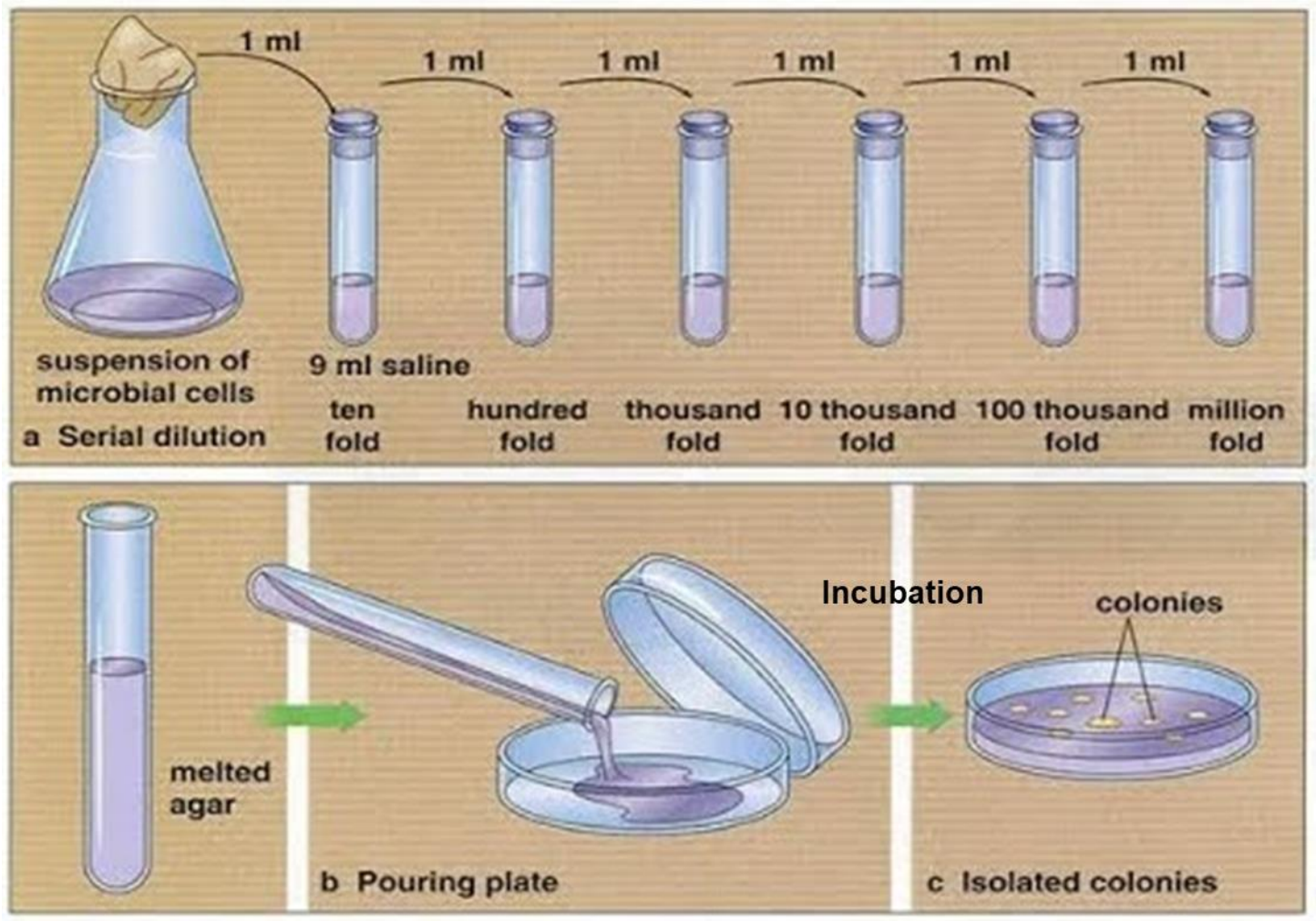
Genomic

Greatest Challenge: History

- Plating methods are considered “The Gold Standard” for live microorganism enumeration
  - Simple to apply
  - Low entry-level cost
  - Historical use
- The link between colony-forming units (CFU) and clinical data

Plating: Simple but Challenging

- Labor intensive
- Selecting appropriate sample and agar preparation
- Providing optimum growth requirements



- Methods lack robustness
- No reference standards
- Slow
  - Time to result can range from 2-7 days
- Variability in results
  - 20-30% or 10-15% RSD

Colony-Forming Units (CFU) & Clinical Data

- **Historic use of plating methods has led to a link between CFU & clinical data**
  - Dosage is either not reported or is not reported as a CFU dosage in ~ 60% of published or registered trials
    - Zuccotti et al. (2008)
      - Probiotic clinical trials published from 1978-2007 found though searching PubMed and EMBASE
        - 74 of 201 (37%) studies report CFU dosage
    - Dronkers et al. (2020)
      - Globally registered probiotic trials from 2000-2019
        - 680 of 1,619 (42%) studies report CFU dosage

Enumeration Opportunities

Technology & Innovation

- Develop technologies/methods to allow strain level counting
- Strive for simultaneous identification and enumeration

Addressing History

- Continue the conversation
  - Involve all stakeholders

Conclusion

- Technologies emerge to address questions and challenges
  - Challenges and opportunities for emerging technologies become cyclic
- Currently, new and additional tools (emerging technologies) are needed to meet strain expectations for identification and enumeration
- Emerging technologies may hold the key to improving overall quality management and quality of live microorganism products

Symposium Goals

- Explore the use of emerging technologies as potential paths to addressing challenges encountered when analyzing probiotics, live biotherapeutic products, and microbiomes.

- For a small selection of emerging technologies:
  - Enhance understanding of the theories driving the science
  - Look at performance
  - Give examples of current and potential uses/applications

- Provide a platform where you can freely ask questions and exchange ideas

Thank You

Enjoy the Symposium

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