PROBIOTICS & NEW TECHNOLOGY

What, why, where and how

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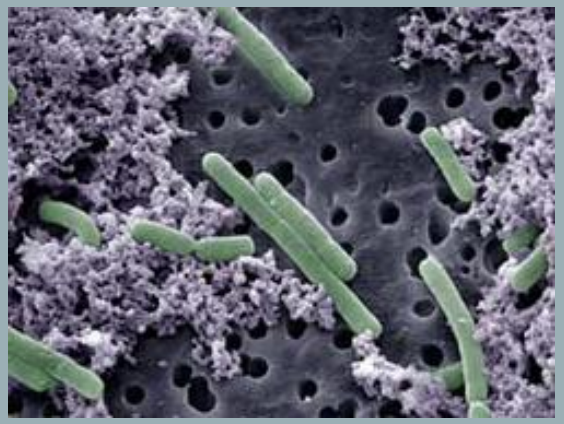


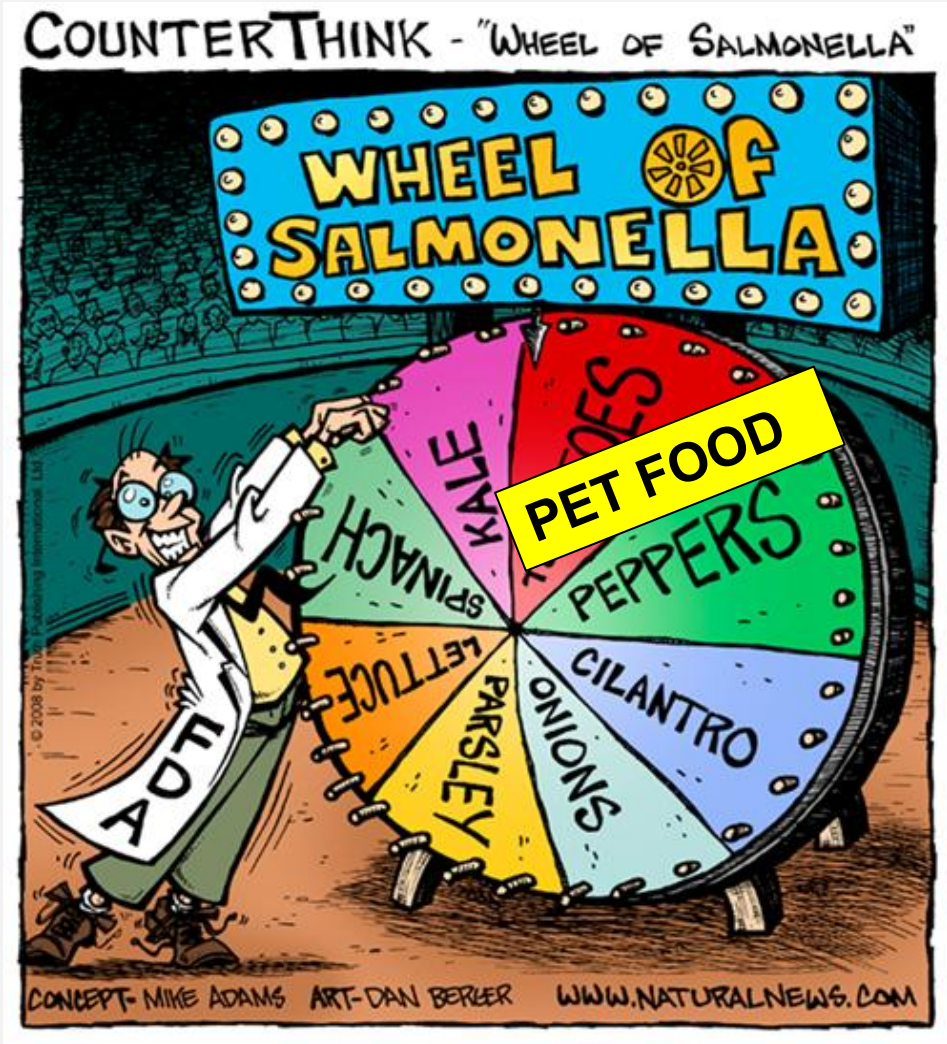
PROACTIVIDAD

- Globalization
- Greater consumer awareness
- Very public outbreaks
- FSMA

❖ Driving forces in the food industry …

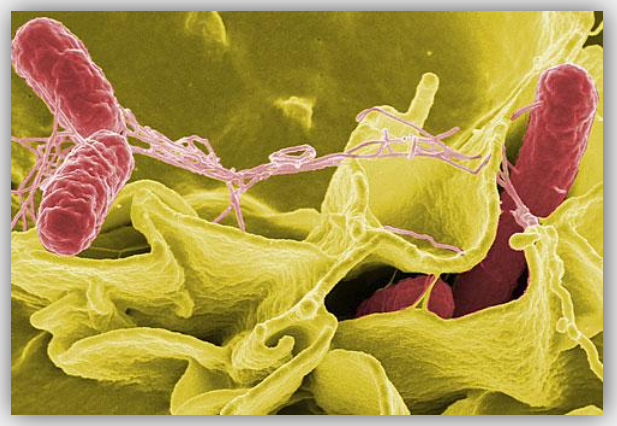
PET FOOD INDUSTRY

- Melamine - 2007
- Salmonella - 2008
- Zero Tolerance
- Risk



PET FOOD INDUSTRY

- *Salmonella* Risk
  - REAL?
  - PERCIEVED?



- Does it really matter?

CONTAMINANTS IN ANIMAL FEEDS

- **Environmental contaminants**
  - Pesticides, radionuclides, and heavy metals
- **Bacterial contaminants**
  - *E. coli* O157:H7 (cattle feed), *Salmonella enterica* and *Campylobacter* spp. (poultry litter), *Listeria monocytogenes* (silages and big-bale silage)
- **Fungal contaminants**
  - *Aspergillus, Penicillium, Fusarium, and Alternaria*
  - Mycotoxins, Aflatoxins, Ochratoxins, Fumonisins
- **Plant toxins**
  - Lectins, Cyanogens, Antigenic proteins, Gossypol, Sapon
- **Weed seeds**
  - Alkaloids, Amino acids, and Proteinase inhibitor
- **Animal toxins**
  - Prion proteins (Bovine Spongiform Encephalopathy)

PROBIOTIC CRITERIA FOR FOOD:

- International Scientific Association for Probiotics and Prebiotics (ISAPP)
  - be alive when administered
  - have undergone controlled evaluation to document health benefits in the target host
  - be a taxonomically defined microbe or combination of microbes (genus, species and strain level)
  - be safe for its intended use (GRAS status)

WHAT ARE PROBIOTICS?

- **Non-Heat Tolerant Strains:**
  - *Lactobacillus*
  - *Bifidobacterium*
  - *Enterococcus*
  - *Saccyromyces*
  - *Pediococcus*
  - *Escherichia*
  - *Bacillus*

WHAT ARE PROBIOTICS?

Lactobacillus	*Bifidobacterium*	*Streptococcus*
acidophilus	*longum*	*thermophilus*
casei	*bifidus*	*salivarius*
fermentum	*breve*	
lactis	*infantis*	
reuterii	*adolecentis*	
bulgaricus	*lactis*	
plantarum		
salivarius		

WHAT ARE PROBIOTICS?

- **Non Heat Resistant Strains**
  - **Pro's**
    - Well documented probiotic characteristics
    - Easy to isolate, identify, culture, grow, and maintain
    - High numbers (10^8 or 10^9) achievable
    - Establish as resident microflora in GI system
  - **Con's**
    - If not selected properly, do not survive processing
    - Bacteriophage or other contaminants

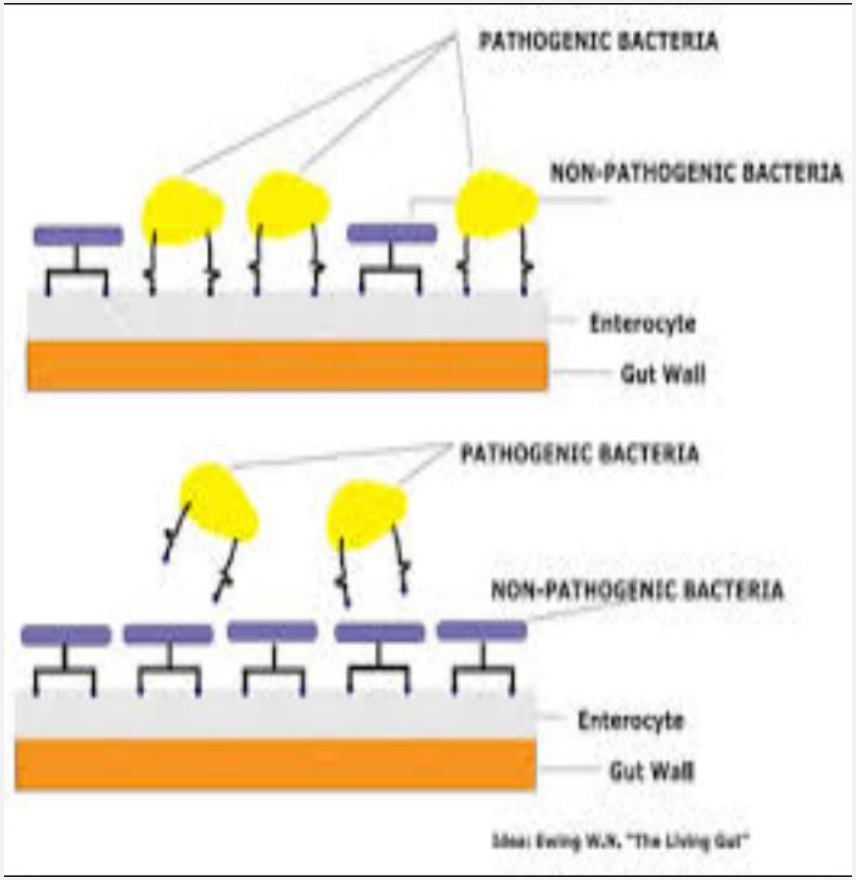
WHAT ARE PROBIOTICS?

Heat tolerant strains

- *Bacillus* spp. (ex, *coagulans*) ,spore-forming, facultative anaerobic, lactic acid-producing
- Pro's
  - Produce some related probiotic effects
  - May survive certain production processes
    - Survival through extrusion distinguishes this process from others that are patented by other manufacturers (Nestec)
- Con's
  - Not as well documented
  - Difficult to isolate, grow, culture and maintain
  - Very susceptible to contamination events due to difficulty in routine QC programs

WHAT DO PROBIOTICS DO?

- **Aid in digestive health**
  - **Competitive inhibition**
  - **Competitive exclusion**
  - **Receptor binding**
  - **Antagonistic behavior**
- **Secondary benefits**
  - **Antimicrobial characteristics in:**
    - **FINISHED PRODUCTS**
    - **INGREDIENTS**



- **Environmental Application**

WHY DO WE WANT PROBIOTICS?

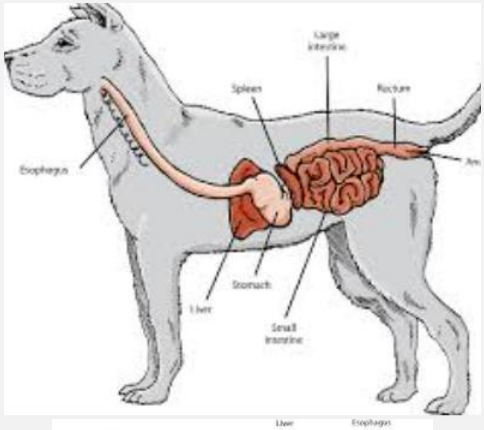
- Animal Health
- Superior Product Lines
- Market Share
- $ and Competition
- Robust Antimicrobial Characteristics--Not a Probiotic!
  - Antimicrobiotic

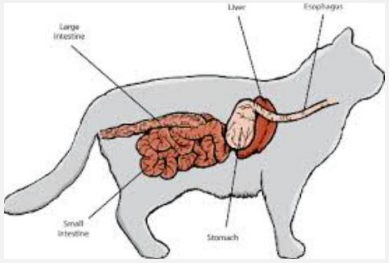
KEY BENEFITS OF PROBIOTICS

- **Improve pet GI heath**

- **Food Safety Effect**
  - Competitive Inhibition of Pathogens (i.e. *Salmonella)*
  - Competitive Exclusion of Pathogens

- **Probiotics are effective against pathogens:**
  - **In the laboratory**
  - **In the human or animal**
  - **In the environment**





WHY DO WE WANT PROBIOTICS?

- Danisco Market Breakdown (May 2009)
  - Asia, North America and Europe: 1.5 Billion/68 Billion
  - 5-25% growth rate
  - Health Claims (Humans and Animals) (2000-2007)
    - Gastrointestinal Health (56%)
      - "Healthy Digestion", "Gut Flora and Pathogens" "Nutrient Absorption" "Transit Time"
    - Immune Health Claims (27%)
      - "General Health" "Allergy"

WHERE DO THEY COME FROM?

- Human to Human
- Swine to Swine
- Bovine to Bovine
- Canine to Canine
- Feline to Feline
- Any animal species

WHERE TO START?

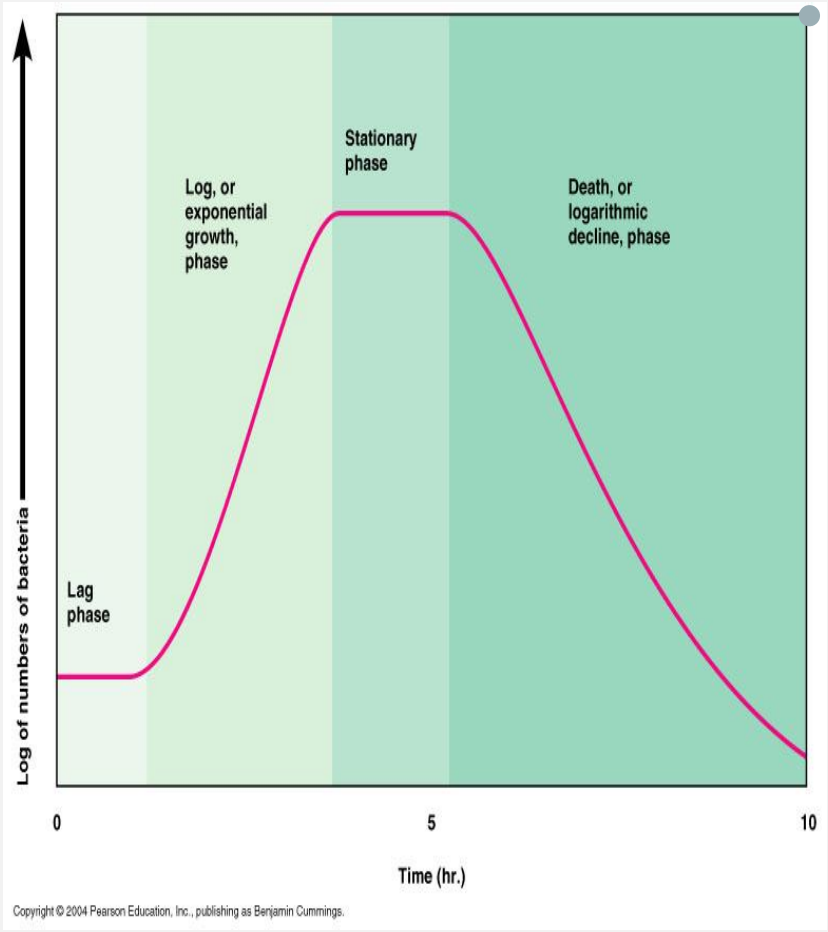
- Find organism
  - Buy or "License" the organism forever
  - Contract for delivery of probiotic for addition to product (Hollison)
  - Nestec (Lactic Acid Bacteria)
  - Procter & Gamble Pet Care (*Bifidobacteria*)
  - AFB International (*Bacillus* spp.)
  - Danisco/Dupont

- Is this organism going to work?

WHERE AND HOW TO START?

- Find organism that works!
  - Screen for healthy animals fed target diet
    - **Obtain** freshly voided feces
    - **Rescue LAB** but uses selective screening for:
      - **Primary** Screening:
        - Bile tolerance, pH tolerance
      - **Secondary** Screening:
        - Pathogen inhibition, heat sensitivity or resistance, other characteristics.
    - Phenotypic and Genotypic **Identification**
    - Strain **lypholyzation and preservation**

HOW:



Grow the organism

- Cell stability depends upon harvest time within the stationary phase.
- Fermentation with continuous gas and pH adjustments allow for most stable harvest time assessment.

HOW:

- Small Batch Formulation:
  - Grow organism with designed parameters
    - pH, temperature, gas control
    - 20-50 Liter Capacity
  - Continuous centrifugation to harvest cells
  - Run QC checks on concentrated cell suspension
  - Apply cell suspension to product (pilot plant)

Concentrated cell suspension (10^{10} cfu/g)

HOW:

- Apply cell suspension to product (lab scale or pilot plant)
- Liquid/Paste (Applied with CWG or Oils or Water Spray)
- Powder (Applied with dry powder carrier) ***viable cells may die***

Concentrated cell suspension (10^{10} cfu/g)

Post Extrusion, Post Drying, Topical Application to Food Product

HOW:

- Liquid/Paste (Applied with CWG or Oils or Water Spray)

Concentrated cell suspension (10^{10} cfu/g)

Need to determine initial viability and stability over time.

Need to conduct feeding trials to verify nutritional claims, if any

Need to screen for antimicrobial properties

HOW:

Large Batch Formulation

- Grow organism
- Extensive "clean-room" environmental program
- Extensive culture maintenance program
- Extensive Quality Control Program
- May have to manage low level contaminants

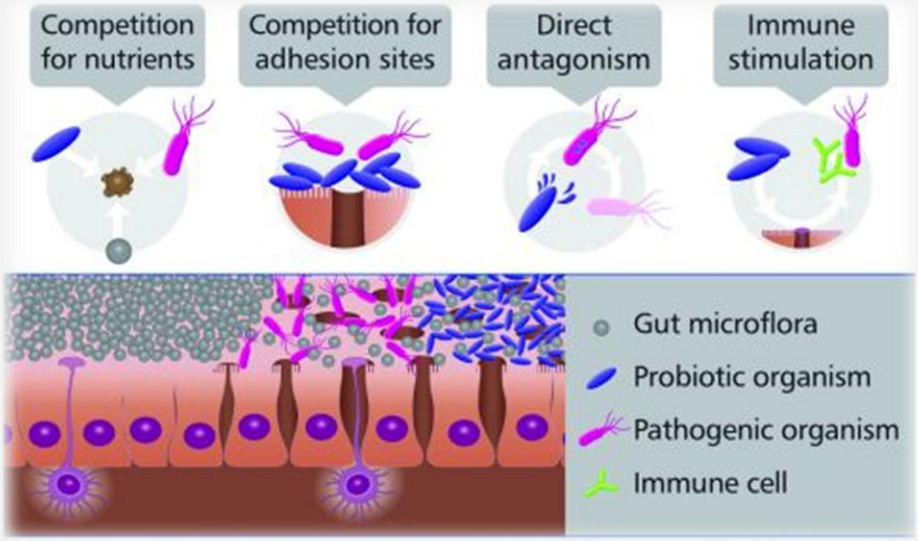
DO WE REALLY NEED THE "PROBIOTIC" EFFECT?

- Probiotic Effect
  - Dietary Adjuncts
  - Direct Fed Microbials
  - Enhanced Microbial Therapy

or

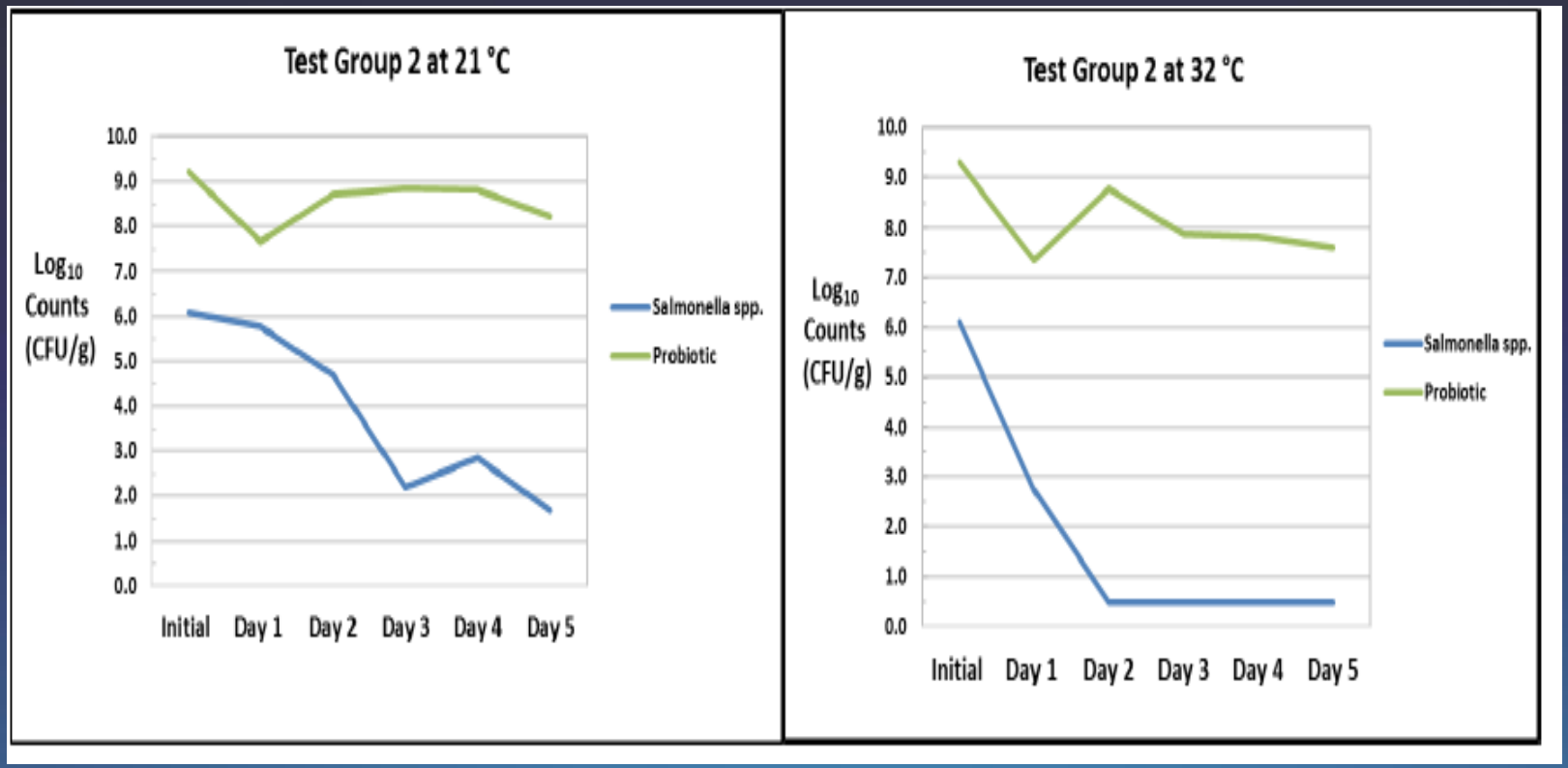
- Food Safety Effect
  - Robust Microbial Inhibition
  - Pathogen Interference Factors

YES



Does it really work?

HOLLISON
ENHANCING THE SAFETY OF FOOD



Notable log reductions of *Salmonella* spp. were achieved for test group 2. A log reduction of 4.41 was achieved for test group 2 when stored at 21 °C and a log reduction of 5.58 was achieved when the organisms were stored at 32 °C. The data indicate that the probiotic cocktail will out compete Salmonella spp. when initial concentrations are high (~ 10^9 CFU/mL).

BUYER BEWARE

- **Bacteriological evaluation of dog and cat diets that claim to contain probiotics** (The Canadian Veterinary Journal)
  - **J. Scott Weese and Luis Arroyo. Department of Clinical Studies, Ontario Veterinary College, University of Guelph, Guelph, Ontario N1G 2W1.**
- 19 products were screened
- No products contained all of the organisms presented on labels
- 9 products contained no probiotic listed
- 0 to 10^5 cfu/g
- Overall, the actual contents of the diets were not accurately represented by the label descriptions.

HOW IS IT USED IN THE U.S.

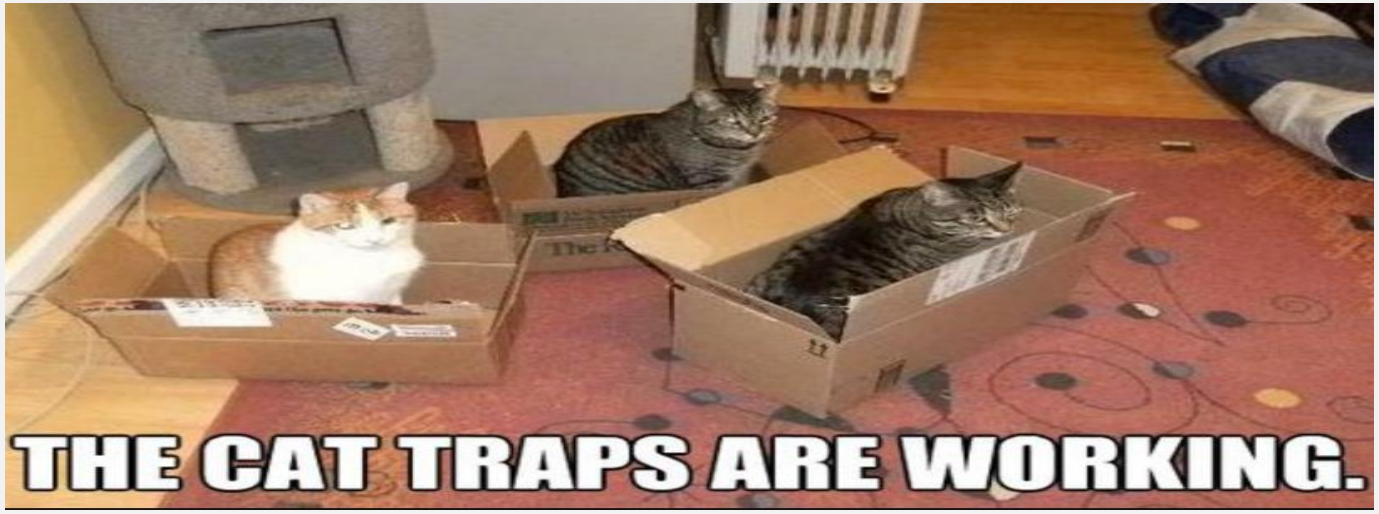
APPLIED DIRECT TO PRODUCT
OR
IN THE ENVIRONMENT

HOW HAS THIS BEEN ACCEPTED BY END USERS?

Our customers that show probiotic on the label have seen increased sales.

What's Next?

- **Outside the box – The Future…**
  - **New Monitoring Technology**
  - **Novel Uses For Ingredients**
  - **Food Safety Partnership and Collaboration**



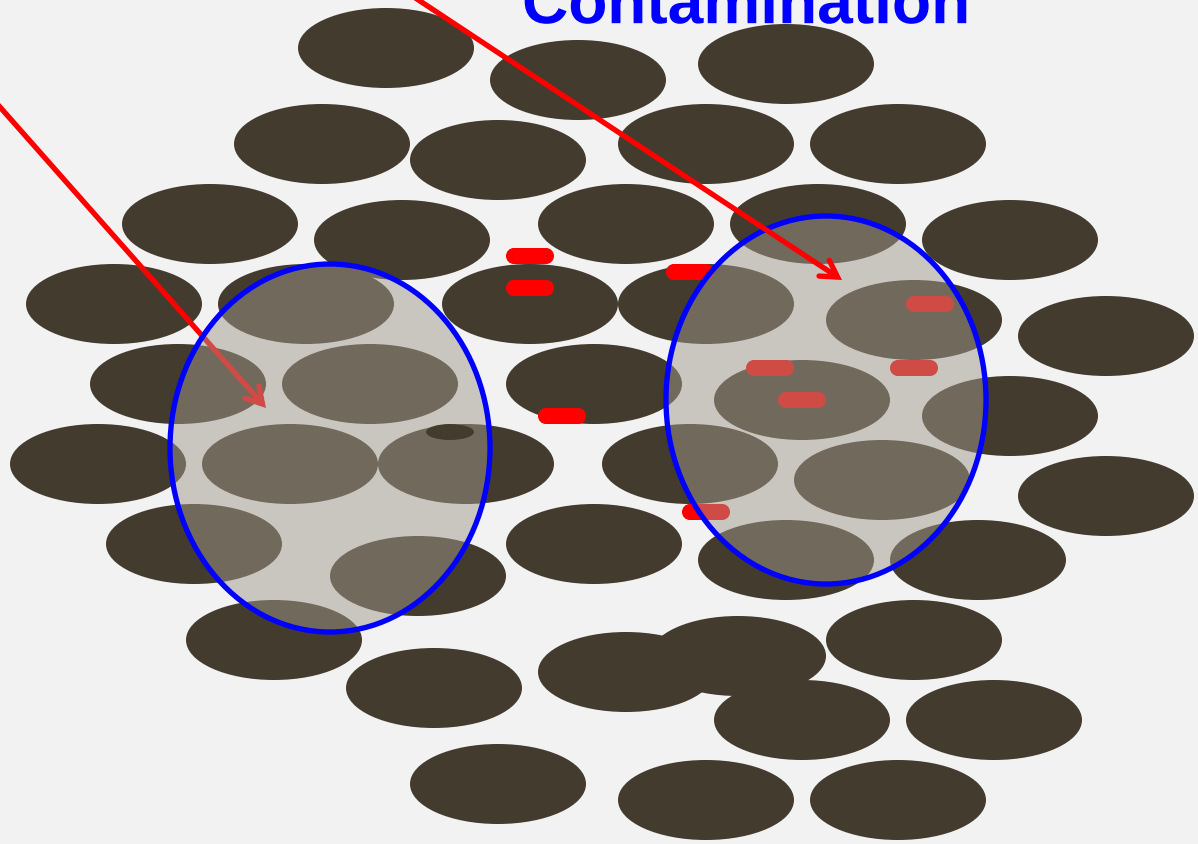
HOLLISON

GRAB SAMPLING

Traditional “Grab Sample”

Positive Contamination

Negative Contamination?



1:100,000 Probability of detection

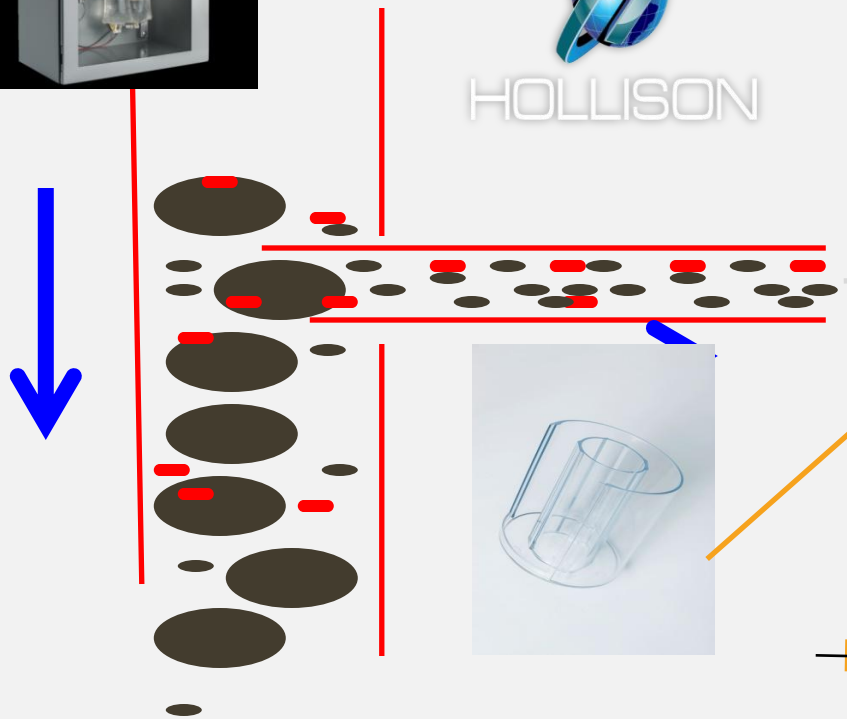
(based on statistical calculation by weight)

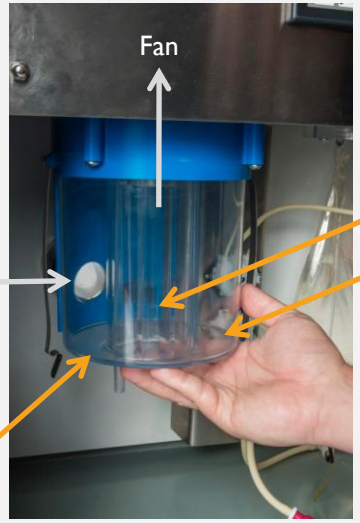
OLD WAY

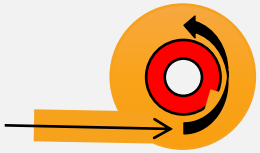
New Monitoring Technology

HOLLISON

Aerosol dispersion 70-80%







SEPARATION / COLLECTION / HYDROSOLIZATION

SAMPLE COLLECTION

"Lab-ready" sample

Culturing

Sequencing

Immunodiagnostics

PCR

qPCR

Bacteriophage

MATRIX TYPES (DRY PARTICULATE FOOD)

Considerations

Moisture / fat content
Size of product
Internal / Surface contamination
Aggregation characteristics
Homogeneity
Porosity

THANK YOU FOR YOUR ATTENTION!

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HOLLISON
ENHANCING THE SAFETY OF FOOD