

Emerging Techniques for Pathogen Control

Siobhan Reilly, PhD

Log10

Fairy Dust or Unicorn Horn

PROBIOTICS



Probiotics

- *Lactobacillus*
- *Bifidobacterium*
- Competitive Inhibitors
 - Space and nutrients
 - Acids, peroxides, extracellular molecules

They Do Other Things!!!

Protein

Lipids

Rna

Carbohydrates
(EPS)

Every food manufacturing facility has biofilm.

Dna

Ions

Water

BIOFILMS



Every food manufacturing facility has a control strategy.
Cleaning and Sanitation Programs

BIOFILMS



Biofilm Control

Detergents

- Time, Temperature, Agitation

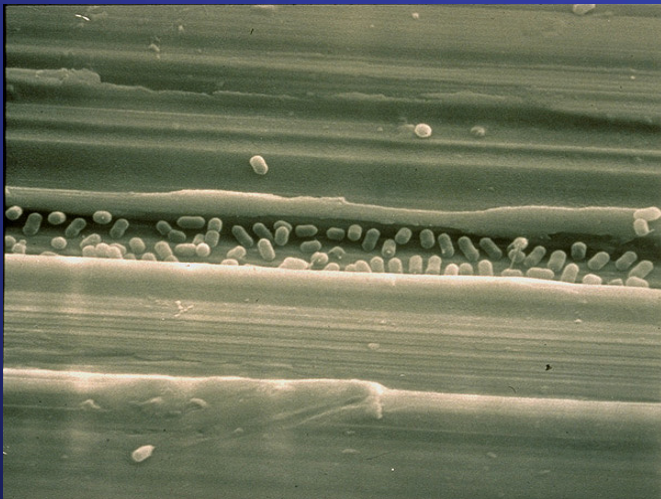


Sanitizers

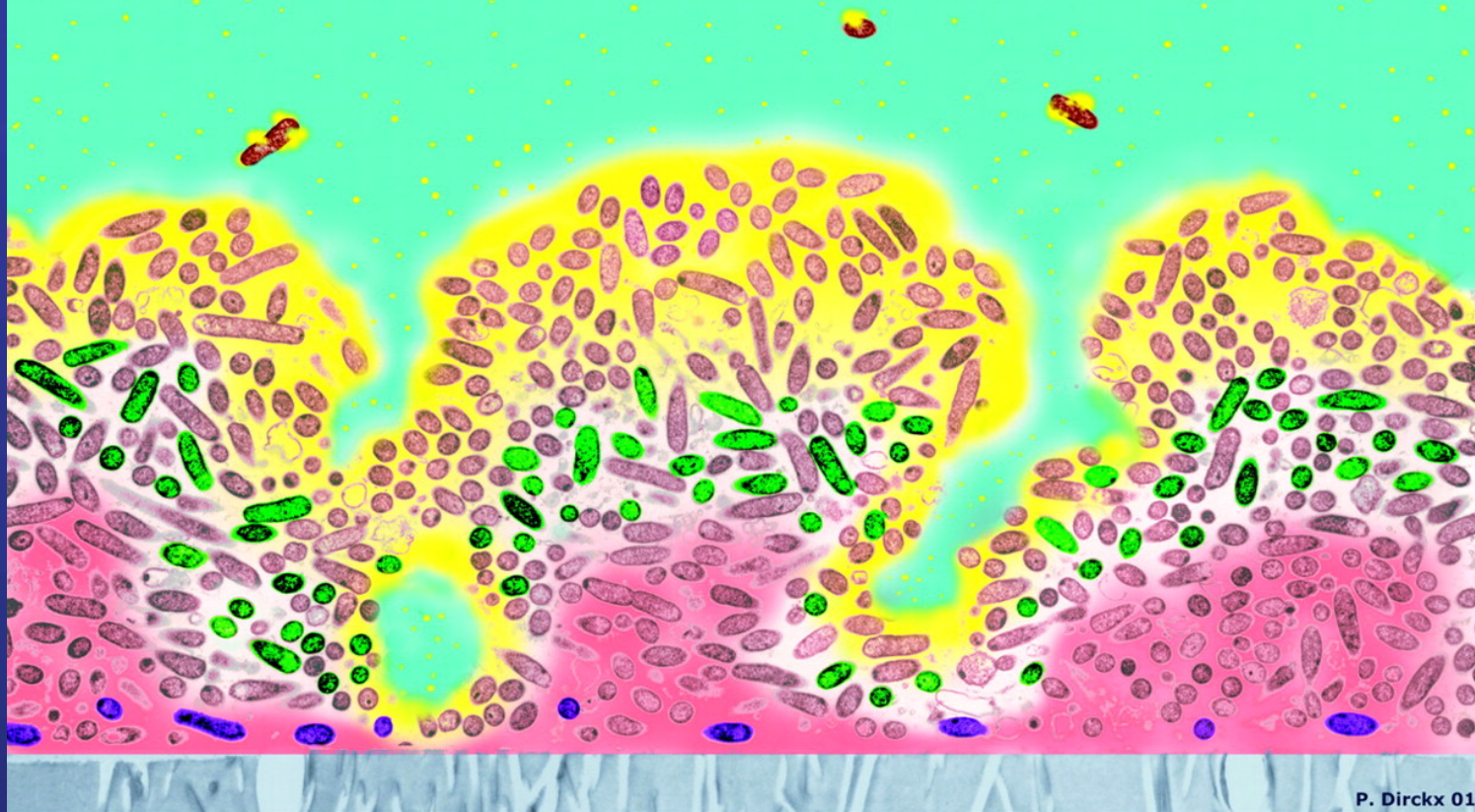
- Time, Temperature, Agitation



Biofilm Control



Mechanisms of Biofilm Tolerance



**Slow
Penetration**

**Stress
Response**

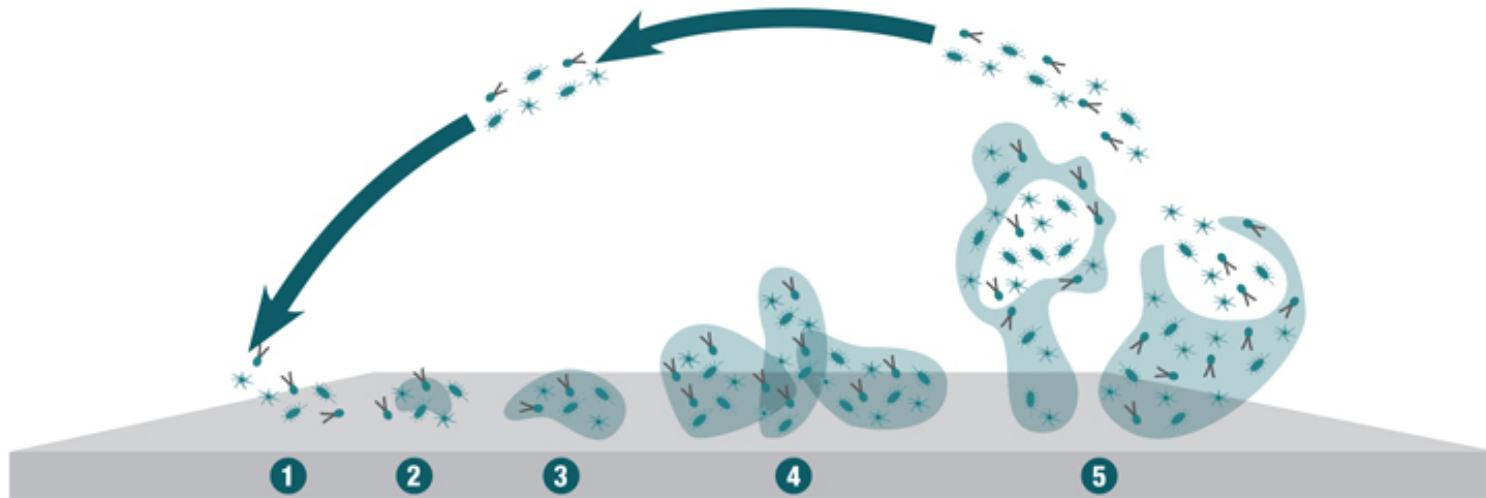
**Altered
Microenvironment**

Persisters

Jason D. Chambless et al. *Appl. Environ. Microbiol.*
2006;72:2005-2013

Applied and Environmental Microbiology

Quantitative Biofilm



The life cycle of a biofilm consists of several events occurring in the following timeframes:

EVENT:

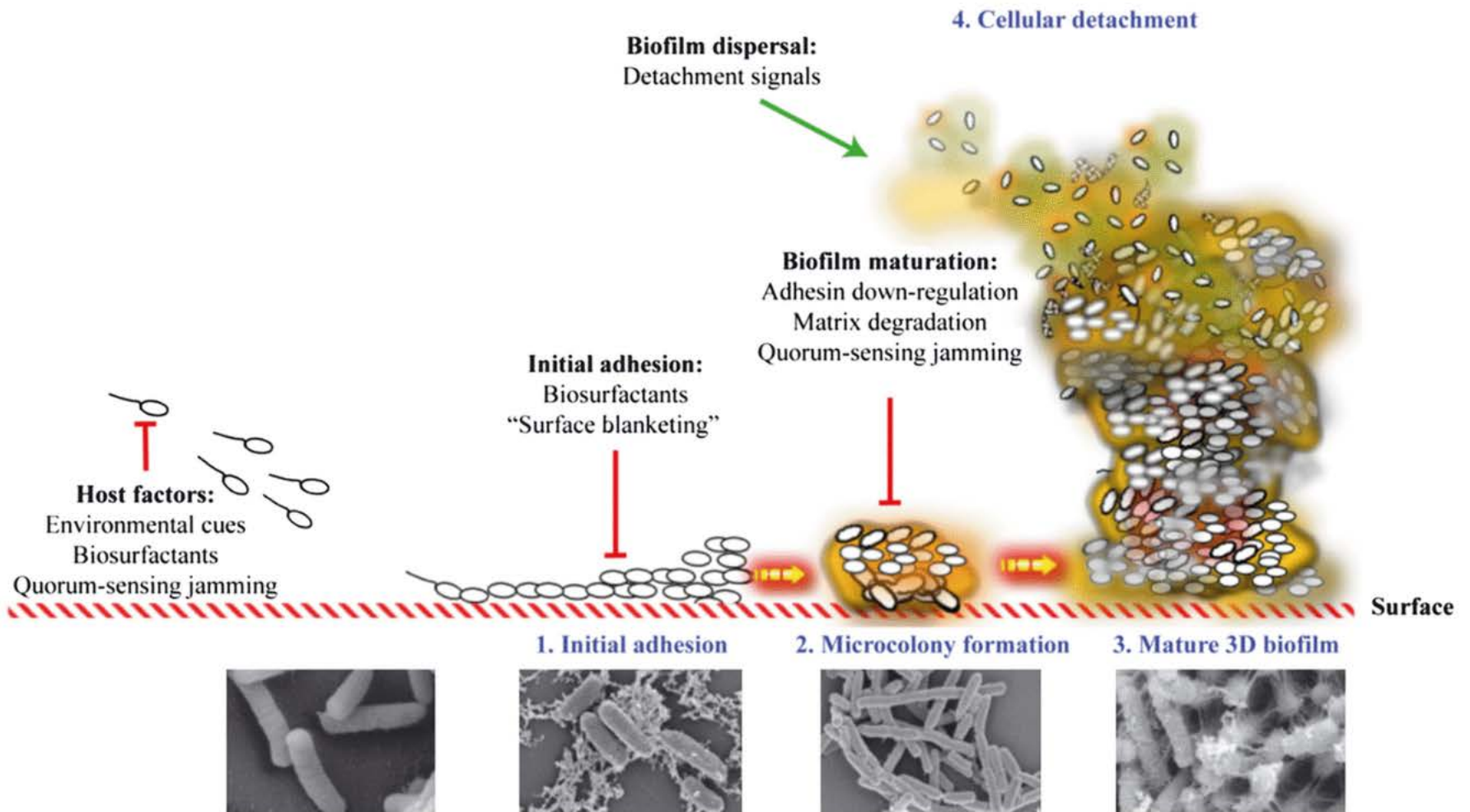
1. Bacteria adhere to a surface
2. Attached bacteria divide and begin to excrete extracellular matrix
3. The matrix-cell mixture expands, forms architectural features of biofilms
4. Further expansion and microbial growth
5. Dispersion of planktonic bacteria

TIMEFRAME:

Reversible, continuous
 Within seconds to minutes
 Minutes to hours
 Days
 Days to weeks

Source: www.oraclinical.com

BIOFILMS



Probiotics

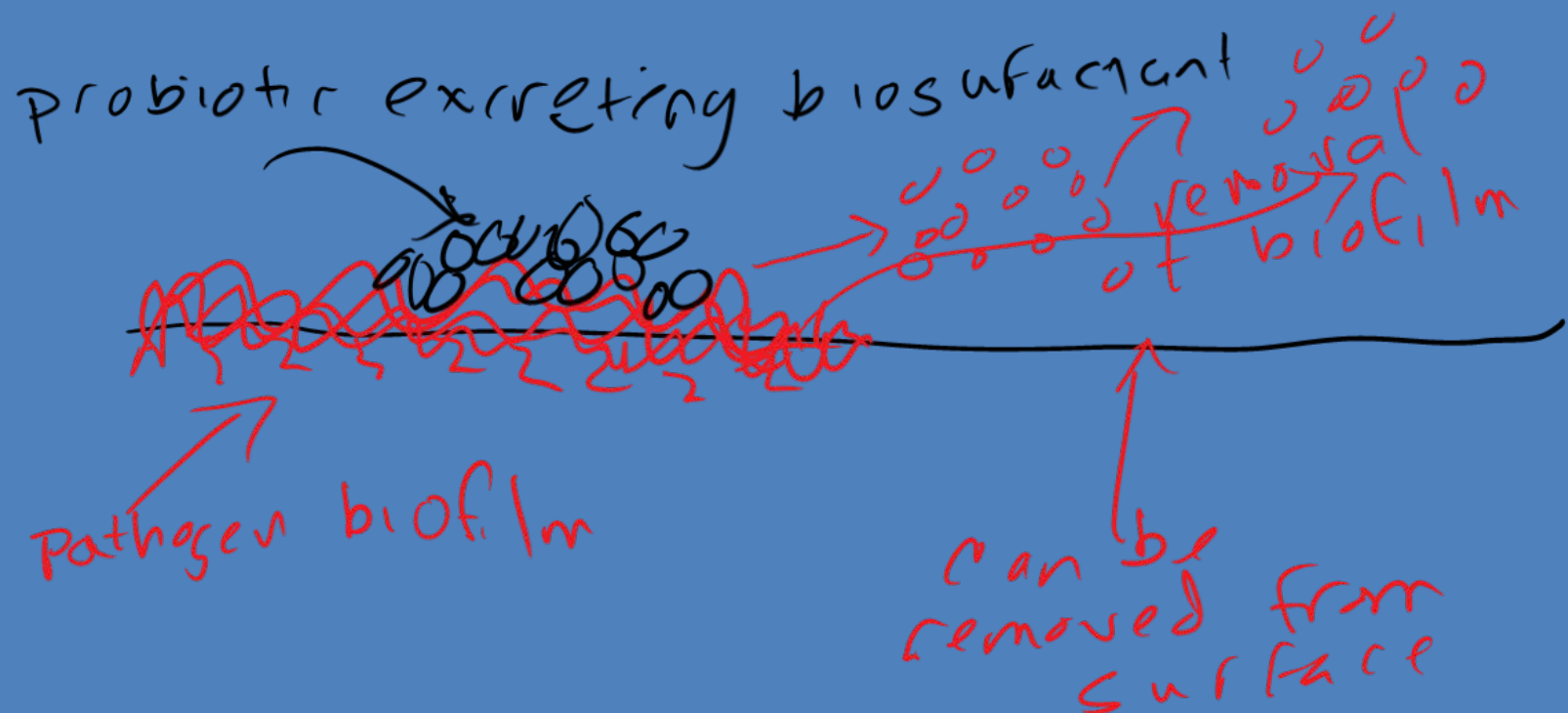
- *Lactobacillus*
- *Bifidobacterium*
- Competitive Inhibitors
 - Space and nutrients
 - Acids, peroxides, extracellular molecules

They Do Other Things!!!

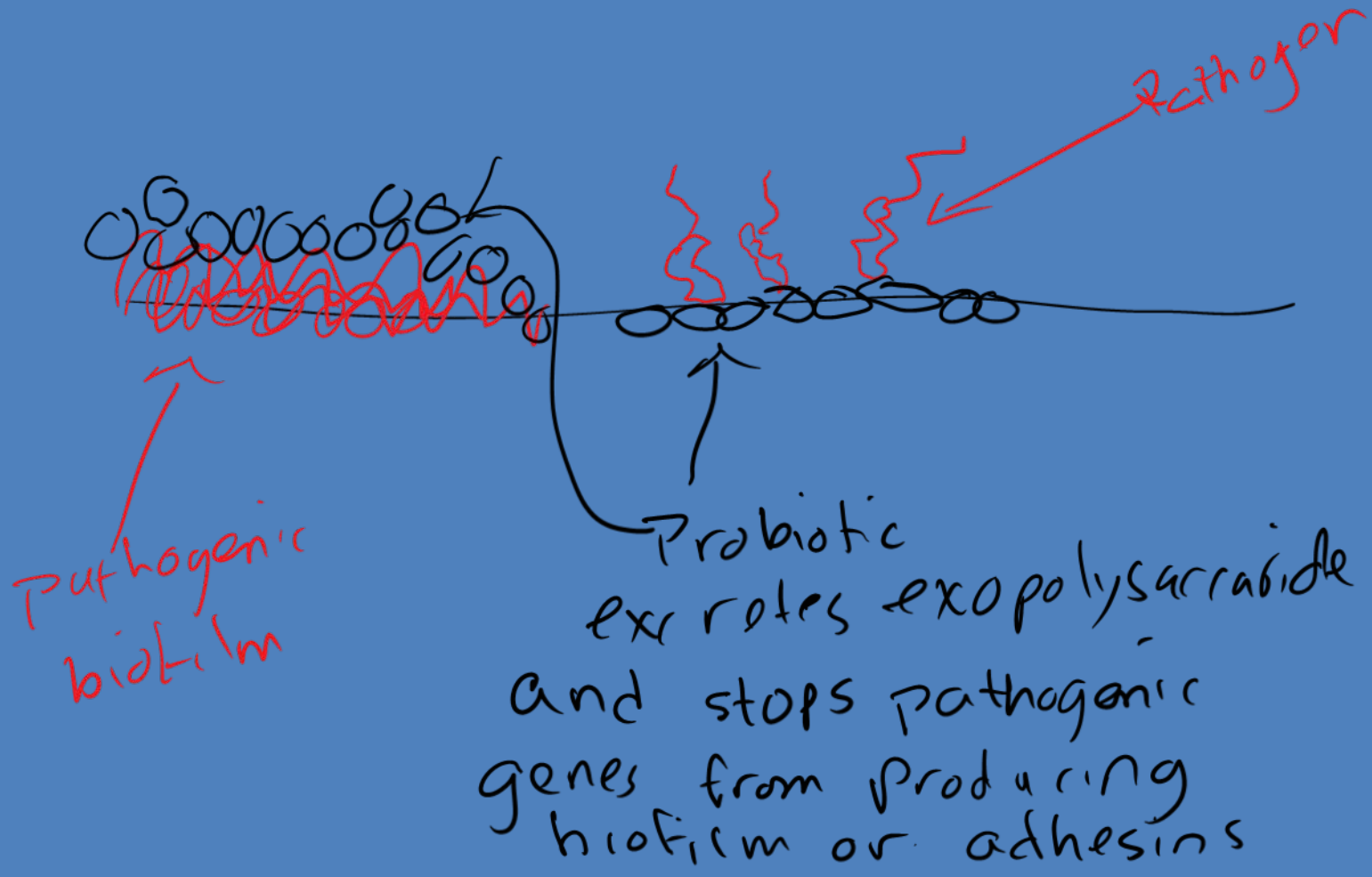
blanketing, the mechanism that can take place is by simply populating the food contact surface and preventing the pathogen from making the bond by taking up all the space.

initial inhibition, if the population is/was there and excreted of “anti-adhesion molecules” that change the molecular charge or the hydrophobicity of the surface preventing pathogen binding.

***Lactobacillus* biosurfactant production**, the “slippery detergent” that changes surface tension and thus allowing the surface to become wet and allow for dispersion of old biofilm or prevent pathogen adherence by letting them slide away, and not have time secrete their own biofilm or dissolve it as it was being produced.



Exopolysaccharide (EPS) production, or also known as anti-adhesion polysaccharides, can modulate the expression of pathogen genes that produce biofilms or surface adhesins. Especially *Bifidobacterium*, which also may use this EPS to build its own biofilm.



Dry Blend, food grade

Food Product

Human/Animal

Bifidobacterium

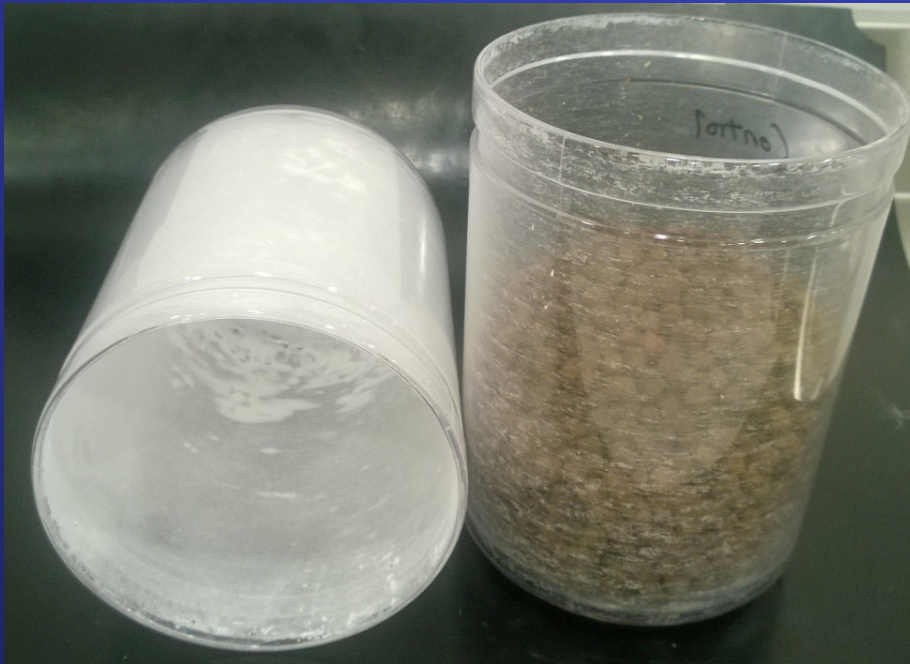
Lactobacillus

Prebiotic

Environment

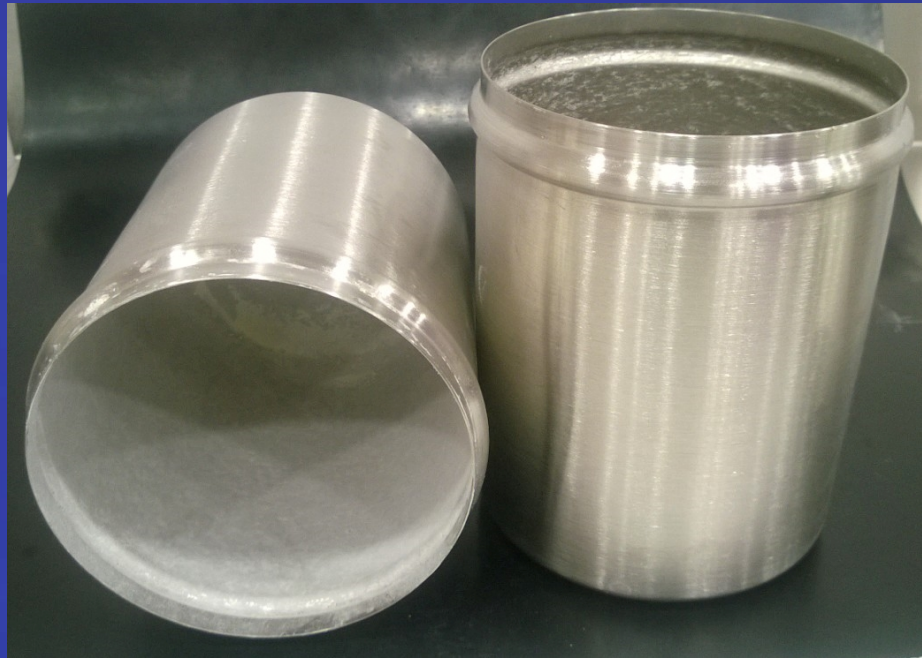
In the Laboratory-Food Contact Surface

- Food Grade Plastic



- *Salmonella* biofilm
- 1000 CFU/ml

- 304 SS



- *Salmonella* biofilm
- 1000 CFU/ml

SS or Plastic inoculated with
Salmonella

Let biofilm develop overnight at room temp

Surface Application of Dry
Powder (Control or
Probiotics)

Added about 3.8 grams of dry product containing:
1) None
2) 1.0×10^{10} CFU/g Lactobacillus and Bifidobacterium

Applied and left overnight at room temp

Sampled Each Day

Added 454 grams of sterile food kibble
to wipe biofilm:

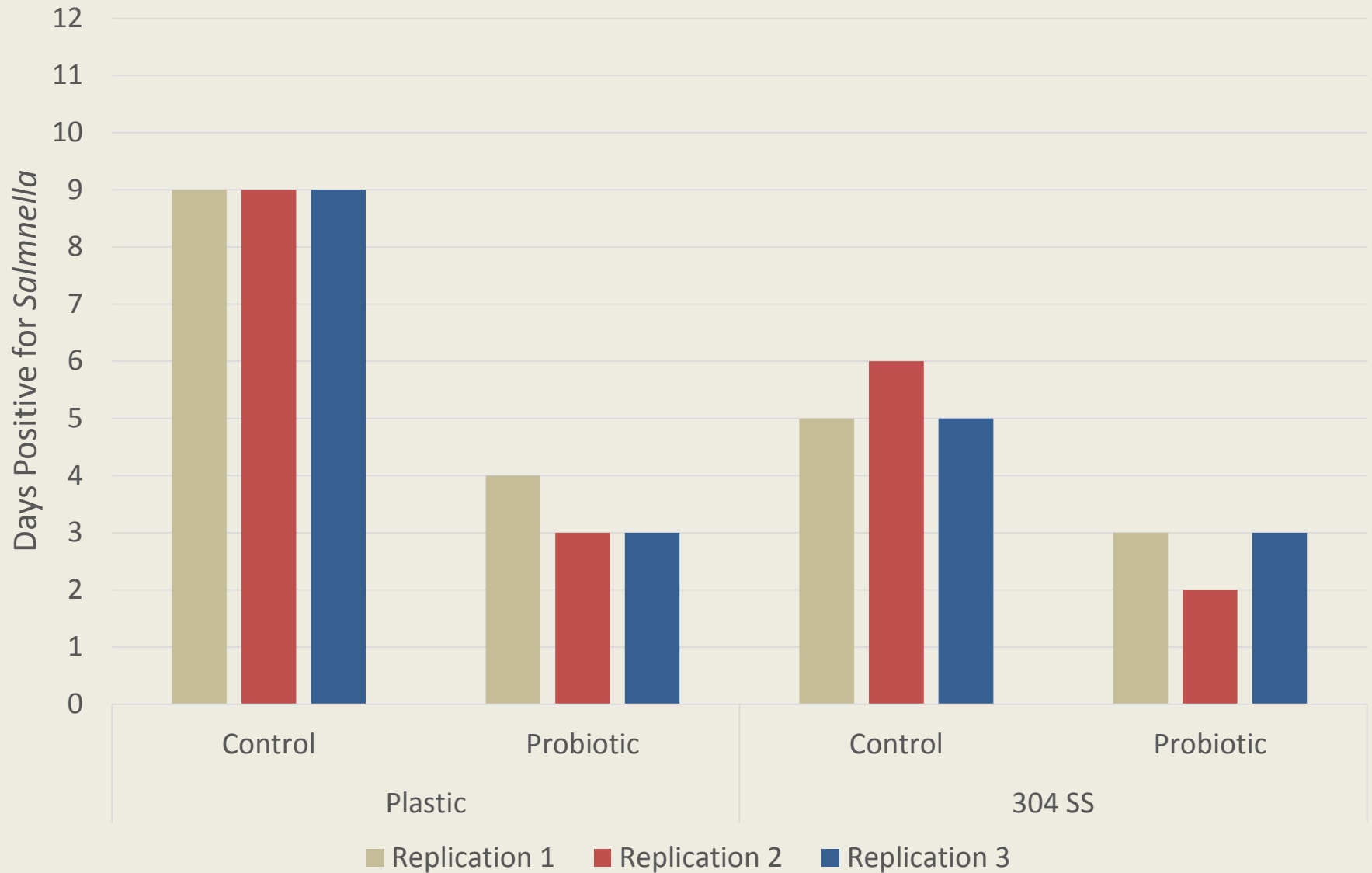
- 1) Everyday
- 2) Used all 454 grams for enriching
- 3) Used AOAC and FDA methods

until 3 consecutive negatives

In the laboratory



Effect of *Lactobacillus* and *Bifidobacterium* Dry Application on *Salmonella* BioFilm



What have we done??

**Isolated host specific strains of Lactobacillus,
Bifidobacterium, Enterococcus**

Selected for anti-pathogen characteristics

BSL2 Laboratory

Pathogen and Culture Bank



What have we done??

FDA-ISO-BioPharma Manufacturing

Added probiotic to over 1 Billion pounds of food

Removed *Salmonella* >12 pet food processing plants



THANK YOU

