Use of Enterococcus Faecium NRRL B-2354 as a Surrogate

December 8, 2015

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The Almond Conference

Speakers

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Validating the use of *Enterococcus faecium* NRRL B-2354 for almond processes

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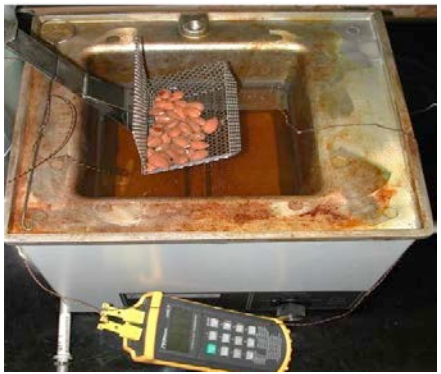
Surrogates: when appropriate or necessary?

- In-plant validation studies
  - usually almost always involve use of surrogates
  - introduction of the pathogen usually considered an unacceptable risk
    - E.g., continuous flat bed dry roaster

Pathogens: when appropriate or necessary?

- When parameters can be mimicked in the laboratory or on pilot scale equipment
  - E.g., oil roast, water blanch

- In very rare cases pathogens may be used for in-plant validation where:
  - No surrogate exists
  - Contamination of equipment/facility is unlikely
  - Precautions are taken/planned in advance
  - Experiments are planned/executed with guidance of experts



What makes an ideal surrogate?

- Non-pathogenic
- In low moisture foods (e.g., almonds)
  - Similar desiccation tolerance
- Represents pathogen of concern or “target organism”
  - For almonds and other tree nuts: *Salmonella*
- Similar inactivation characteristics and kinetics
  - Predict target organism
- Easy preparation
  - Stable high-density populations
  - Easy to enumerate

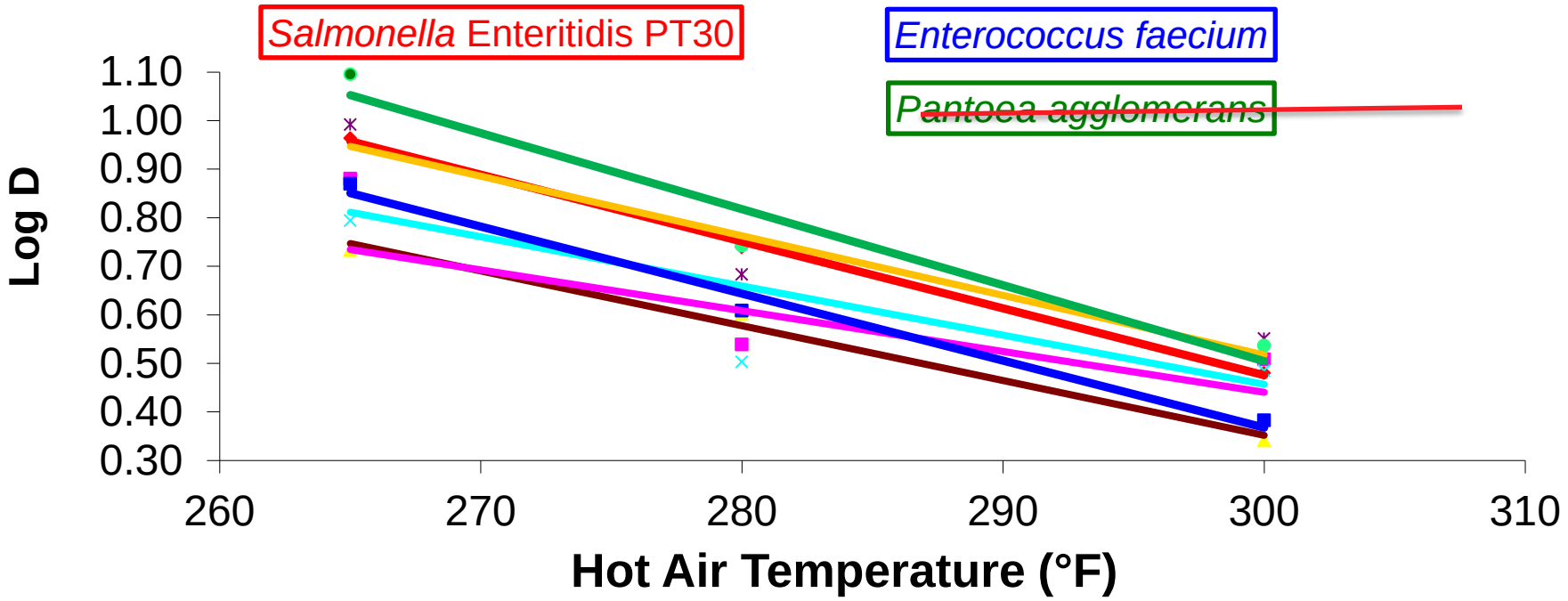
Salmonella

See: http://www.fda.gov/Food/ScienceResearch/ResearchAreas/SafePracticesforFoodProcesses/ucm094141.htm

Almond Board Microbial Surrogate Development 2004-2007

Potential Surrogates for *Salmonella*	
	Culture Names and Source ID
Salmonella culture	*Salmonella* Enteritidis Phage Type 30 (ATCC BAA-1045) (SE PT 30)
Surrogate cultures	*Pantoea agglomerans* SPS2F1 (PA)
	Pediococcus spp. NRRL B-2354 (Reclassified as *Enterococcus faecium*) (PD)
	Enterococcus faecalis (ATCC 49452) (EF)
	Lactobacillus plantarum (ATCC 14917) (LP)
	Lactobacillus fermentum (ATCC 9338) (LF)
	Streptococcus faecalis (ATCC 33186) (SF)

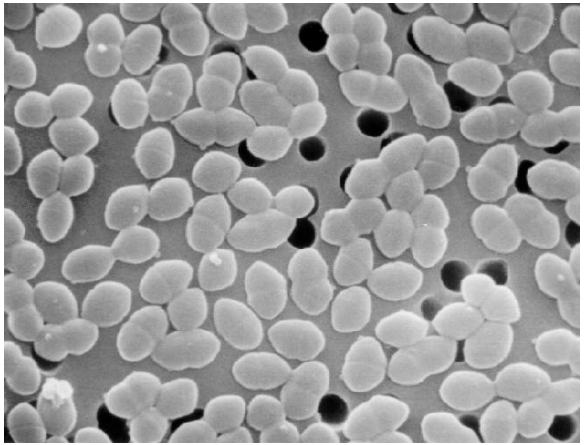
Potential Surrogates Screened (Dry Heat)



Pantoea agglomerans and *Enterococcus faecium* were chosen for further study

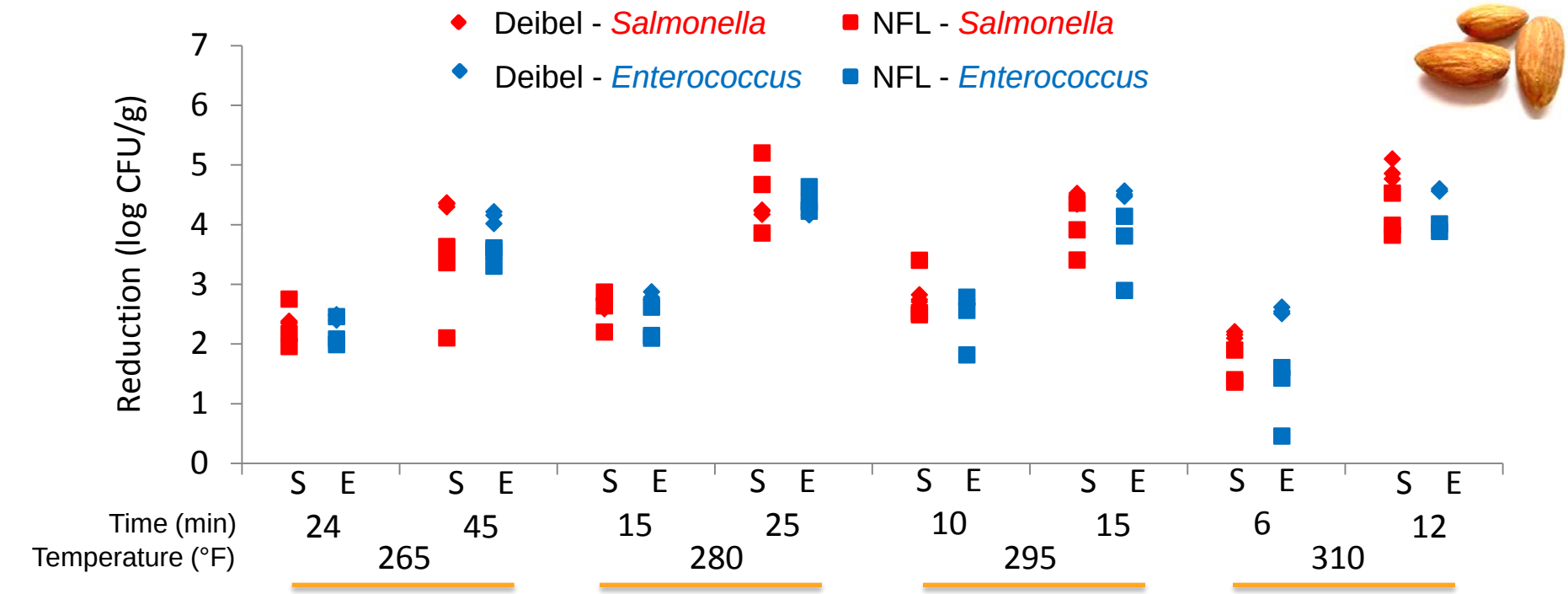
E. faecium NRRL B-2354/ATCC 8459

- Originally *Micrococcus freudenreichii* Guillebeau
  - Isolated from milk and dairy utensils
  - Used as a surrogate for thermal processing of dairy products (1947)
- Renamed *Pediococcus* sp.
- Reclassified as *Enterococcus faecium* (Ma et al., 2007)
  - Evaluating use as a surrogate for meat
- Deposited to
  - American Type Culture Collection ATCC 8459
  - Northern Regional Research Laboratory NRRL B-2354
    - USDA National Center for Agricultural Utilization Research

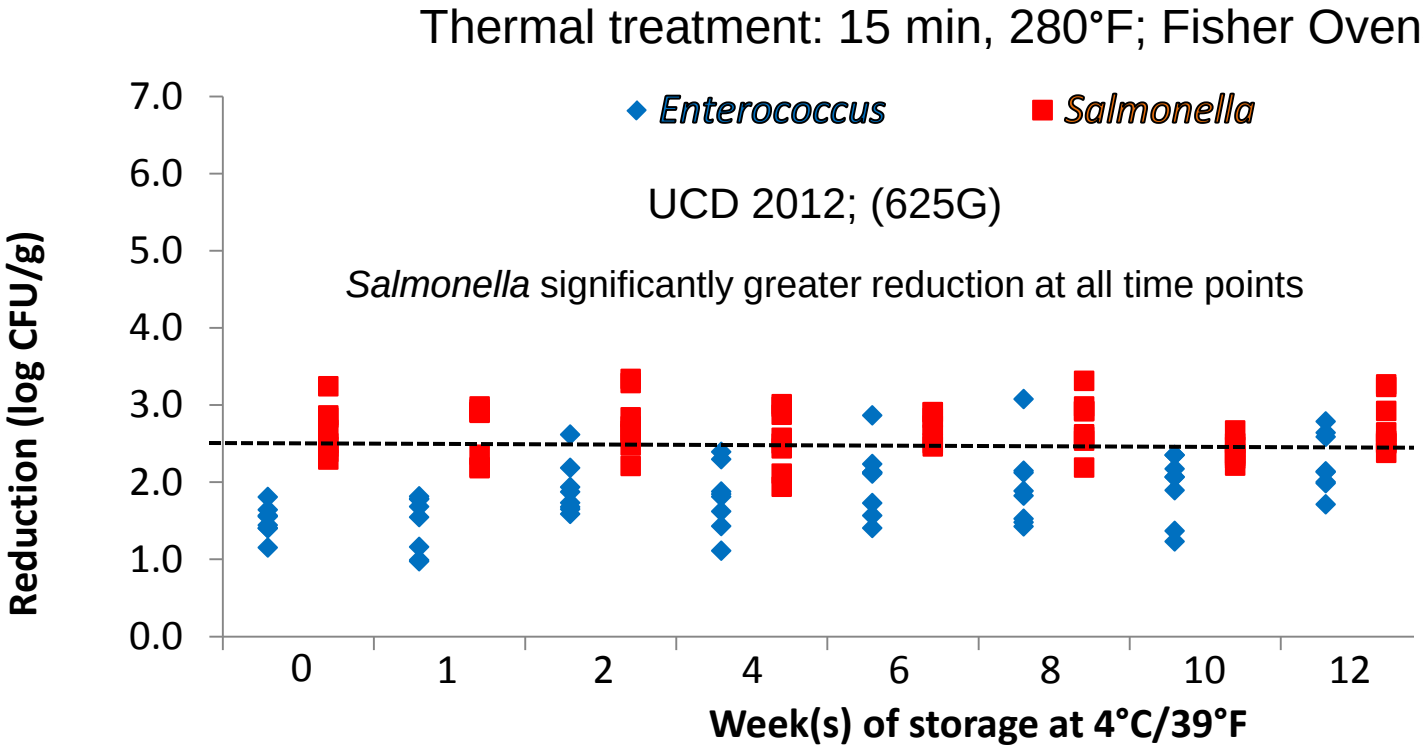


http://atcc.org/Products/All/8459.aspx
Annous and Kozempel. 1998. J. of Food Protection 61(5):578
Ma et al. 2007. J. of Food Protection 70(4):952
Kornacki, J. L. 2012. Food Safety Magazine.
https://commons.wikimedia.org/wiki/File:Entercoccus_sp2_lores.jpg

Reduction of *Salmonella* Enteritidis PT30 and *E. faecium* NRRL B-2354 (laboratory ovens, 2007)

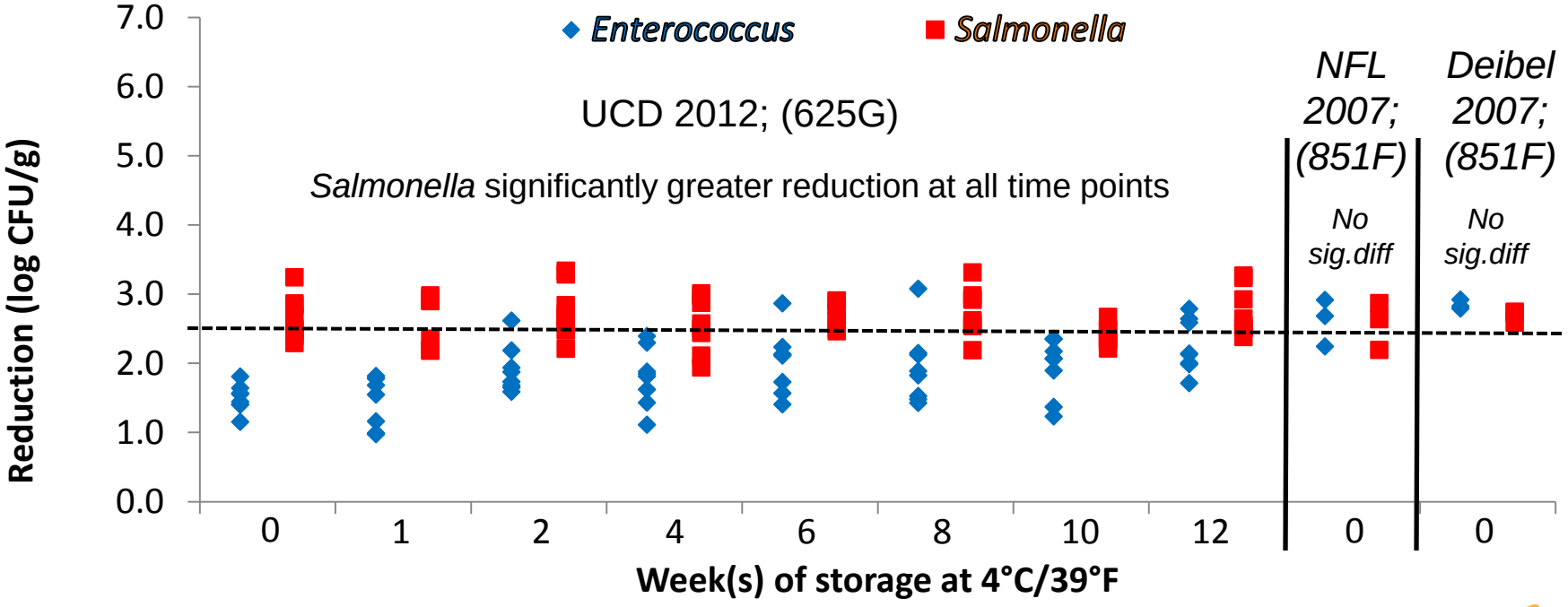


Influence of storage time at 4°C on thermal tolerance of *E. faecium* NRRL B-2354 and *Salmonella* Enteritidis PT30



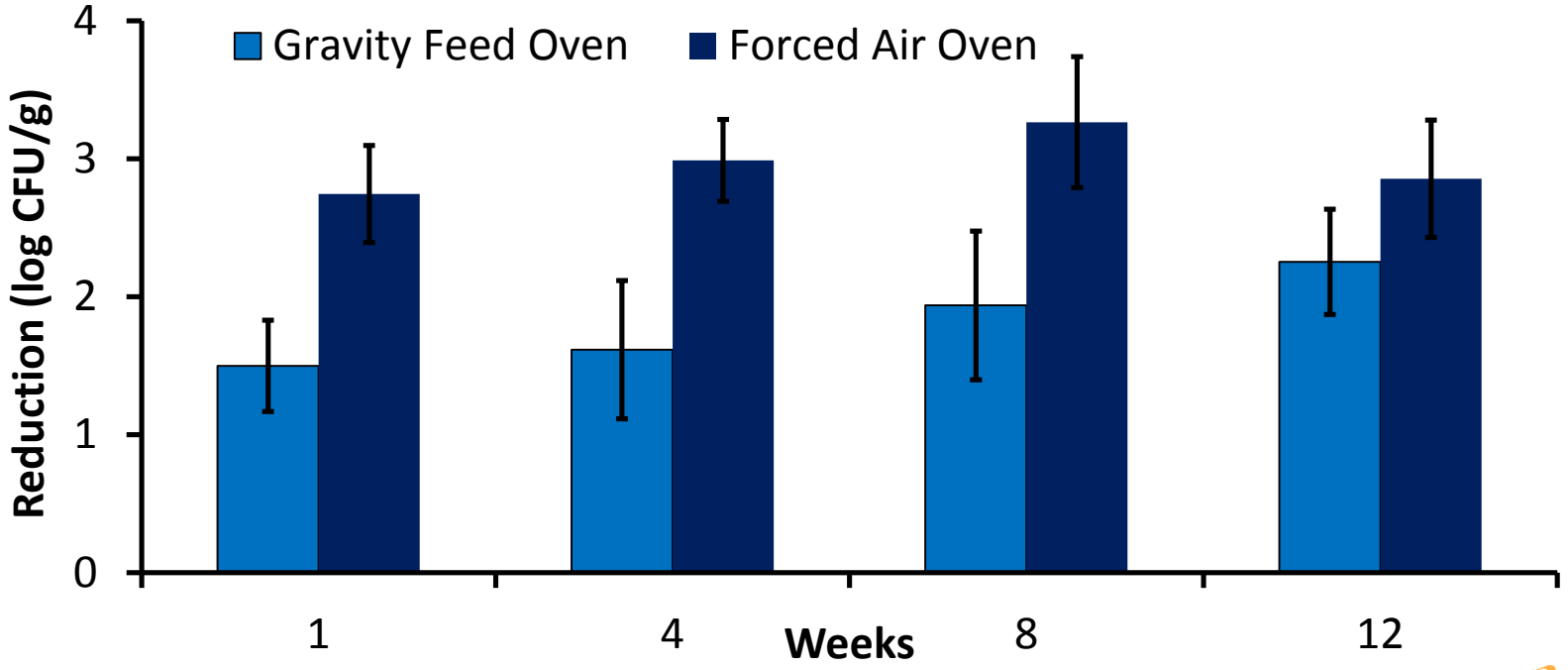
Influence of storage time at 4°C on thermal tolerance of *E. faecium* NRRL B-2354 and *Salmonella* Enteritidis PT30

Thermal treatment: 15 min, 280°F; Fisher Ovens

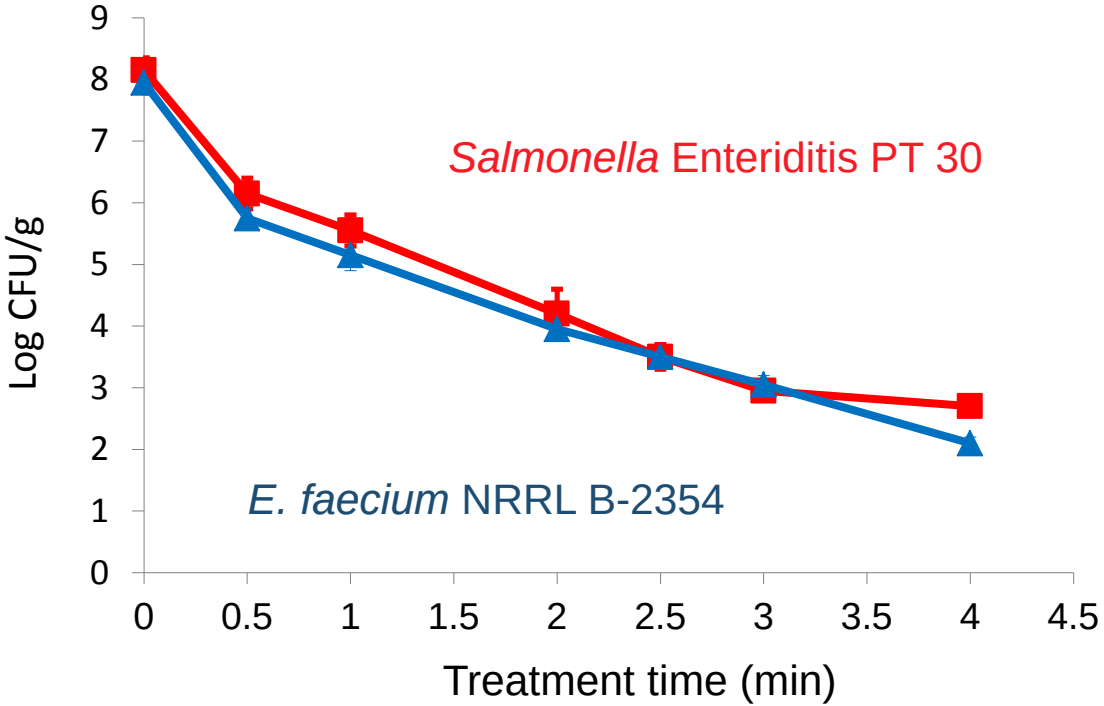


Ovens are not equal - *Enterococcus faecium* NRRL B-2354

15 min, 280°F in gravity feed (Fisher 625 G) or forced air (Fisher 725 F) oven

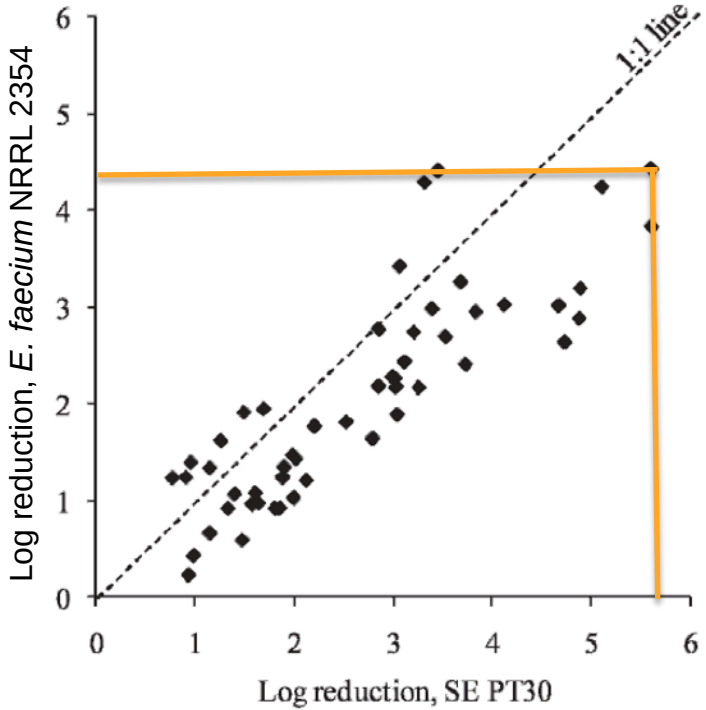


Hot oil 121°C/250°F



Kaur and Harris, unpublished

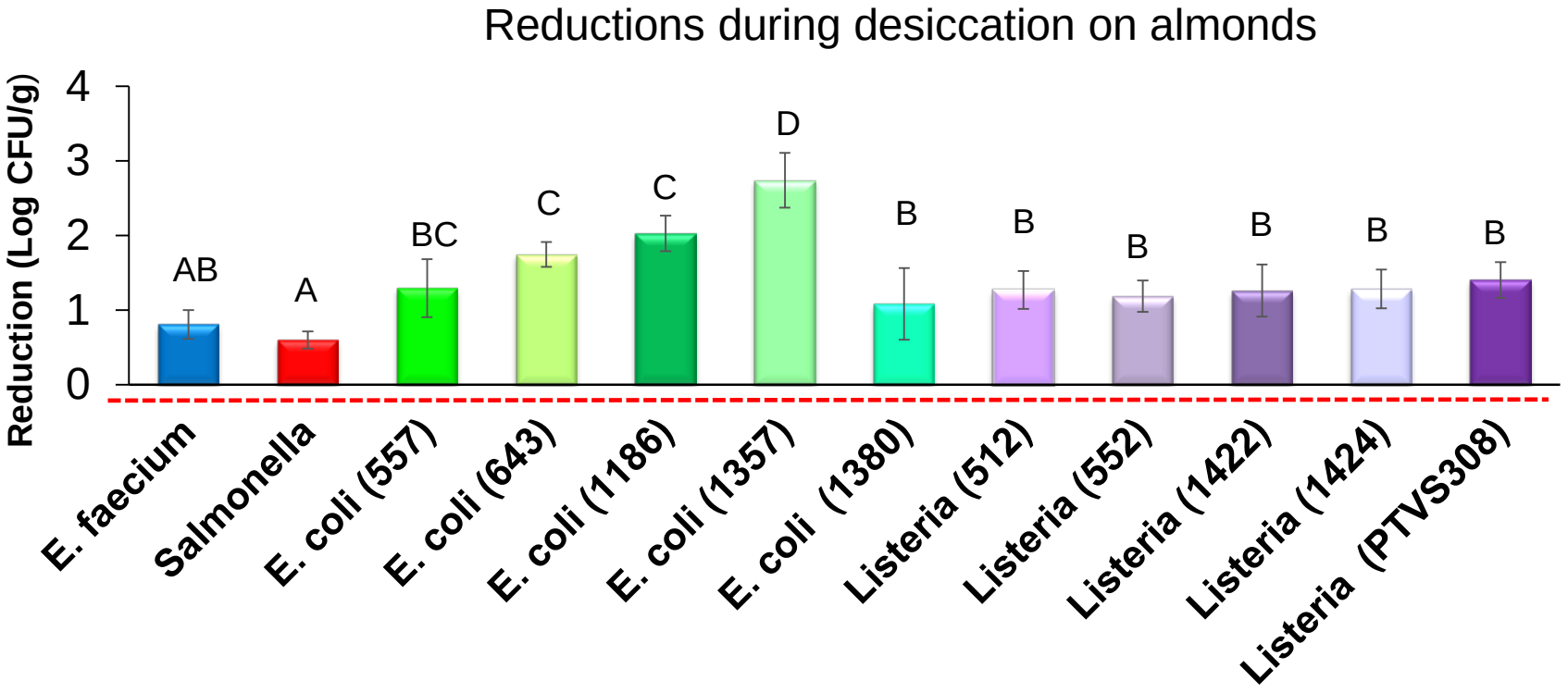
E. faecium NRRL B-2354 a useful surrogate in moist air heating



- Conclusions
  - NRRL B-2354 is a conservative surrogate for SE PT30 in the targeted reduction range of 4 to 5 log
  - a potentially useful surrogate for steam heating

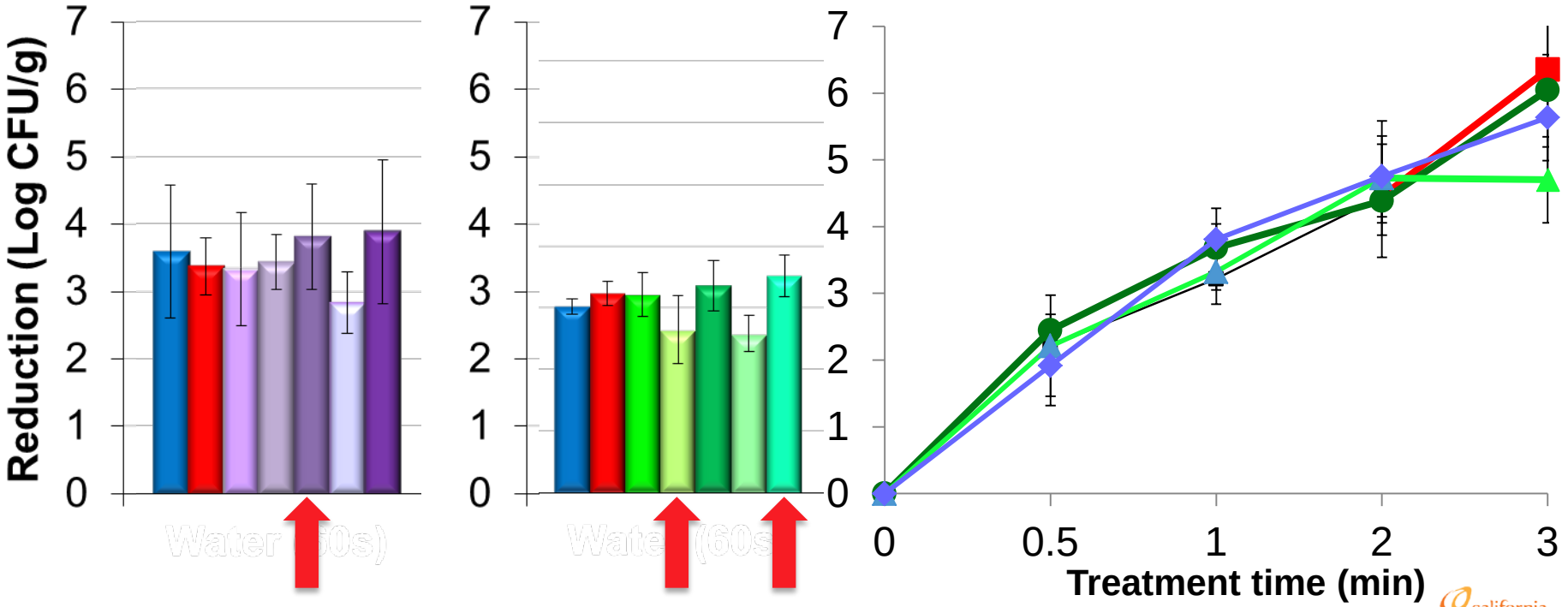
Jeong et al. 2011. Journal of Food Protection 74(4):603.

Comparisons to other pathogens



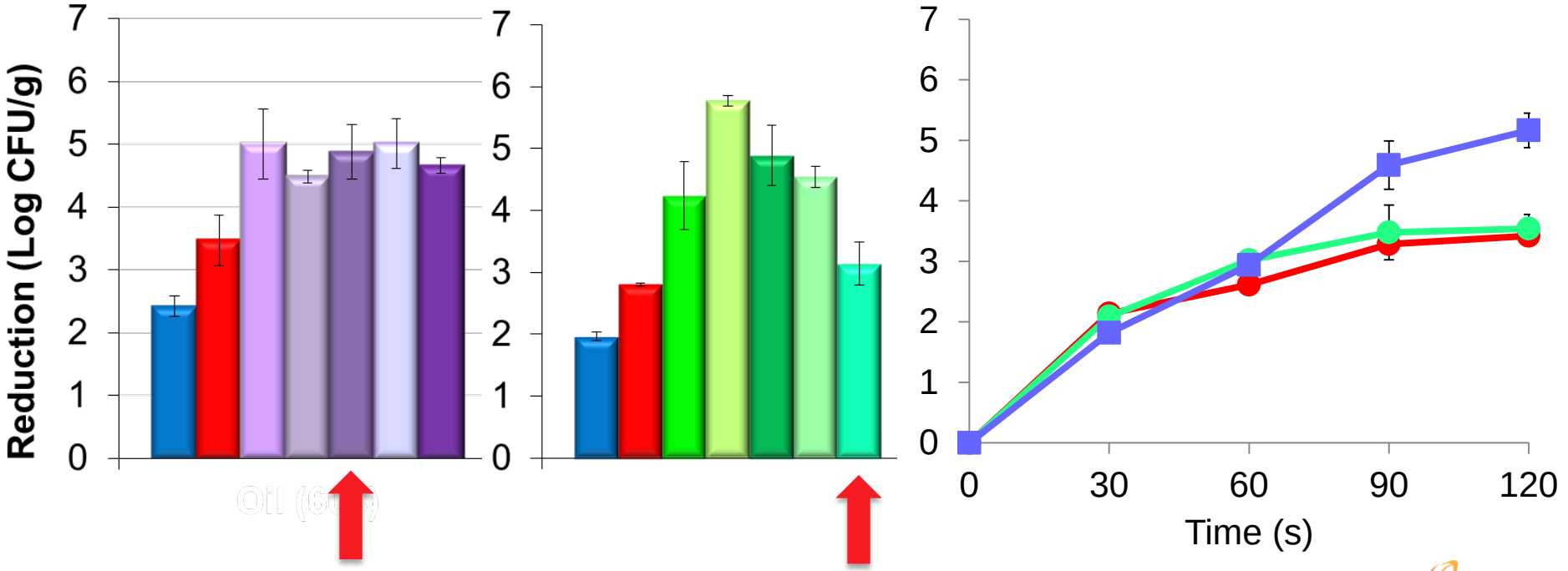
Moussavi and Harris, unpublished

Reduction of in hot water (176°F/80°C) *E. faecium* NRRL B-2354, *E. coli* O157:H7, *Listeria monocytogenes,* and *S.* Enteritidis PT30



Moussavi and Harris, unpublished

Reduction in oil (250°F/121°C) *Listeria monocytogenes*, *E. coli* O157:H7, *E. faecium* NRRL B-2354 and *S.* Enteritidis PT30 on almonds



Moussavi and Harris, unpublished

E. faecium NRRL B-2354 is an appropriate surrogate for *Salmonella* in almonds

- Jeong et al., 2011 Journal of Food Protection 74(4):603
  - Moist air or steam processes (ambient or vacuum)

- NFL and Deibel Laboratories, 2007
  - Dry-heat processes:
    - dry roast,
    - brine and pre-wet dry roast,
    - dry roast flavoring,
    - dry plasticizing

Guidelines for Using *Enterococcus faecium* NRRL B-2354 as a Surrogate Microorganism in Almond Process Validation

ABC, 2014

http://www.almondboard.com/Handlers/Documents/Enterococcus-Validation-Guidelines.pdf

Jeong et al. 2011.

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Maria Marco,
University of California, Davis

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Biosafety of *Enterococcus faecium* NRRL B-2354

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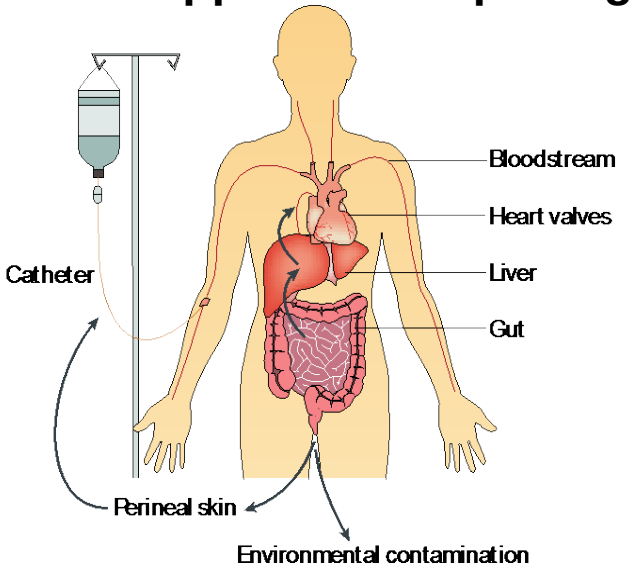
UCDAVIS
UNIVERSITY OF CALIFORNIA

E. faecium: impact on human health

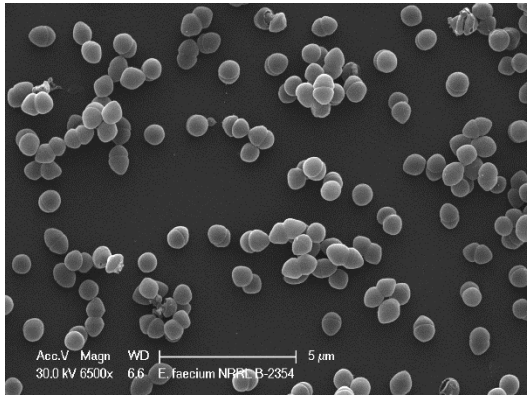
Used in some food fermentations

Sold as a probiotic (humans and animals)

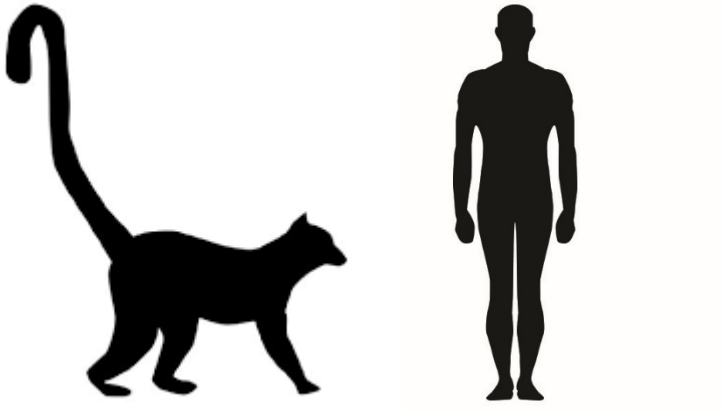
Antibiotic-resistant, opportunistic pathogen



Strain specificity of *E. faecium*



70% genetically related



78% genetically related

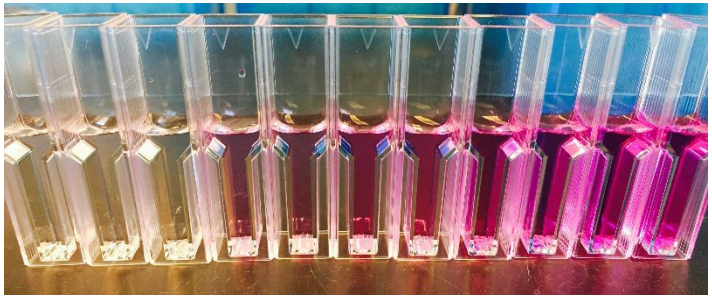
Each bacterial strain is genetically distinct

Thermal tolerance
Antibiotic resistance
Virulence

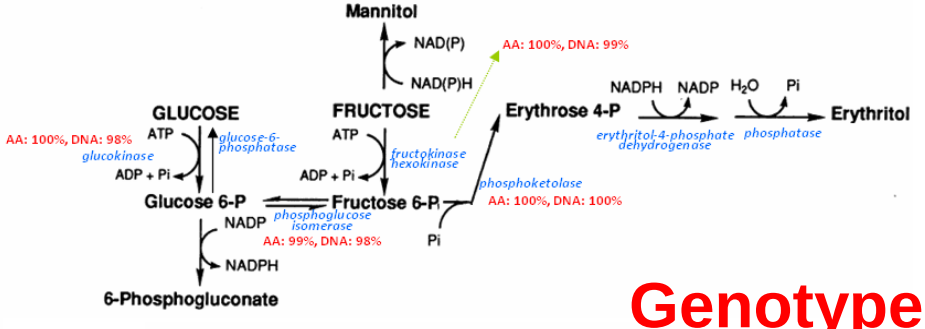
Staley 1997 Cur Op Biotechnol

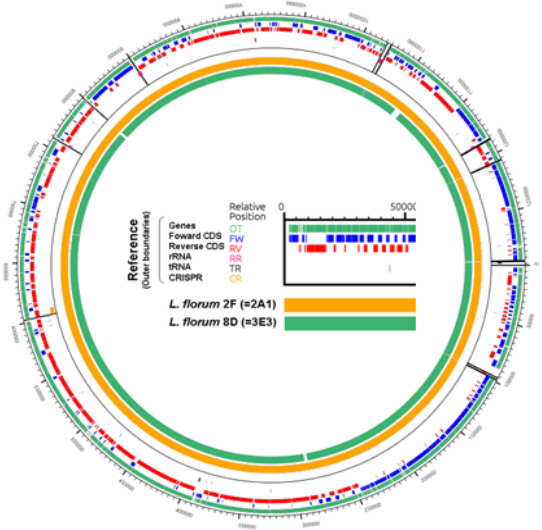
How to evaluate bacterial strains for safety

Phenotype



Genotype

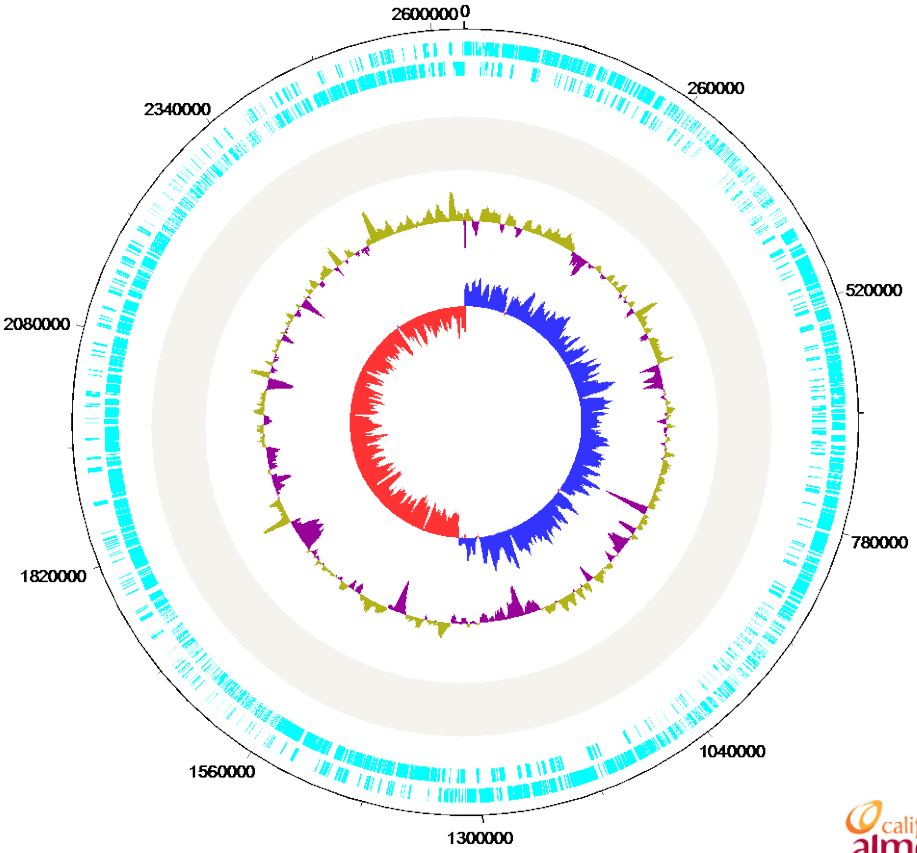




Genome sequence of NRRL B-2354

Complete genome:
Chromosome (2.6 Mbp)
One plasmid (214 kbp)

Genome of
E. faecium ATCC 8459
is over 99% identical

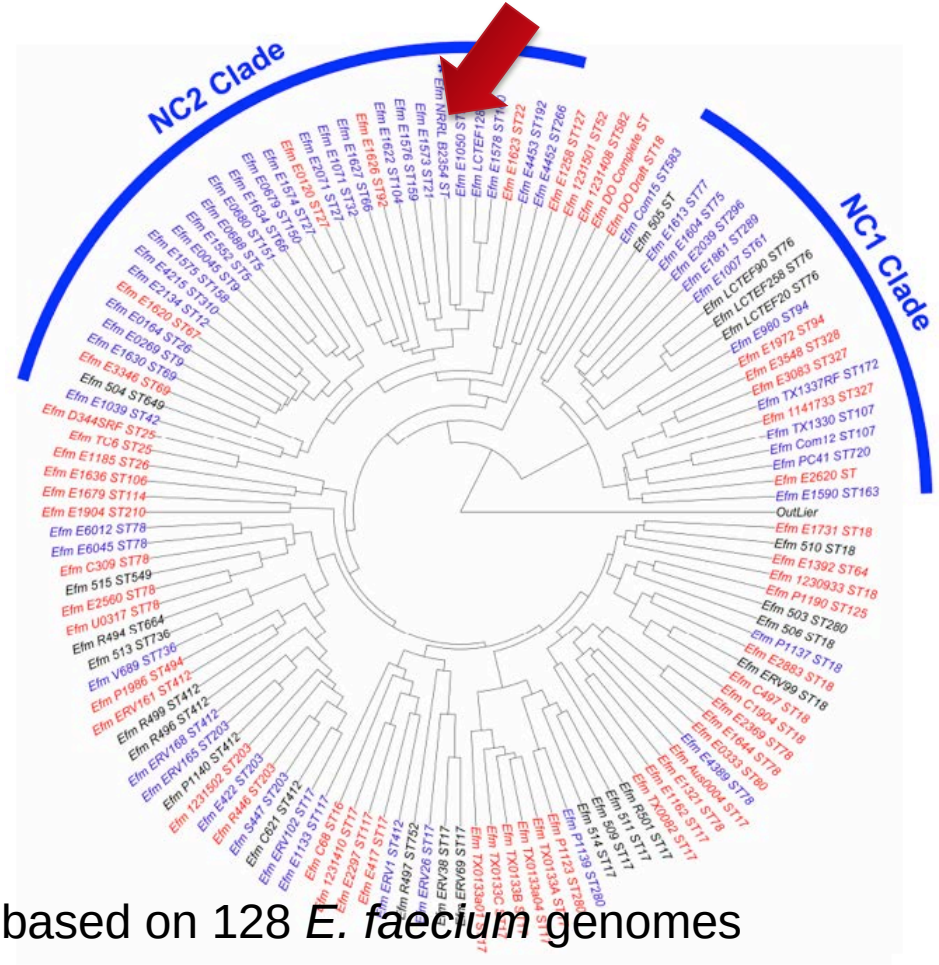


Kopit et al (2014) Appl Environ Microbiol
Kim et al (2013) Appl Environ Microbiol

NRRL B-2354 is a “community” strain

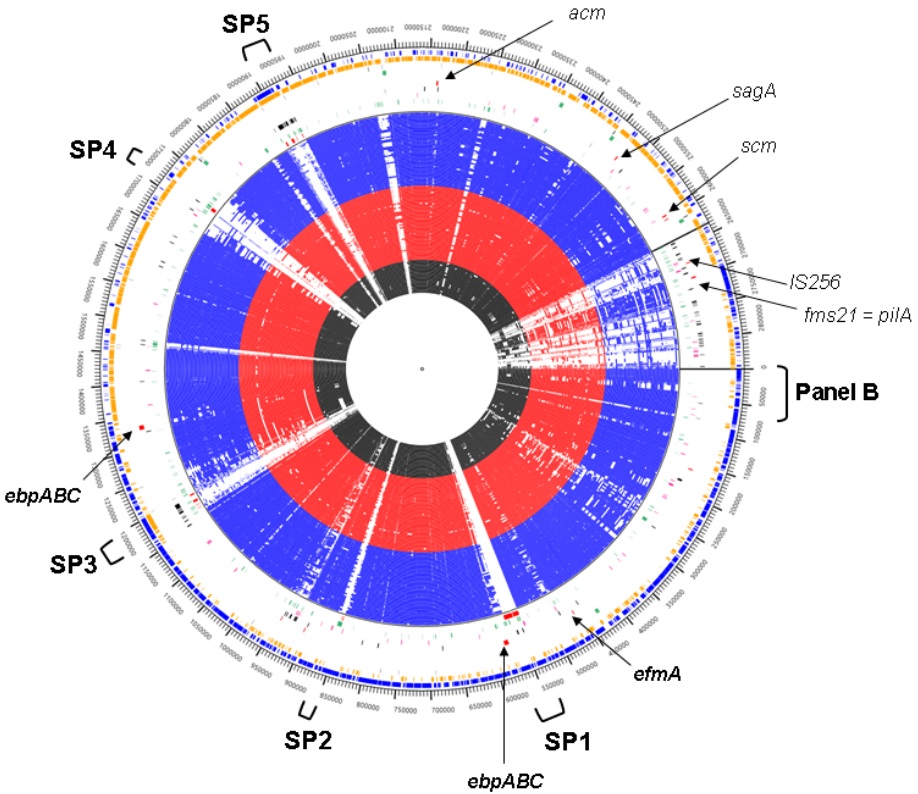
Clinical

Community



Hierarchical clustering based on 128 *E. faecium* genomes

NRRL B-2354 is distinct from other strains



- 45 genes not found in >100 other *E. faecium* genomes. Contained in loci (SPs)
- Lacks *cas* –CRISPRs for viral recognition
- Fewer mobile elements, virulence genes, and genes for antibiotic resistance than clinical strains

NRRL B-2354 does not produce hemolysin

Negative control:
***E. coli* DH5α**

NRRL B-2354

Positive Control:
***E. faecalis* ATCC 29212**

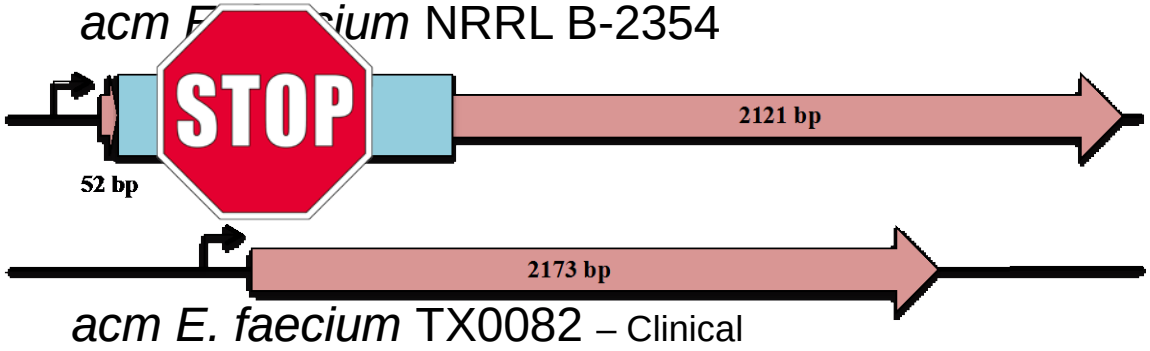
***cyl* genes are not present in NRRL B-2354**

Cultures grown on tryptic soy agar with 5% defibrinated horse blood

Virulence Factor: adhesion to human proteins

E. faecium NRRL B-2354 contains:

- *scm* (collagen I and IV adhesin)
- *sagA* (fibrinogen, fibronectin, collagen I adhesin)
- *acm* (collagen I adhesion)

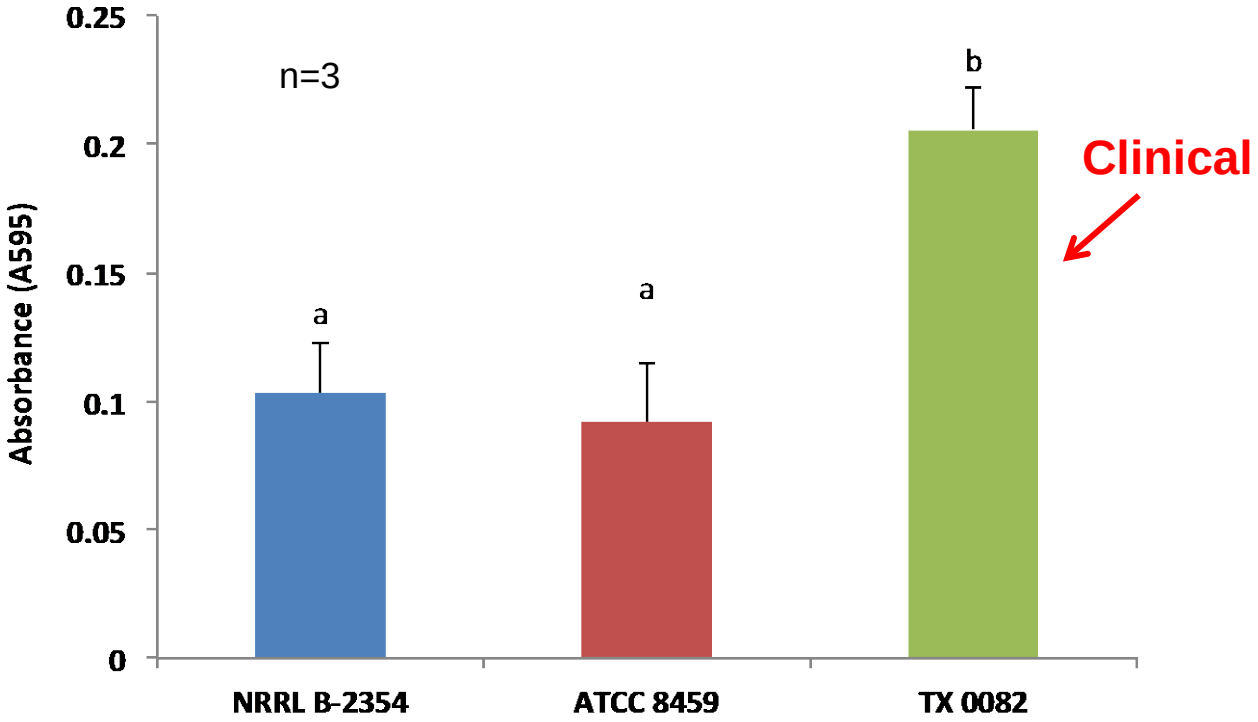


acm E. faecium NRRL B-2354

acm E. faecium TX0082 – Clinical

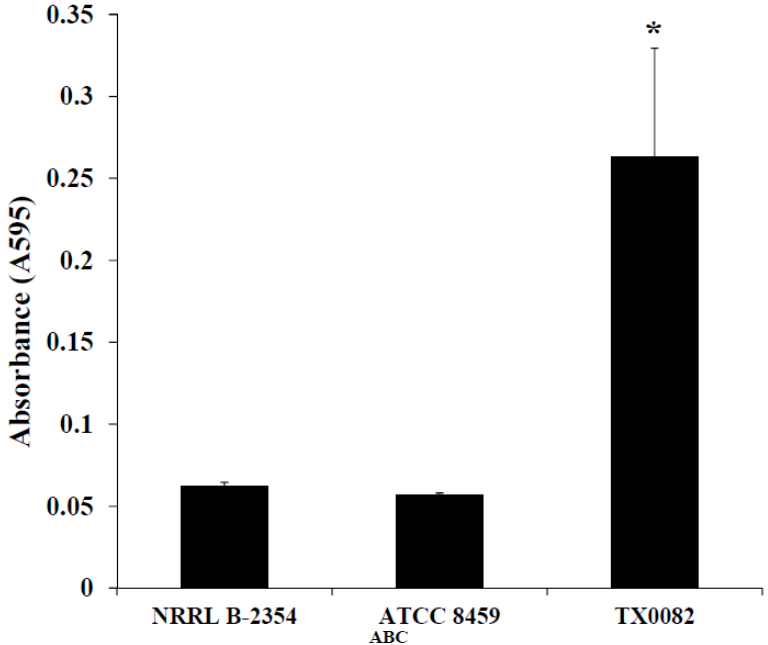
100% coverage, 99% nucleotide identity

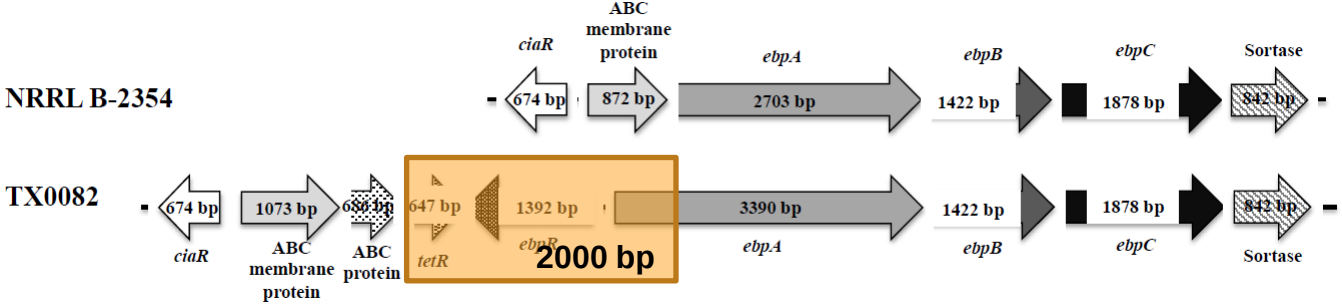
NRRL B-2354 does not bind collagen



Adherent cells were stained with crystal violet, positive control: clinical strain *E. faecium* TX0082, Tukey's HSD, $P < 0.05$

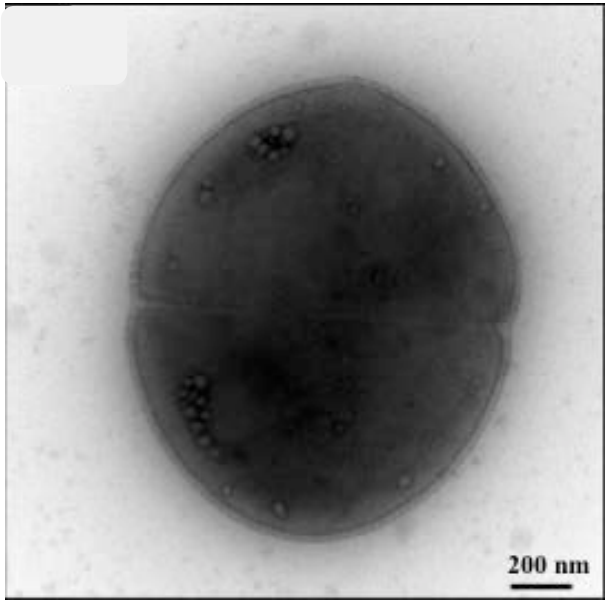
NRRL B-2354 does not form biofilms



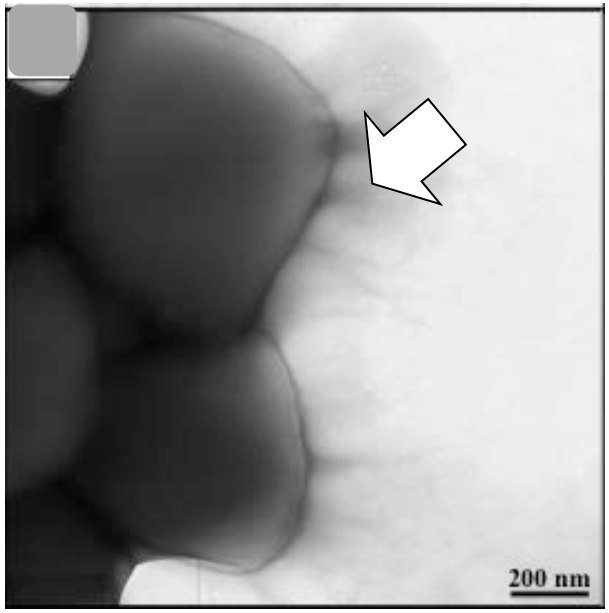


NRRL B-2354 does not make pili

NRRL B-2354



TX0082



Transmission electron microscopy

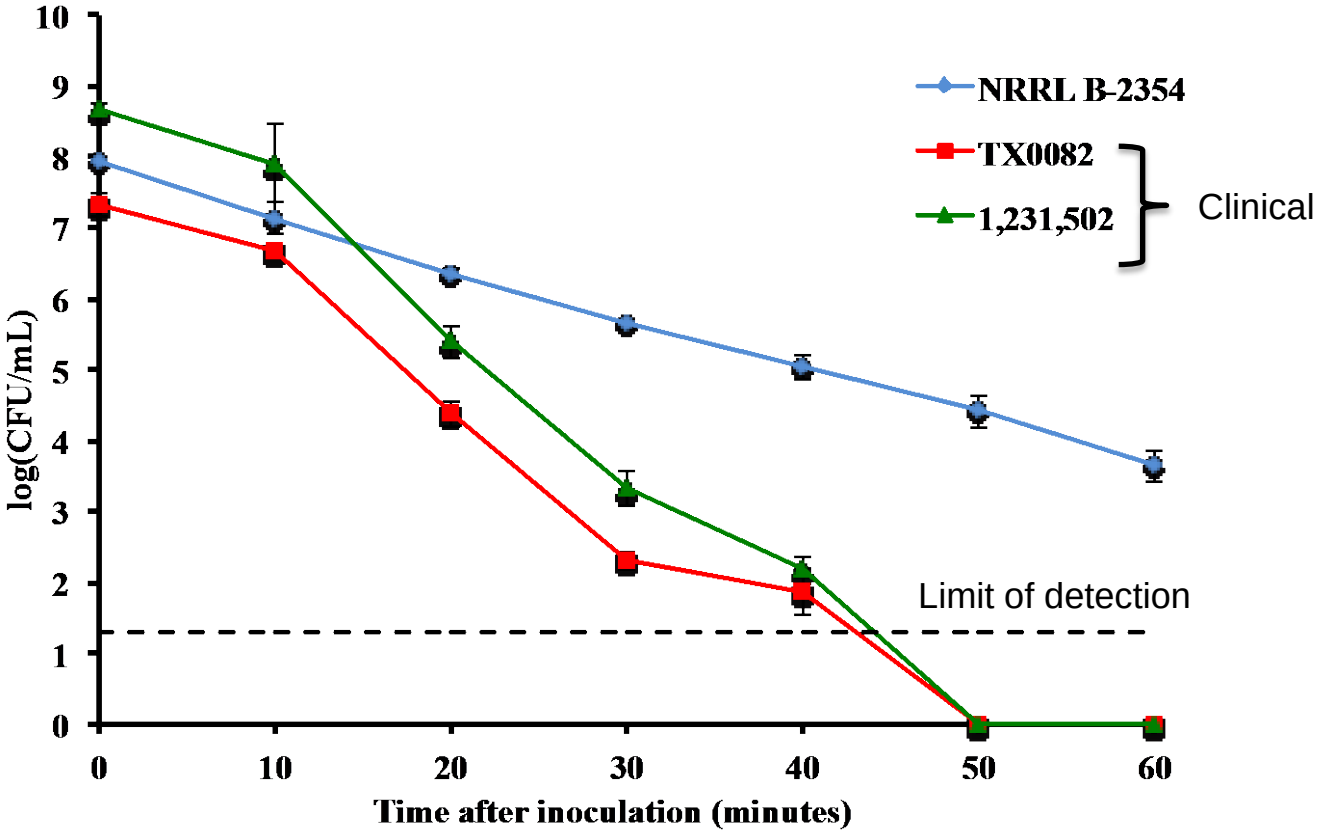
B-2354 contains *pil* operon and *efaA* but lacks *ebp* genes

NRRL B-2354 is sensitive to antibiotics

Class	Antibiotic	NRRL B-2354	ATCC 8459	Result
Aminoglycosides	Gentamycin	Sensitive	Sensitive	Sensitive
	Streptomycin	Sensitive	Sensitive	Sensitive
Cephalosporins	Cefazolin	< 2*	< 2*	Sensitive
	Cefoxitin	8	8	Sensitive
Glycopeptides	Vancomycin	< 0.5	< 0.5	Sensitive
Macrolides	Erythromycin	2	2	Intermediately sensitive
Penicillins	Ampicillin	< 0.125	< 0.125	Sensitive
	Penicillin	< 1	< 1	Sensitive
Quinolones	Levofloxacin	2	2	Sensitive
Tetracyclines	Minocycline	< 1	< 1	Sensitive
	Tetracycline	< 0.5	< 0.5	Sensitive

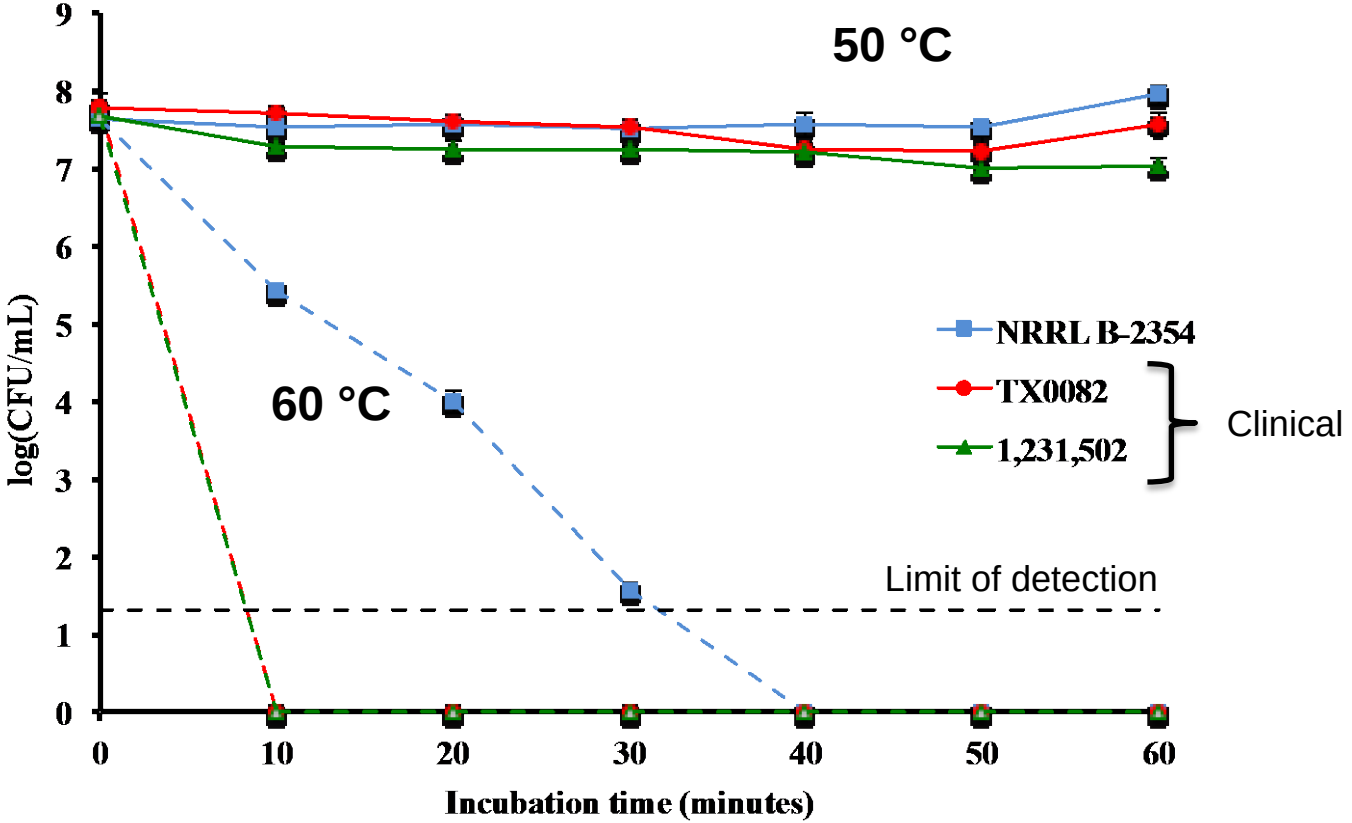
Minimum inhibitory concentration, BD Phoenix system, UC Davis Medical Center

NRRL B-2354 is acid tolerant



Incubated in physiological saline, pH 2.4

NRRL B-2354 is thermal tolerant



Incubated in physiological saline, LOD = 20 CFU/mL

Regulatory advice: *E. faecium* strain biosafety

European Food Safety Authority (2012)*

- Absence of *esp, hyl,* IS16 (marker of hospital-associated strains)
- Sensitive to ampicillin

* Safety evaluation of *E. faecium* strains intended as additives in animal feed

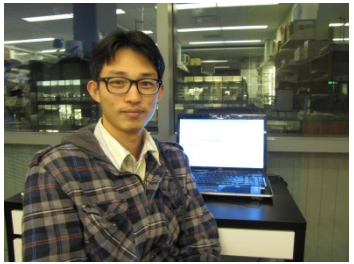
Conclusions

E. faecium NRRL B-2354 (ATCC 8459) lacks phenotypic and genotype features of clinical strains

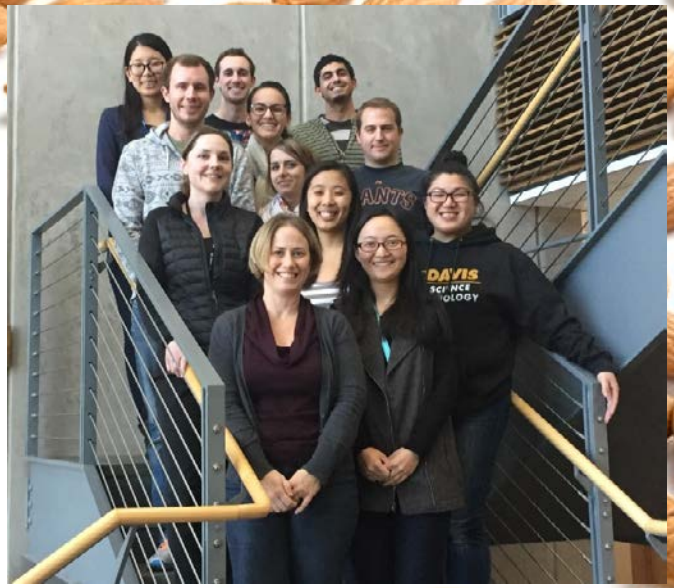
According to our assessments *E. faecium* NRRL B-2354 (ATCC 8459) is safe for use in thermal process validation tests

Acknowledgements

Lauren Kopit



Eun Bae Kim



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california almonds
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Validation of Thermal Processes Applied to Almonds

Continuing Investigations to Support the Use of *E. faecium* NRRL B-2354 as a Surrogate

Almond Board of California

December 8, 2015

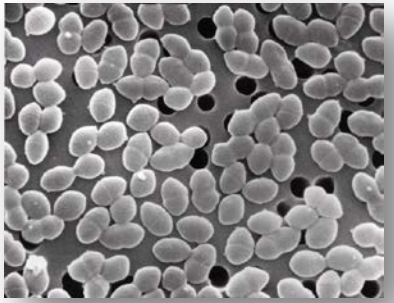
Overview

- Review criteria to select a surrogate organism
- Review ABC guidelines for preparation and evaluation of surrogate heat resistance
- Summary of ongoing research at The National Food Laboratory to evaluate the appropriateness of *Enterococcus faecium* NRRL B-2354 as a surrogate for *Salmonella* Enteritidis PT30 on almonds
  - ✓Extended storage of surrogate-inoculated almonds
  - ✓Thermal resistance of surrogate on different almond types

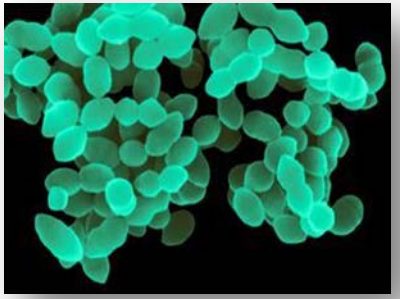
Selection of a Surrogate

How is the appropriateness of a surrogate organism determined?

- Identify the target organism
  - ✓Based upon food safety plan
  - ✓Outbreaks in similar ingredients/products
- Food matrix composition/parameters
  - ✓Worst case scenario
- Thermal process parameters
- Select potential surrogate(s)
  - ✓Non-pathogenic
  - ✓Stable
  - ✓Easy to grow to high levels
  - ✓Similar thermal resistance to the target organism



Enterococcus faecium



Pediococcus acidilactici

Selection of a Surrogate

Main goals when evaluating a surrogate:

- Understand the thermal resistance of target organisms in the selected food matrix
- Understand the thermal resistance of the surrogate organism to the same conditions

Surrogate organism should demonstrate similar or greater resistance to heat

Selection of a Surrogate for Almonds

Consideration

- ✓Identify the target organism
- ✓Food matrix
- ✓Process parameters
- ✓Selection of a surrogate

Almonds

- ✓*S.* Enteritidis PT30
- ✓Whole kernel almonds
- ✓Dry roasting
- ✓*E. faecium* NRRL B-2354

ABC Surrogate Evaluation Guidelines

ABC published a protocol, “Guidelines for Using *Enterococcus faecium* NRRL B-2354 as a Surrogate Microorganism in Almond Process Validation”, to provide guidance to the industry on the following points:

- ✓ Preparation of *E. faecium* NRRL B-2354 culture for inoculation
- ✓ Inoculation of almonds with the prepared *E. faecium* NRRL B-2354 culture
- ✓ Heat resistance verification for *E. faecium* NRRL B-2354 on the inoculated almonds
- ✓ Storage of the inoculated almonds prior to validation testing

Can the protocols be refined further to provide more flexibility in their use for the validation of almond processes?

Reevaluate ABC Surrogate Evaluation Guidelines

Reevaluate the following limitations of the protocol:

The almonds inoculated with the surrogate should be used within **two weeks** of preparation and **stored at 38-40°F (refrigeration temperatures).**

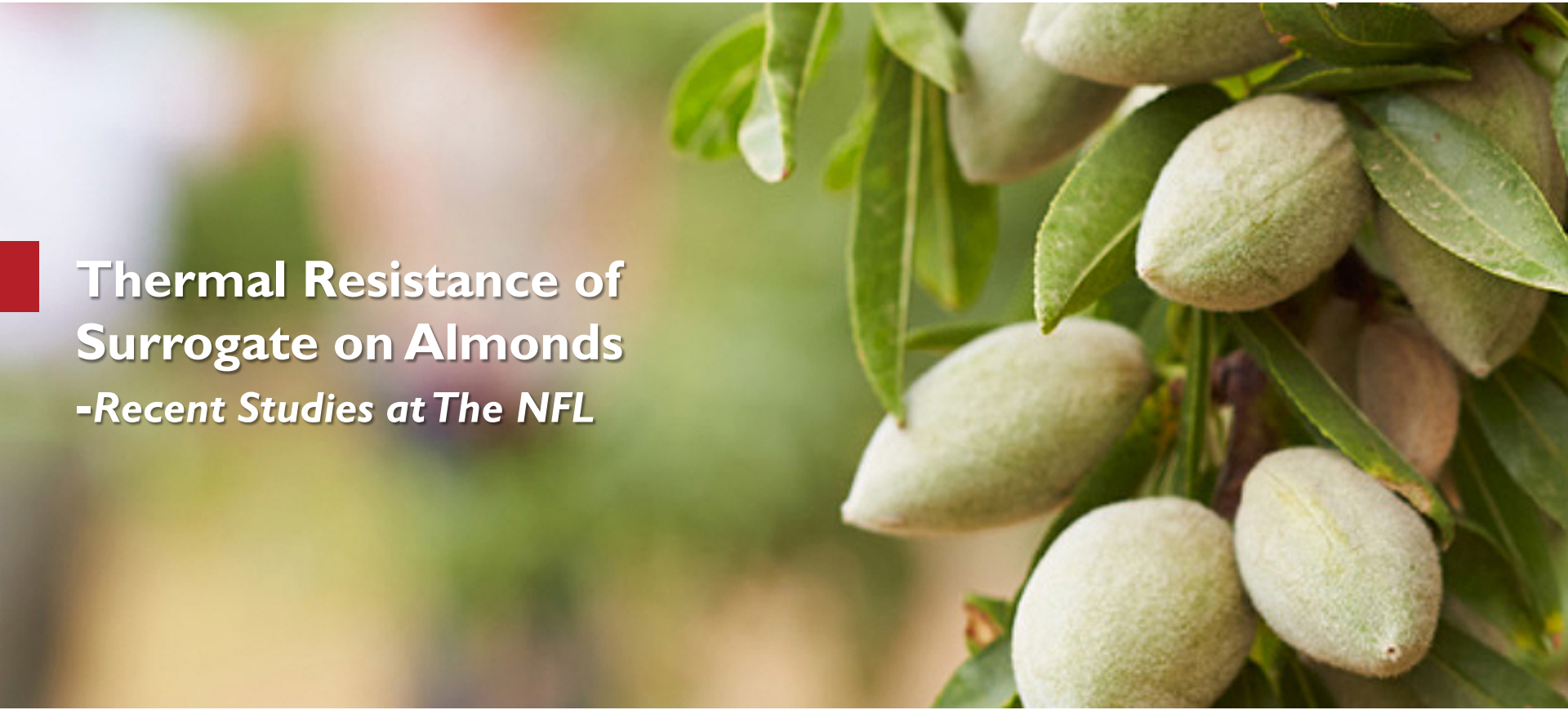
- ✓ Can inoculated almonds be stored beyond 14 days without sacrificing heat resistance?
- ✓ Do inoculated almonds need to be stored at refrigeration temperatures?

The heat resistance of *E. faecium* NRRL B-2354 and its correlation to *S.* Enteritidis PT30 was completed using **whole intact almonds.**

- ✓ Can whole kernel inoculated almonds be used in plant validation studies for other almond types?

Thermal Resistance of Surrogate on Almonds

-Recent Studies at The NFL



california almonds
Almond Board of California

Study Design Overview

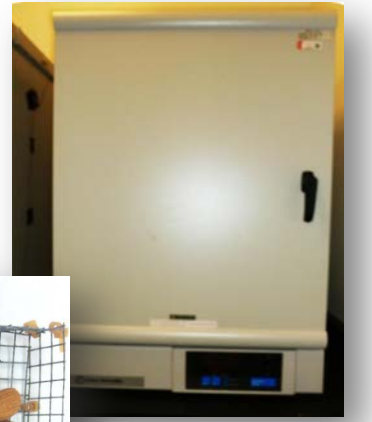
S. Enteritidis PT30
E. faecium NRRL B-2354

Refrigeration
Room temperature

Forced Air Oven

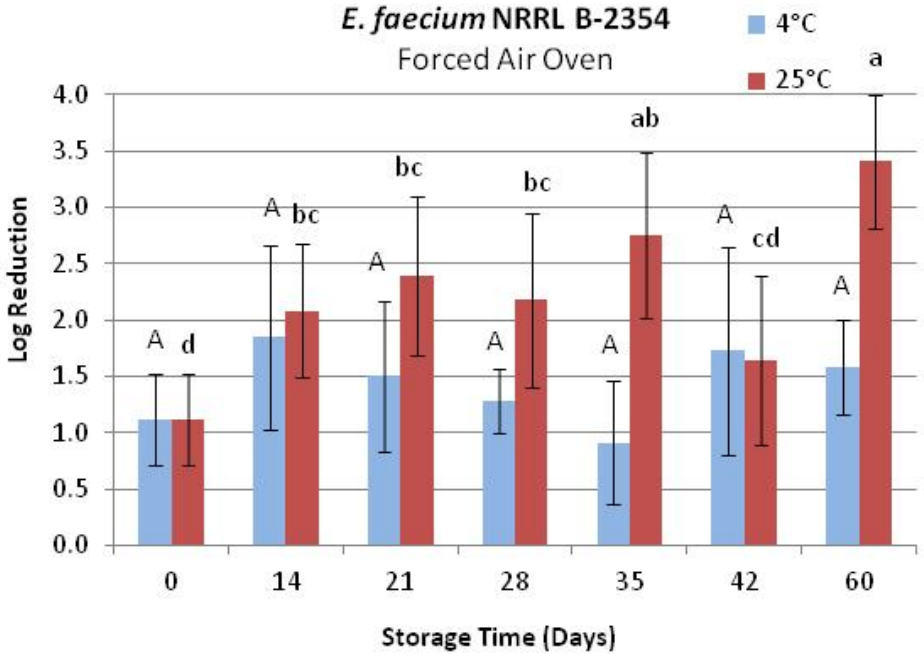
Up to 60 days

280°F for 15 minutes



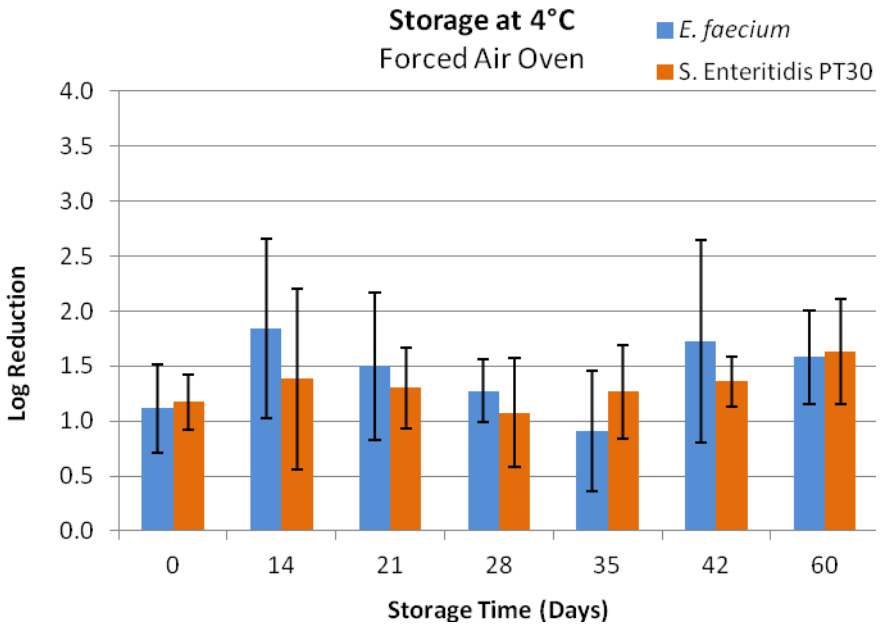
Calculate the log reduction to evaluate the thermal resistance of organisms on almonds

Extended Storage of Surrogate-Inoculated Almonds



The current shelf life of almonds inoculated with *E. faecium* NRRL B-2354 can be extended from 14 to 60 days when stored at 4°C.

Extended Storage of Surrogate-Inoculated Almonds



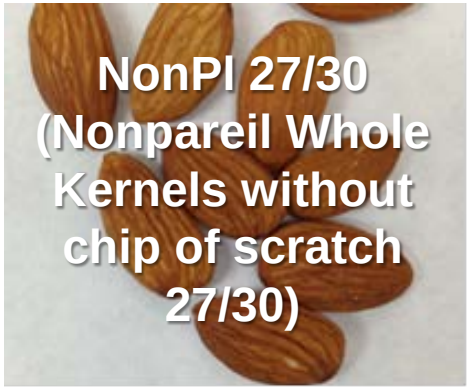
E. faecium NRRL B-2354 is an appropriate surrogate for *S.* Enteritidis PT30 when the inoculated almonds are stored at 4°C.

Extended Storage of Surrogate-Inoculated Almonds

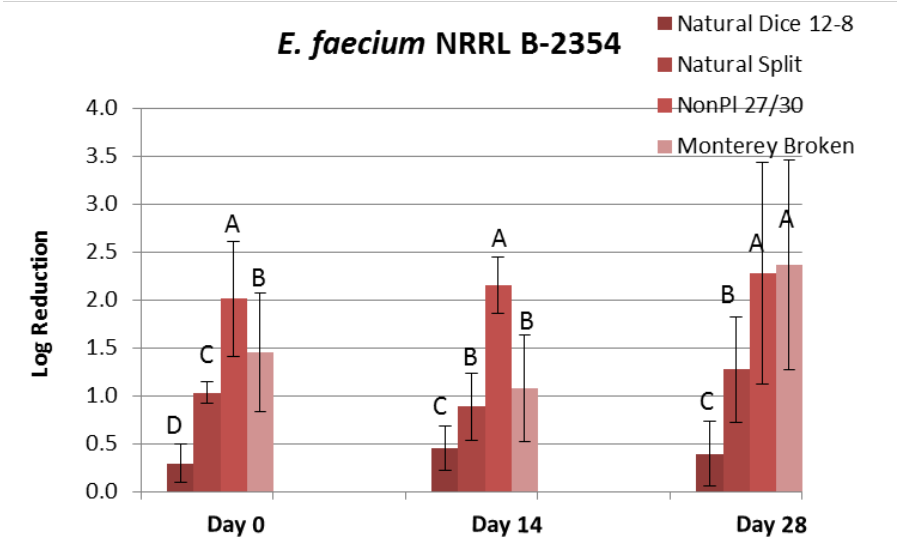
The almonds inoculated with the surrogate should be used within **two weeks** of preparation and **stored at 38-40°F (refrigeration temperatures).**

- ✓ Can inoculated almonds be stored beyond 14 days without sacrificing heat resistance? **YES**
- ✓ Do inoculated almonds need to be stored at refrigeration temperatures? **YES**

Thermal Resistance of Surrogate on Different Almond Types

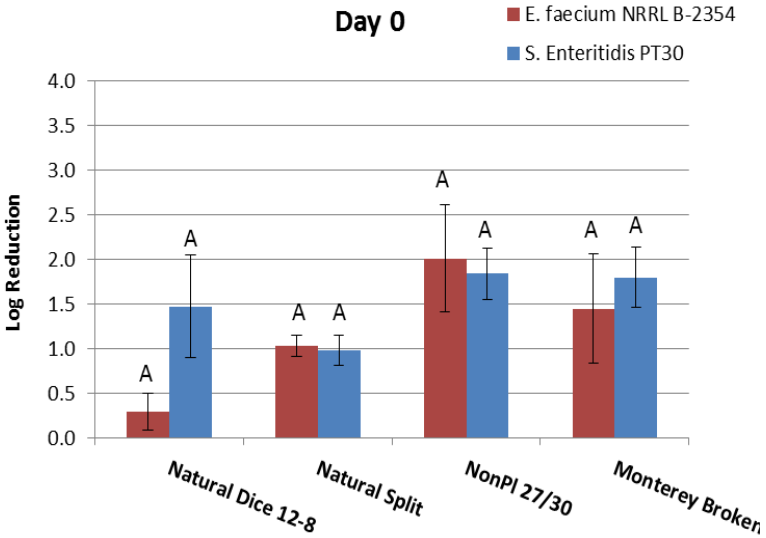


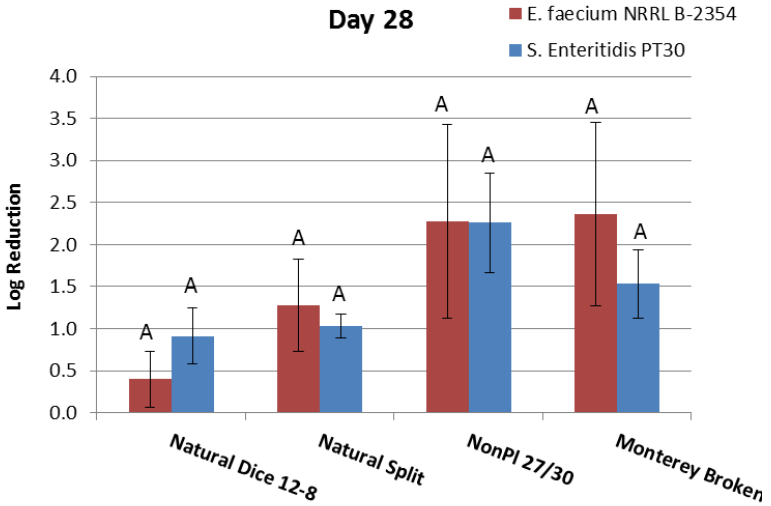
Thermal Resistance of Surrogate on Different Almond Types



Almond type has an impact on the heat resistance of *E. faecium* NRRL B-2354

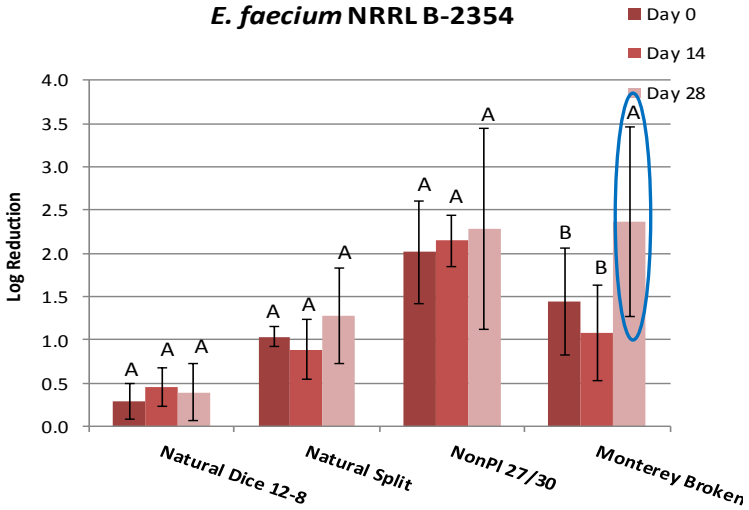
Thermal Resistance of Surrogate on Different Almond Types





Heat resistance of *S.* Enteritidis PT30 is comparable to *E. faecium* NRRL B-2354 on all four almond types.

Thermal Resistance of Surrogate on Different Almond Types



The current shelf life of almonds inoculated with the surrogate can be extended beyond 14 days at refrigeration temperature.

Thermal Resistance of Surrogate on Different Almond Types

The heat resistance of *E. faecium* NRRL B-2354 and its correlation to *S.* Enteritidis PT30 was completed using **whole intact almonds.**

- ✓ Can whole kernel inoculated almonds be used in plant validation studies for other almond types? **NO**

Conclusions

- Shelf life of almonds (whole kernel) inoculated with *E. faecium* NRRL B-2354 can be extended from 14 to 60 days when stored at 4°C.
- Almonds inoculated with *E. faecium* NRRL B-2354 should not be stored at 25°C, or heat resistance will change.
- Shelf life of the Natural Dice 12-8 and Natural Split almonds inoculated with *E. faecium* NRRL B-2354 can be extended to 28 days at 4°C.
- Heat resistance of *E. faecium* NRRL B-2354 or *S.* Enteritidis PT30 is significantly different depending on almond type.

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Questions?

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