Raw Milk Quality Tests – Do They Predict Fluid Milk Shelf-life

or

Is it time for new tests?

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Fluid milk shelf life

- What defines shelf life
  - Microbiological criteria
    - Time until regulatory limit is reached: 20,000 CFU/ml
  - Sensory quality/consumer acceptance ("the ultimate shelf life test")
- What affects shelf life
  - Raw milk quality (presence of bacteria, enzymes, and off flavors that are not eliminated by pasteurization)
  - Post-pasteurization microbial contamination and chemical degradation
  - Post-pasteurization handling (e.g., temperature, kight exposure)

Microbial quality of raw milk

- Sources of bacteria in raw milk include:
  - Natural flora of healthy udder
  - Flora of mastitic cows
  - Exterior of cow
  - Dairy barn environment, air, water
  - Equipment milk contact surfaces
- Bacterial growth in raw milk influence by:
  - Milk residue on equipment
  - Prolonged milking time
  - Milk storage time/temperature

Raw Milk Quality
Important Types of Bacteria in Raw Milk

- Cause Spoilage
  - fermentative/acid producers (LAB - lactic acid bacteria; coliforms)
  - proteolytic, lypolytic, etc, (breakdown proteins, fats, etc.)
  - gas producers (coliform bacteria; some LAB)
- Grow under refrigeration
  - psychrotolerant (e.g., Pseudomonas)
- Survive pasteurization
  - thermoduric or thermo-tolerant
  - includes spore-formers, some psychrotolerant species and strains
- Cause mastitis infections in cows

  - Staphylococcus, Streptococcus, coliforms, others

Raw Milk Quality

- Traditional raw milk quality tests include:
  - Standard Plate Count (SPC)
  - Psychrotrophic Bacteria Count (PBC)
  - Coliform Count (CC)
  - Laboratory Pasteurization Count (LPC)
  - Preliminary Incubation Count (PI)
  - Somatic Cell Count (SCC)

Microbiological Tests Used as Indicators of Pasteurized Milk Shelf-Life

- Raw milk tests:
  - PI count
    - 13ºC/18 h pre-incubation
  - Lab pasteurization count
    - 62.8ºC/30 min pasteurization
  - HR3 test
- Pasteurized milk tests:
  - Moseley keeping quality
    - SPC obtained for fresh samples and samples stored at 7ºC for 5 to 7 days
  - HR1, HR2 and HR3 test
  - PI count
    - 13ºC/18 h incubation
  - MicroFoss

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Results from raw milk microbiological tests do not predict the shelf-life performance of commercially pasteurized fluid milk

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Study Goals

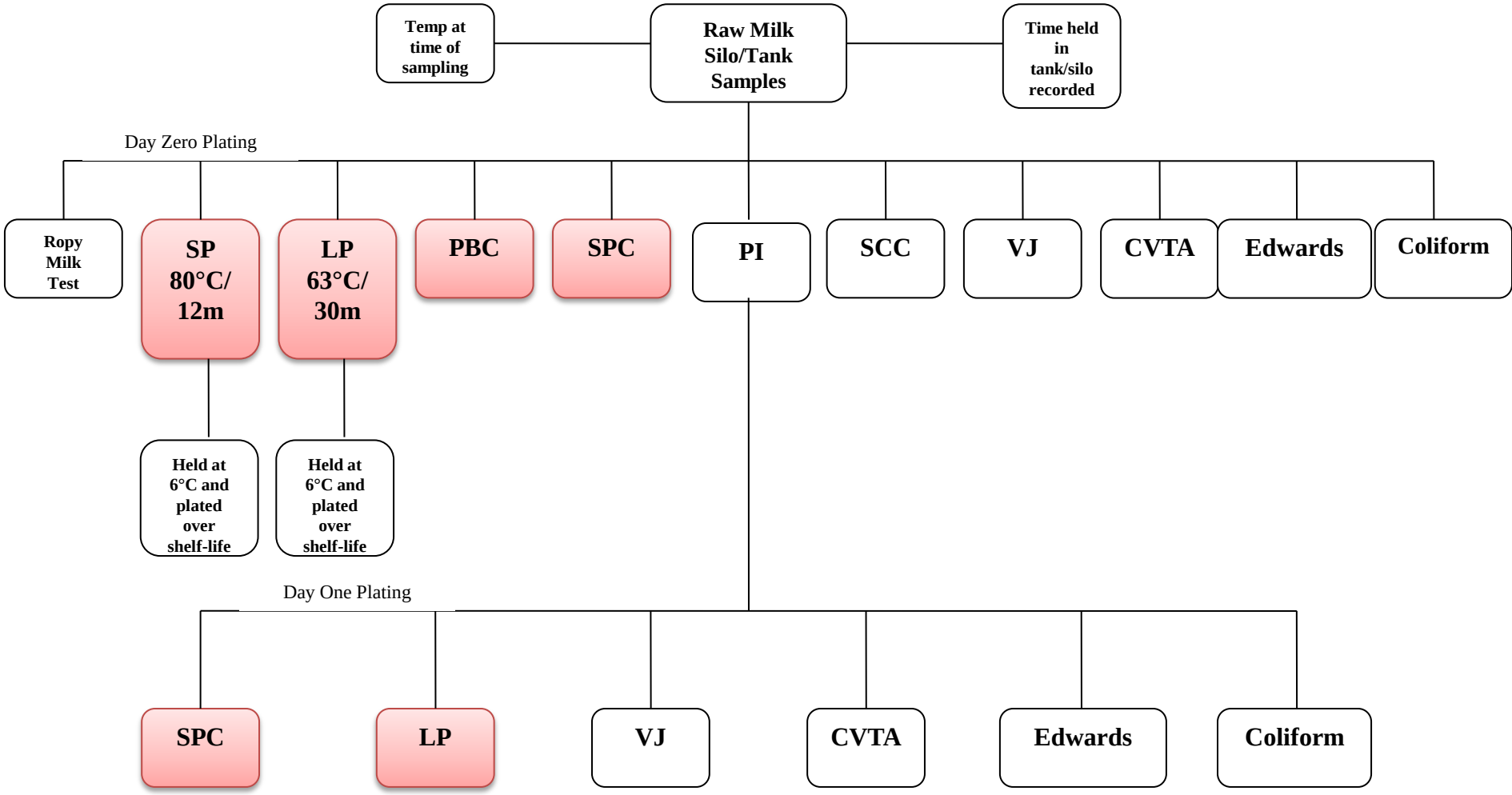
- Measure statistical correlations between raw milk microbiological tests and pasteurized milk shelf life as defined by both sensory evaluation and microbiological evaluation

Study Design

- Raw milk and corresponding 2% pasteurized milk samples were collected once a month for 12 months from four NYS processors (with the exception of plant C, which closed after month 7)

Study Design

- Raw milk was evaluated using the following tests:
  - Prior to PI incubation:
    - Somatic Cell Count (SCC),
    - Psychrotrophic Bacteria Count (PBC)
    - Ropy Milk Test
    - Spore Pasteurization (SP) (80ºC/12min)
  - Both before and after PI incubation:
    - SPC,
    - Coliform
    - Laboratory Pasteurization (LP 63ºC/30min)
    - Vogel-Johnson medium (Staphylococci)
    - Edwards medium (Streptococci)
    - Crystal Violet Tetrazolium agar (CVTA) (Gram Negatives)
- Bacterial isolates were collected from SP, PBC, LP (from both before and after PI incubation), and SPC (before and after incubation)



Red boxes indicate samples from which isolates were collected

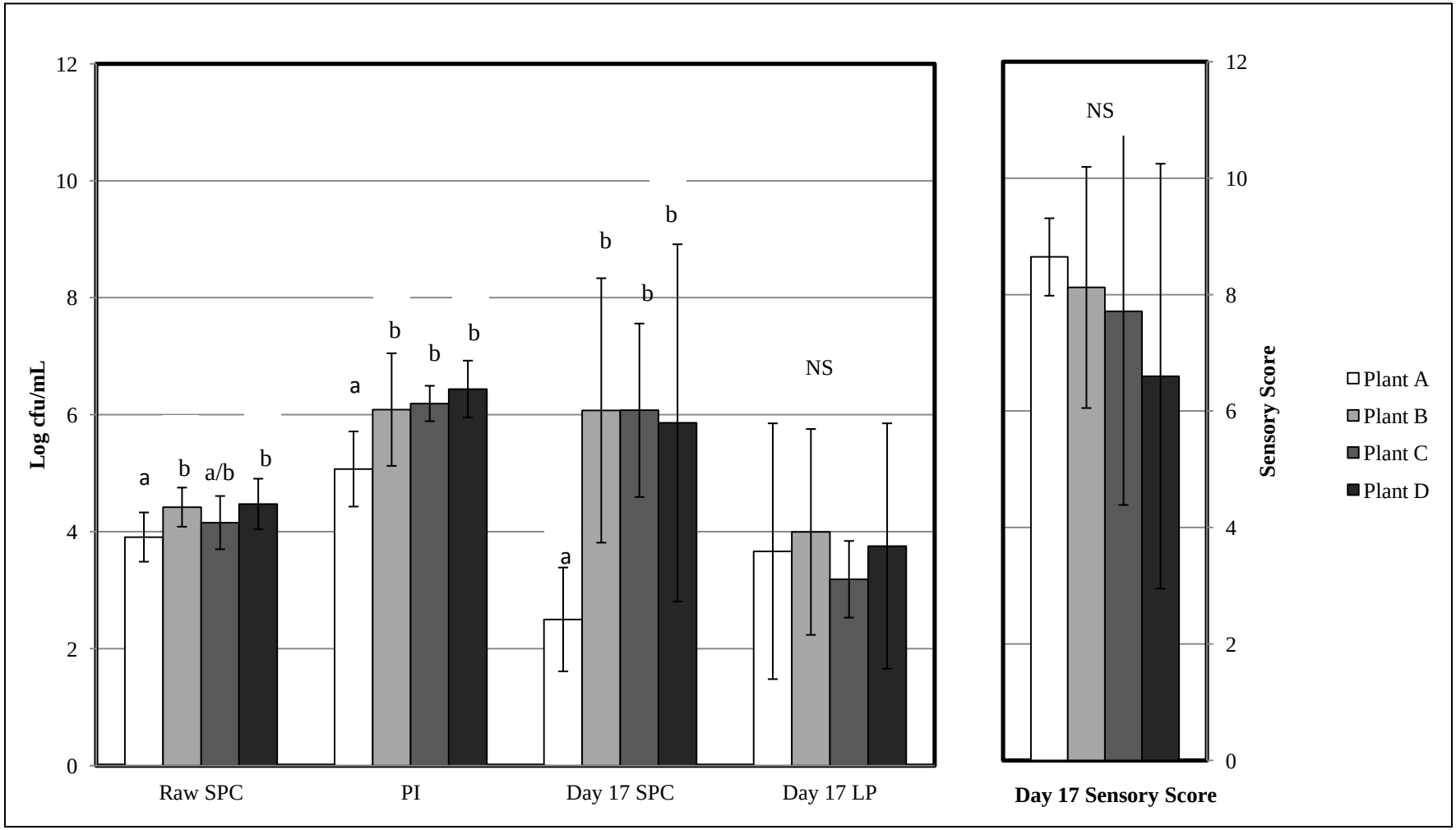
Raw Milk Study Design

- Corresponding 2% pasteurized milk from processors were evaluated as follows:
  - SPC and coliform testing performed on days initial, 7, 10, 14, 17 and 21 post-pasteurization and storage at 6C
  - Sensory evaluation performed on days initial, 10, 14 and 17 post-pasteurization
  - Isolates collected either when the milk sample reached the PMO limit for pasteurized milk (20,000 cfu/mL) or on the last day of the study (21 d post-pasteurization)
    - Data were used to identify post-pasteurization contamination (PPC)

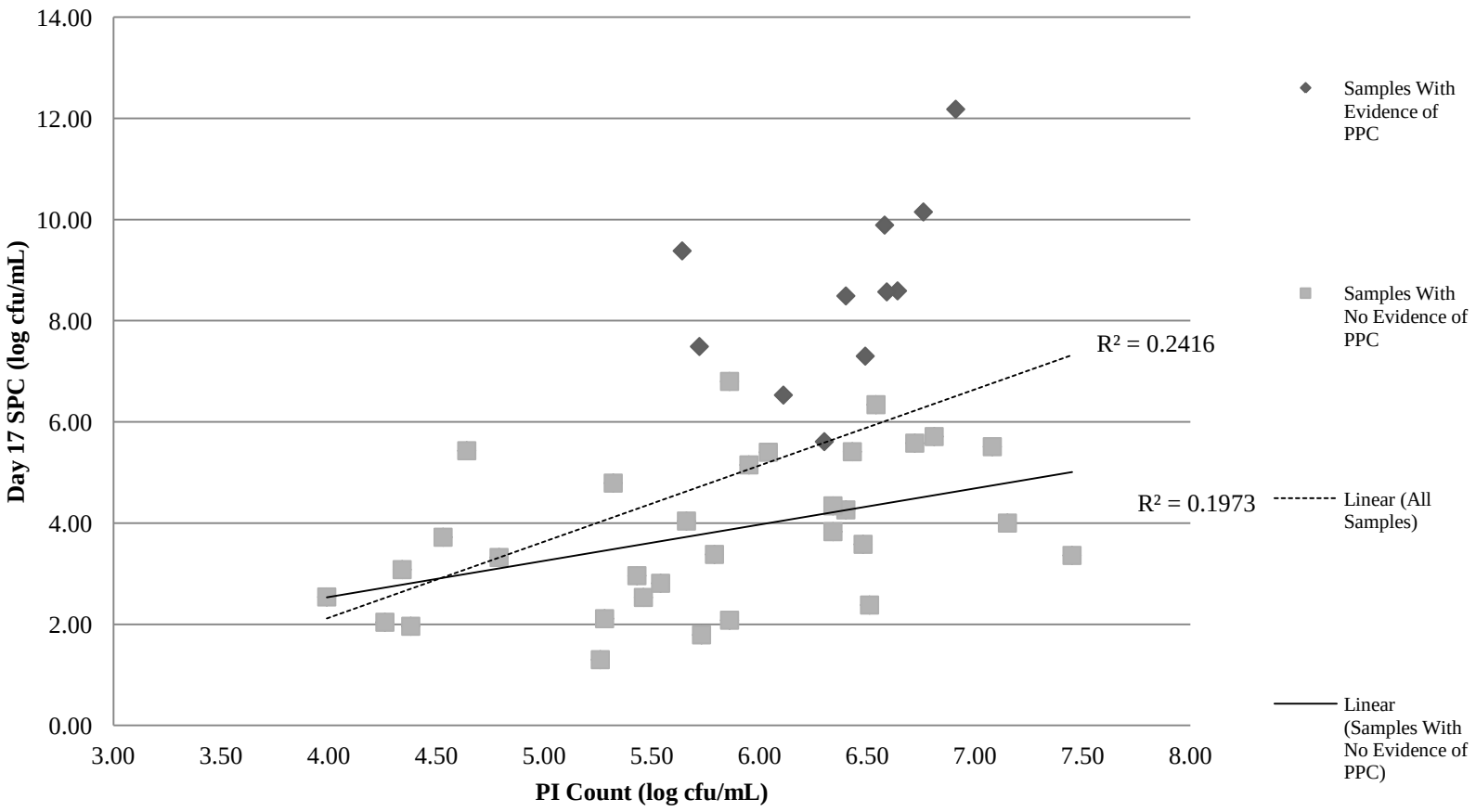
Raw Milk Study Plant Processing Parameters

	Processing Parameters	
Plant	**Temperature (°F)**	**Time (sec)**
A	170	25
B	176.5	33
C	176	30
D	171.7	30

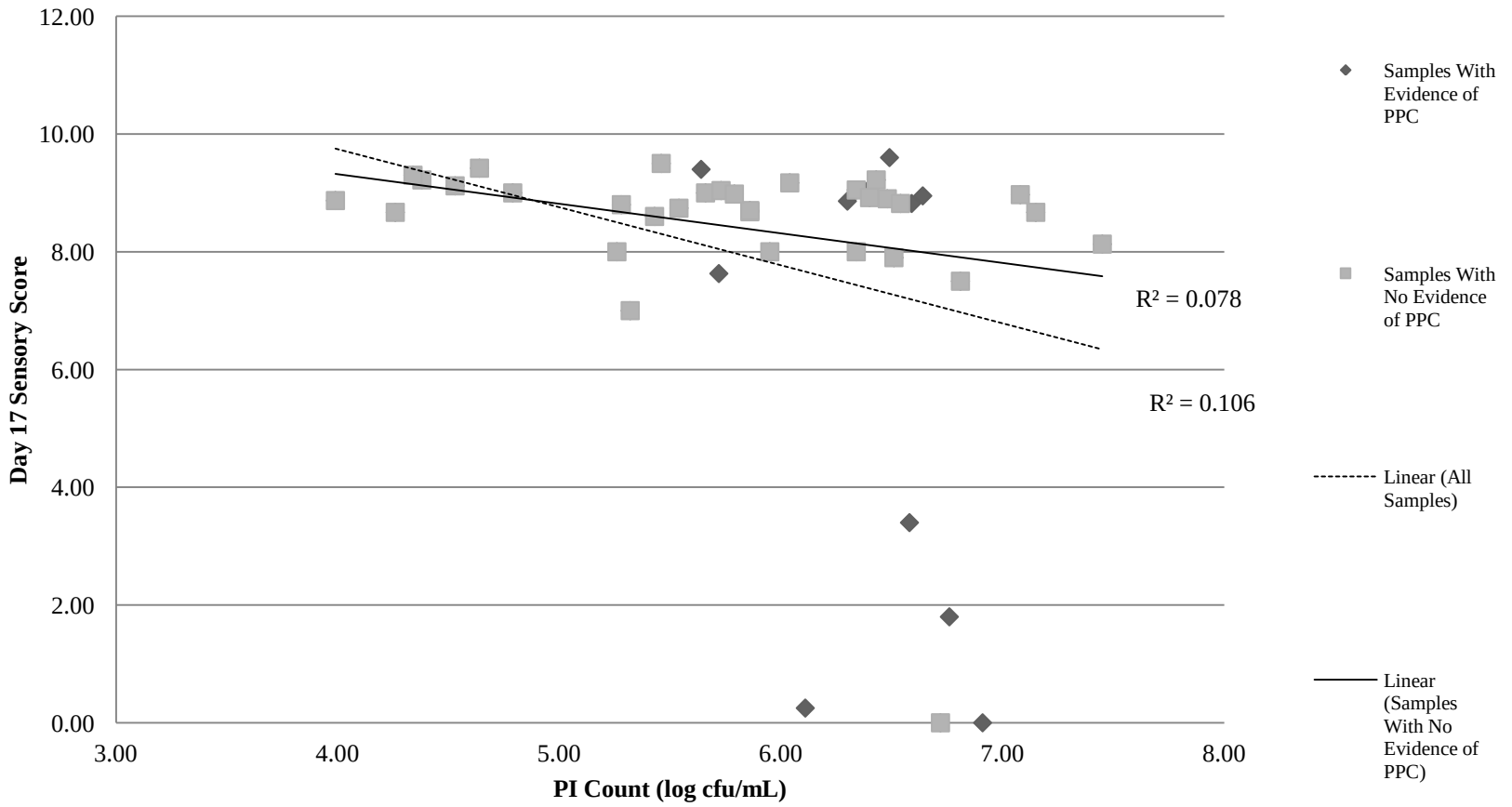
Results–average quality parameters by plant



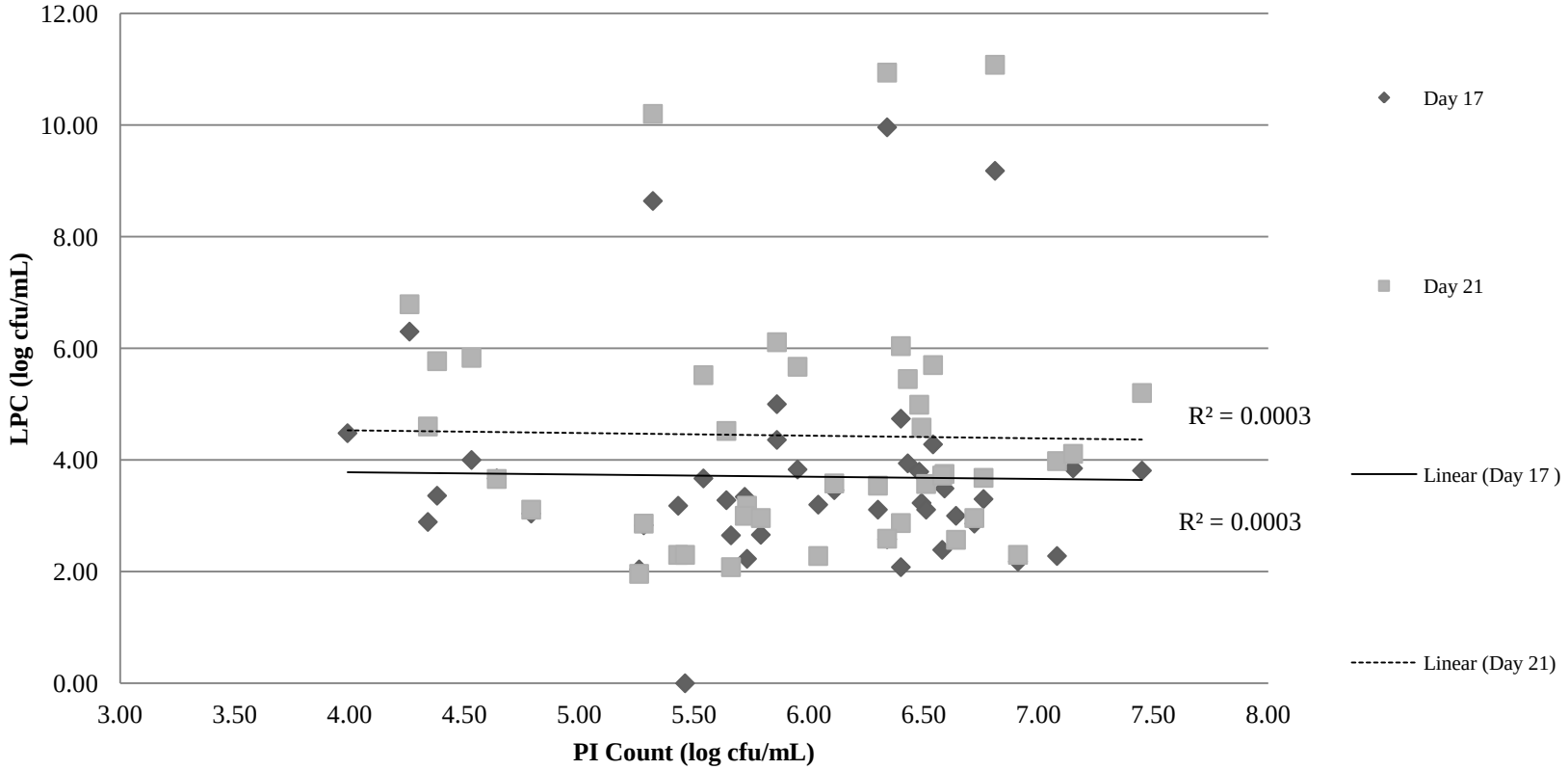
Results – correlation between day 17 SPC and PI count



Results – correlation between day 17 sensory score and PI count



Results – correlation between day 17 and day 21 LPC and PI count



Study Results – Summary of all tests

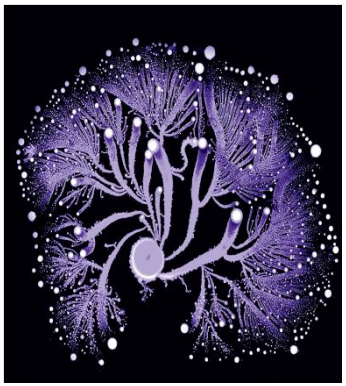
Raw milk tests	R^2 Values							
	D17 SPC	D17 SPC (w/o PPC)	D21 SPC	D21 SPC (w/o PPC)	D17 Sensory Score	D17 Sensory Score (w/o PPC)	D17 LP	D21 LP
SCC	0.0221	0.0005	0.0235	0.0016	0.1132	**0.1571***	0.0014	0.0092
SP	0.0031	0.0001	0.0000	0.0058	0.0301	0.0223	0.0002	0.0140
Edwards	0.0092	0.2637	0.0002	0.2186	0.0283	0.0212	0.0109	0.0180
VJ	0.0011	0.0950	0.0003	0.1274	0.0145	0.0229	0.0569	0.0266
LP	0.0299	0.1009	0.0365	0.1122	0.0004	0.0060	0.0882	0.0509
Raw SPC	0.0544	0.2095	0.0463	0.1877	0.0000	0.0000	0.0896	0.0523
PBC	0.1280	0.2173	0.0876	0.1228	0.0097	0.0038	0.0601	**0.0798***
CVTA	0.1238	**0.4169***	0.1125	**0.3681***	0.0003	0.0092	**0.0907***	0.0678
Coliform	0.1301	0.2641	0.1471	0.2413	0.0565	0.0461	0.0399	0.0064
PI	**0.2416***	0.1973	**0.2211***	0.1874	0.1060	0.0725	0.0003	0.0003
ΔPI	0.1807	0.1524	0.1554	0.1291	**0.1314***	0.0628	0.0436	0.0270

Study Conclusions

- None of the tests commonly used for raw milk screening have the ability to predict the bacterial or sensory quality of pasteurized milk
- Processing plant factors, such as post-pasteurization contamination and processing parameters (e.g., temperature) play a large role in pasteurized fluid milk quality
- New approaches are needed
  - Cold-growing spore-forming organisms, such as *Paenibacillus*, that are known to limit the shelf life of pasteurized milk are a logical target to pursue

Paenibacillus spp.

- Gram-positive, aerobic or facultatively anaerobic, rod-shaped sporeforming bacteria
- *Paenibacillus* only recently recognized as distinct genera from Bacillus (*Paeni* translates to 'almost')
- Spores can survive multiple stresses including: broad ranges of pH, temperature and water activities
- Commonly isolated by heat treatment (80°C for 12 min) to destroy non-spore forming microbes and stimulate spore germination
- Found in soil, rhizosphere, and insect larvae
- Used in a broad range of industrial applications for:
  - Production of extracellular degrading enzymes
  - Antimicrobial and antifungals
  - Biofertilizers and biopesticides of root pathogens
- Genome of *P. vortex* sequenced to better understand social behavior



Paenibacillus vortex

Sirota-Madi et al., 2010

Diversity of Microbes in Milk

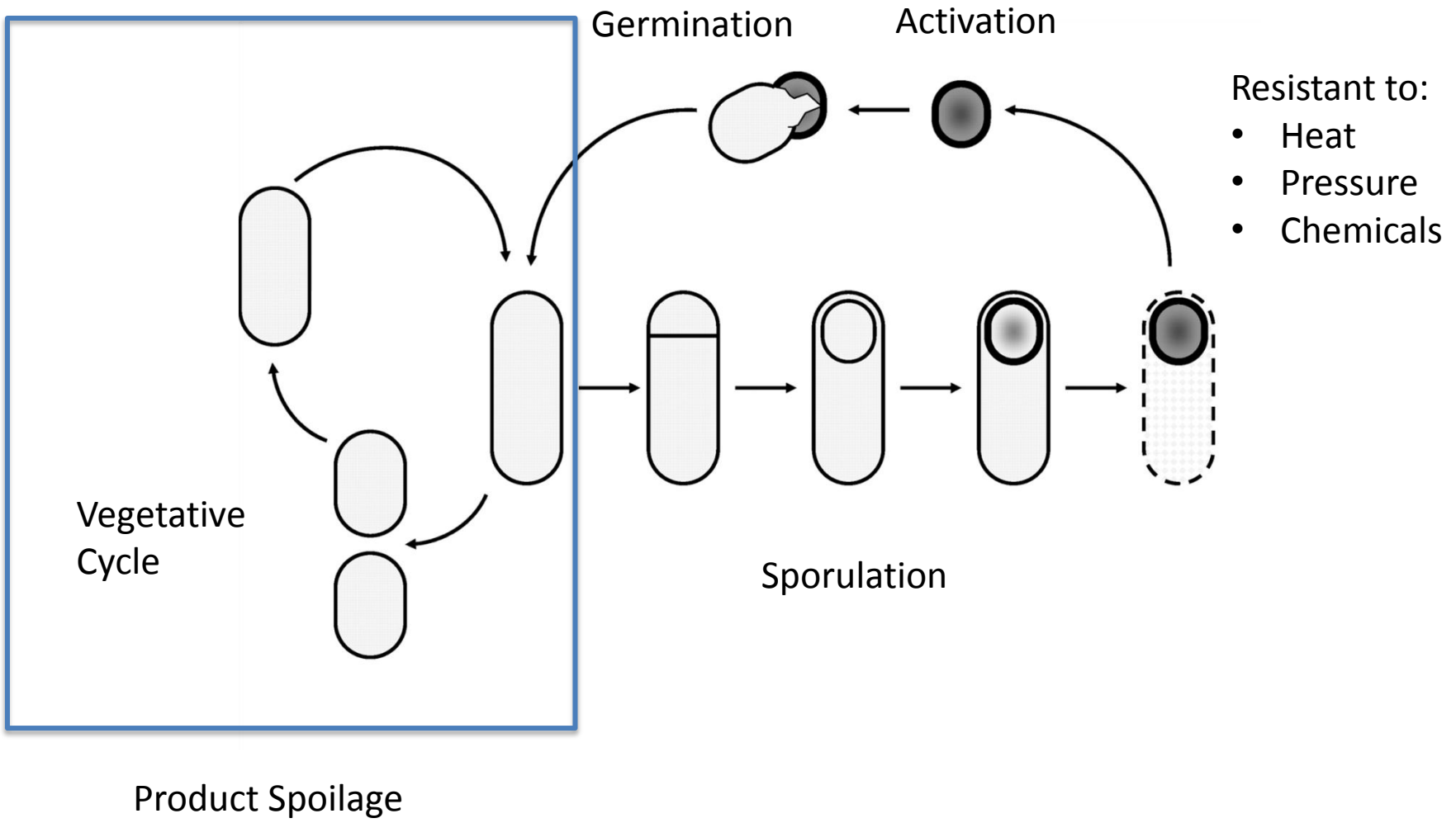
Table 4. Summary of bacterial isolates collected from commercial fluid milk samples throughout shelf life

Bacterial isolate	initial day	day 7	day 14	day 17	Total
Paenibacillus	**1**	**11**	**33**	**30**	**75**
amylolyticus	0	3	6	5	14
spp	1	8	27	25	61
Bacillus	**26**	**15**	**14**	**8**	**63**
cereus	6	7	4	1	18
licheniformis	11	5	0	0	16
mycoides	1	0	3	4	8
stearothermophilus	3	1	0	0	4
pumilus	3	0	0	0	3
lentus	1	1	0	0	2
circulans	0	0	2	0	2
subtilis	1	0	0	0	1
spp	0	1	5	3	9
Microbacterium lacticum	**16**	**10**	**1**	**0**	**27**
Micrococcus varians	**4**	**5**	**0**	**0**	**9**
Gram-negatives	**1**	**1**	**0**	**6**	**8**
Unidentifiable	**1**	**2**	**1**	**3**	**7**
TOTAL	**49**	**44**	**49**	**47**	**189**

Bacillus and *Paenibacillus* spp. predominate in fluid milk when post-pasteurization contamination is controlled

Fromm and Boor, 2004

Sporeformer life-cycle



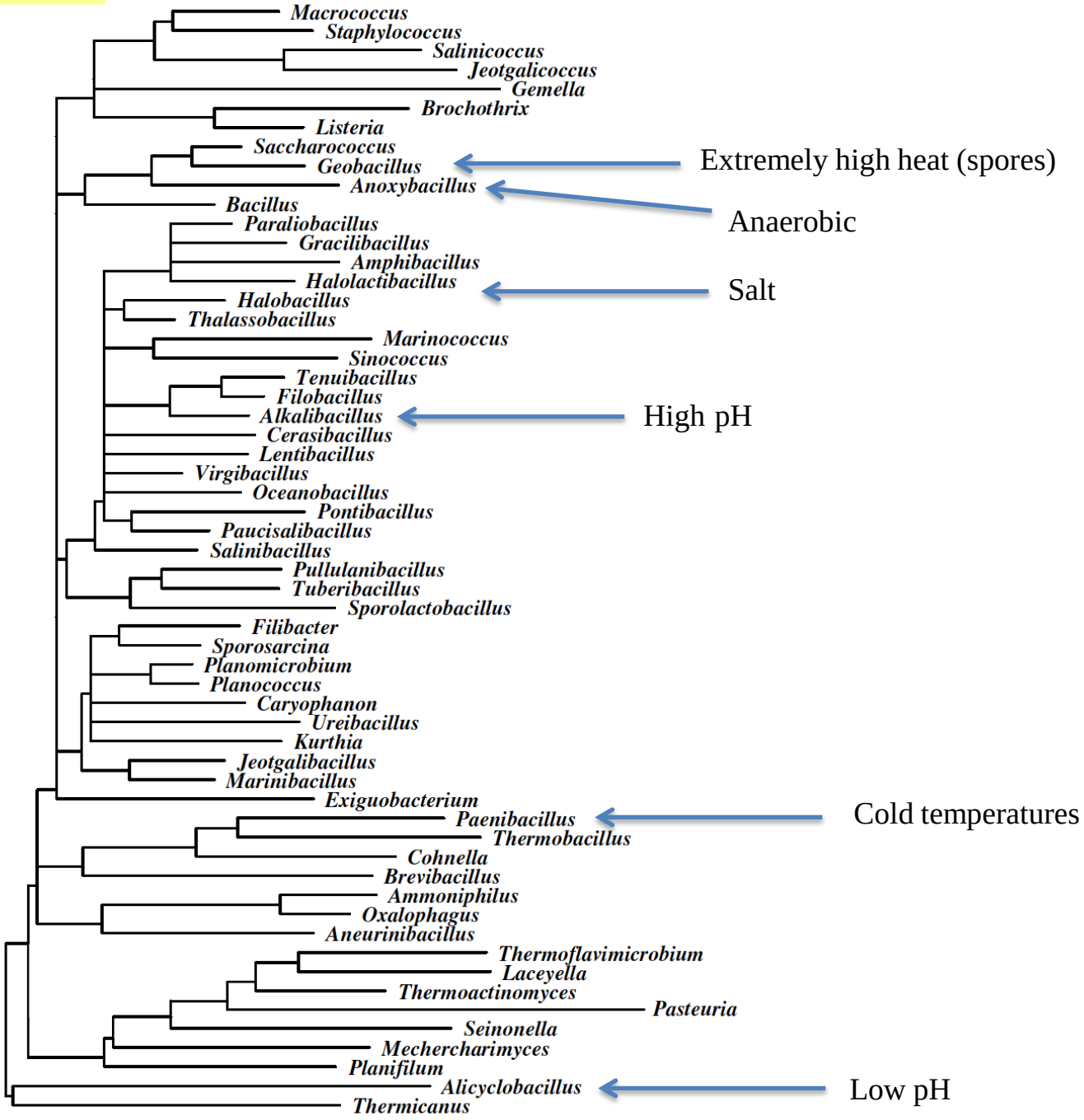
Adapted from Errington, 2010

Figure 2.

Class “Bacilli”

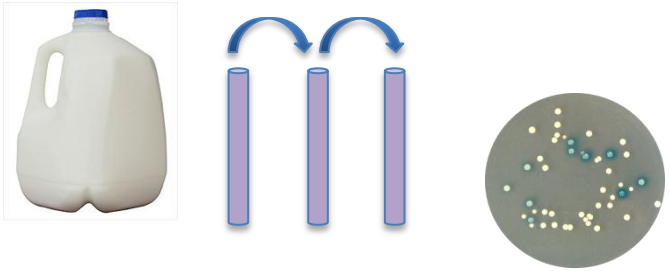
Many endospore forming bacteria

Genetic diversity allows survival in wide range of environments



Ludwig et al., 2009

DNA Sequence Based Characterization

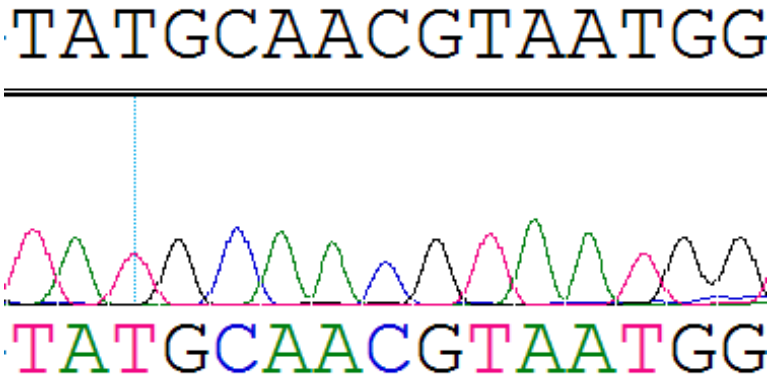


1. Plate sample

2. Representative colony morphologies picked from plate

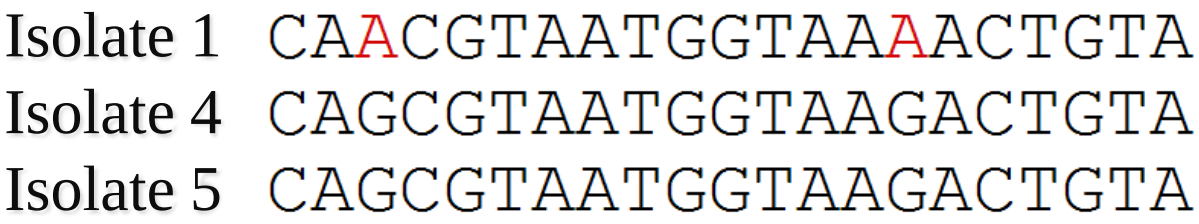
3. Prepare lysate

4. Genetic target amplified with polymerase chain reaction (PCR), then sequenced



DNA Sequence Based Subtyping

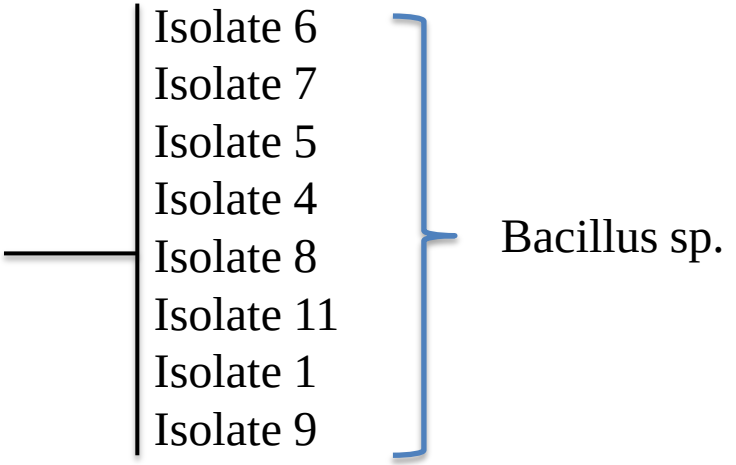
5. Sequences are aligned and compared



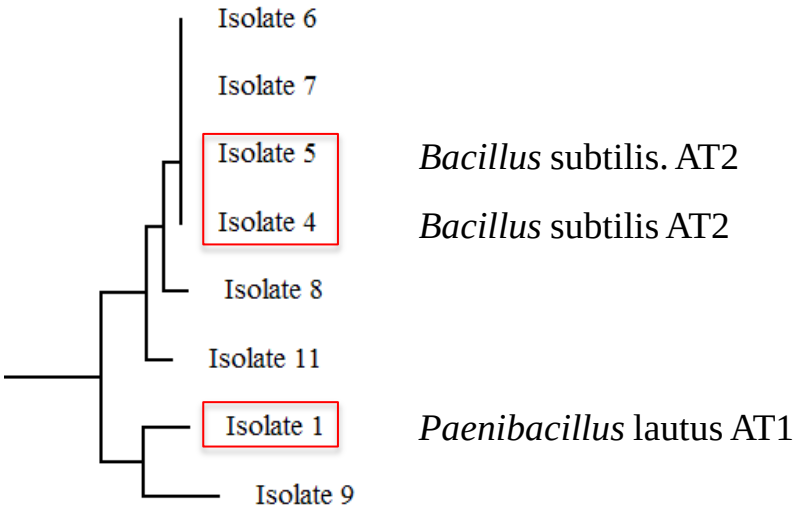
- Traditional sequence based identification targets 16S rDNA
- Sequences can be compared to databases to identify sporeforming or other bacteria
- Sequence based identification is more reliable and faster than traditional phenotypic (biochemical) characterization
- Results for a single culture generally reported by percent similarity to top matches:
  - Example: 99.79% *Bacillus vallismortis*, 99.71% *Bacillus atrophaeus*, 99.48% *Bacillus amylolquefaciens*

DNA Sequence Based Subtyping

- Comparison of sequences can allow for discrimination between spoilage organisms
- Level of discrimination dependent on nucleotide differences
  - 16S rDNA is good for genus identification, but may not discriminate between *Bacillus* species
  - *rpoB* provides appropriate number of polymorphisms (unique bases for a single nucleotide site) for discrimination beyond genus level



16S Sequence Comparison

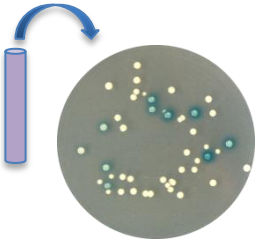


rpoB Sequence Comparison

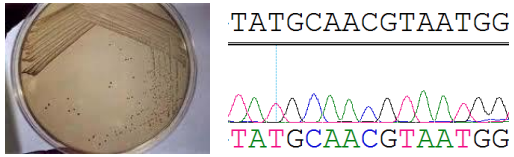
Paenibacillus Assay – Raw Milk Screening Tool

Heat-Treat Raw Milk (80° C for 12 min)

Enrichment at 6° C (17-21 days)



Sample Enumeration (1 day)

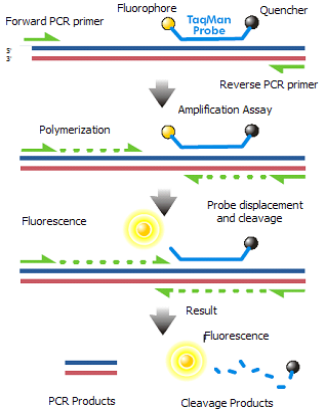


Colony Selection and Sequence Based Identification (3 days)

Heat-Treat Raw Milk (80° C for 12 min)

Enrichment at 13° C (2 days)

Sample Enumeration and Colony Selection (1 day)



Paenibacillus Quantitative PCR (4 hours)

Overview of Quantitative *Paenibacillus* Assay

1. Raw Milk Sample Collection

2. Raw milk spore-shock: 80°C for 12 minutes to kill vegetative cells and induce spore germination

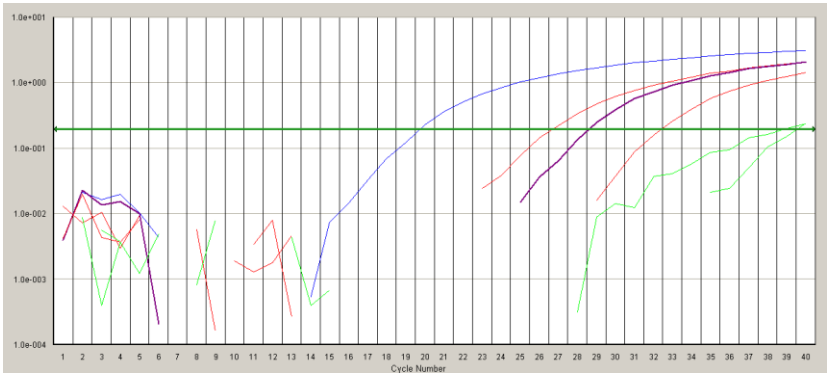
3. Enrichment (incubation) at 13°C for 48 hours

4. Bacterial DNA extraction from 1mL milk sample

5. TaqMan PCR

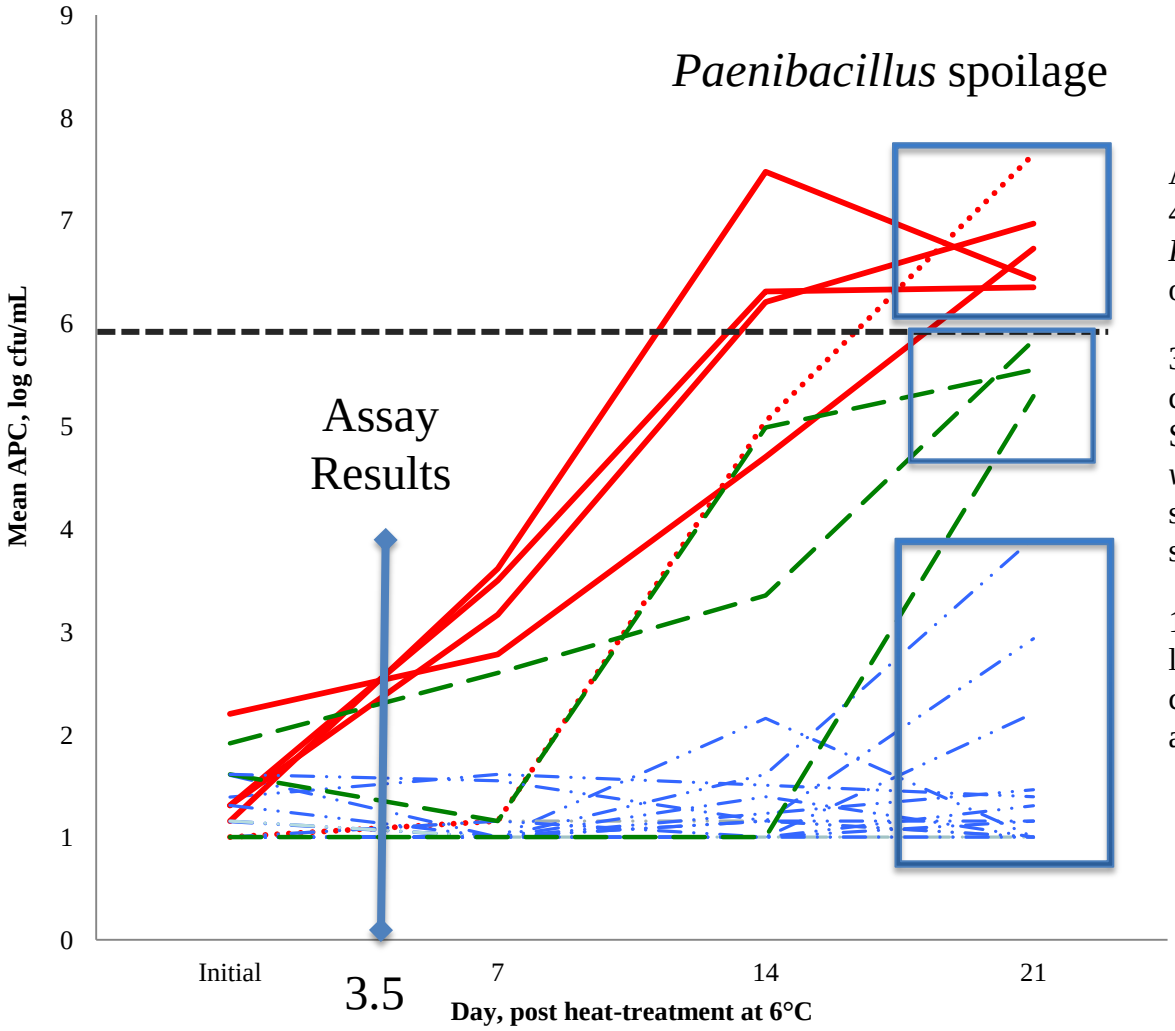
2uL sample added to each well with PCR reagents

96 well-plate



Visual output of Pb target amplification

Evaluation of raw milk quality



Assay predicts spoilage in 4/5 samples with *Paenibacillus* growth to over 1 x 10^6 cfu/mL

3 samples approached 1 x 10^6 cfu/mL but were not detected Spoilage determined to be *B. weihenstephanensis* (2 samples) and *Paenibacillus* (1 sample)

16 of 16 raw milk samples with low bacterial growth (< 20,000 cfu/mL) were not detected with assay (no false positives)

Conclusions

- Spores are an important hurdle to the extension of shelf-life and quality of dairy products
  - Only some sporeformers can grow at refrigeration temperatures
- With a high quality raw milk supply traditional raw milk tests have limited value
- There is a need for new tests in the dairy industry for both raw milk and shelf life prediction of finished products