In-Container Spoilages: Trouble Shooting Starts with Microbiological Analysis

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Presentation Outline

- When would one see in-container spoilage?
- Objectives of in-container spoilage analysis
- Why is one reluctant to conduct spoilage analysis?
- Different levels of microbial spoilage analysis
- Ideal outcome of in-container spoilage analysis
- Examples of in-container spoilage analysis

When Would One See In-Container Spoilage?

- Routinely incubated samples of production
- Taptone rejects – swollen, leakers, etc.
- R&D new product qualification trials
- Retain samples
- Samples retrieved from consumer complaints
- Samples from recalls
- False alarm – e.g., lab error in micro plating
- others

Objectives of In-container Spoilage Analysis

Spoilage microbial analysis can serve many purposes

Objectives of In-container Spoilage Analysis

Objective 1: Pure Spoilage vs Food safety

- Is it caused by non-pathogenic or pathogenic organisms?

- Can one assume that “spoilage organisms” are not pathogenic?
  - Hard to determine - without characterization /identification of causative agents

- Slim Fast recall: *Bacillus cereus* was the “spoilage organism”

Objectives of In-container Spoilage Analysis

Objective 2: Sporadic vs Systemic

- Is it a **sporadic** (isolated) or **systemic** (chronic) issue?

Sporadic

- Single isolated case
- No assignable causes
- Non-repetitive
- Still need investigation to determine it is truly “sporadic”

Systemic:

- Repeated failures – multiple occurrences in one or multiple runs
- Cluster of failures
- Same organisms in multiple spoilages
- Same type of organisms in the failures – e.g., sporeformers
- Require trouble-shooting & corrective actions

Objectives of In-container Spoilage Analysis

Systemic:

- If a systemic issue exists and has manifested itself as spoiled containers, knowing the nature of the spoilage organism will help greatly on trouble-shooting

Objectives of In-container Spoilage Analysis

What would FDA do if they get hold of spoiled containers?

FDA Bacteriological Analytical Manual (BAM): Examination of Canned Foods

- **Medium**: Support both aerobic & anaerobic microorganisms
  - Ex: Cooked meat medium (CMM)
- **Incubation temperature**: 35°C vs 55°C
- **Aerobic** vs **anaerobic incubation**

Objectives of In-container Spoilage Analysis

What would FDA do if they get hold of spoiled containers?

FDA Bacteriological Analytical Manual (BAM): Examination of Canned Foods

- "**….** If rods are included among mixed microflora in CMM, **test CMM for toxin** as described in Chapter 17."

- "If Gram-positive or Gram-variable rods typical of either *Bacillus* or *Clostridium* organisms are found in the absence of other morphological types…. **Test culture for toxin** according to Chapter 17."

Objectives of In-container Spoilage Analysis

What would FDA do if they get hold of spoiled containers?

FDA Bacteriological Analytical Manual (BAM): Examination of Canned Foods

Characterize the spoilage organisms - serving for at least two purposes:

- Is the spoilage micro pathogenic or non-pathogenic?
- Would characterization of spoilage micro reveal anything about the process?

- **FDA has very sophisticated tools to isolate and identify the organisms to species and strain level**.

Do you want to know what

FDA would like to know?

Objectives of In-container Spoilage Analysis

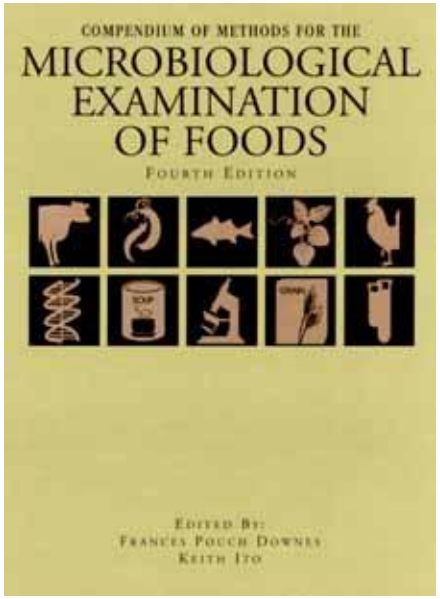
Why microbiologically analyze the spoiled packs?

Compendium of Methods for the Microbiological Examination of Foods (CMMEF)

Chapter 61 – Canned Foods – Tests for Commercial Sterility

Chapter 62 – Canned Foods – Tests for Cause of Spoilage

- Characterize organisms to determine if they are pathogenic or not.
- Trouble shooting for systemic issues



Why Analyze In-Container Spoilages?

Part of “Due Diligence”

- Chance for a plant to re-examine & improve its Pre-Requisite & food safety programs, as well as manufacturing line capability
- Not knowing the nature of spoilage organisms, the plant may miss a “correction /improvement” opportunity

“Rome wasn’t built in one day, but they were laying bricks every hour.”

Why Analyze In-Container Spoilages?

Part of "Due Diligence"

Prerequisite Programs:
Current Perspectives in Food Manufacturing
S. E. Mortimore, B. R. Warren, *Perspectives in Public Health 2014, 134 (4): 191-3*

- "Recalls associated with pathogens such as *Listeria* or *Salmonella* are more often driven by failure of PRPs (e.g. post-process recontamination and/ or unsanitary production environments) rather than failure of critical control points within a HACCP plan."

Why Analyze In-Container Spoilages?

Part of “Due Diligence”

What is below “Tip of The Iceberg”?

- A systemic spoilage incident – rarely results from a single cause
  - One almost always finds multiple potential causes during trouble-shooting
- Recall often occurs when plant repetitively ignores “early warnings”

Why Analyze In-Container Spoilages?

To Clear In-container spoilages as Process Deviations

- Routinely incubated samples of production
- Taptone rejects – swollen, leakers, etc.
- R&D new product qualification trials
- Retain samples
- Samples retrieved from consumer complaints
- Samples from recalls
- False alarm – e.g., labor error in micro plating

Why Analyze In-Container Spoilages?

A statistical perspective

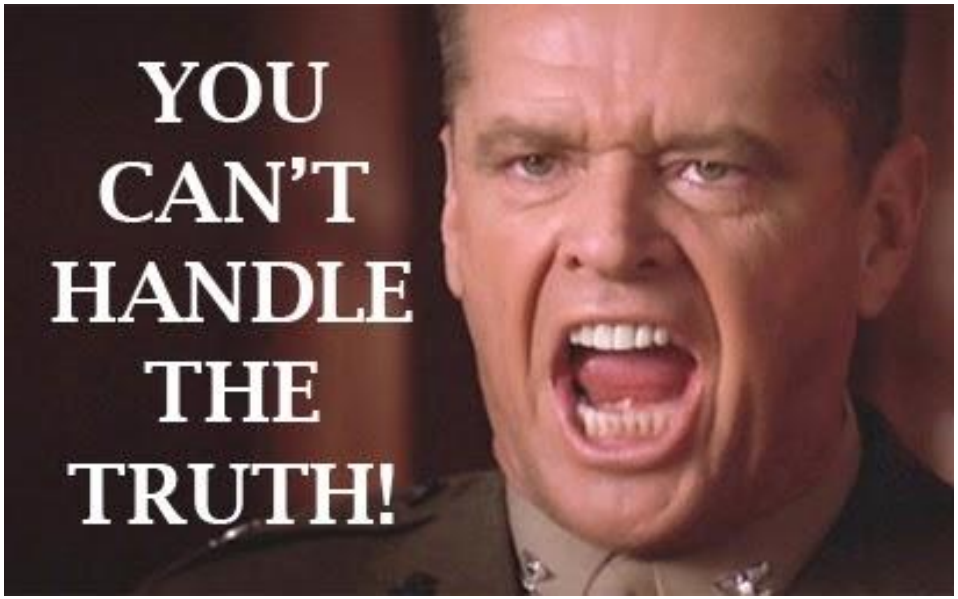
- **Commercial sterility:**
  - Usually 0.01% spoilage rate (1:10,000) at 95% confidence level
  - Sampling 30,000 samples without finding a spoilage

- **Routine production sampling:**
  - 100 – 1000 samples
  - If one spoilage container is found, what does it imply?

可能

Possibly

Reluctant to Conduct Spoilage Analysis?



What if the isolated spoilage organisms indicate food safety concerns?

To Enable In-Container Spoilage Analysis

- **Fence off the lot** - Completely segregate the affected lot from other lots via a clear lot definition
  - e.g., Lot = from CIP to CIP

- **Positive release program**
- **Make a better use of R&D trials /qualification runs**
  - Incubate many samples & analyze spoiled packs to learn more about line

To Enable In-Container Spoilage Analysis

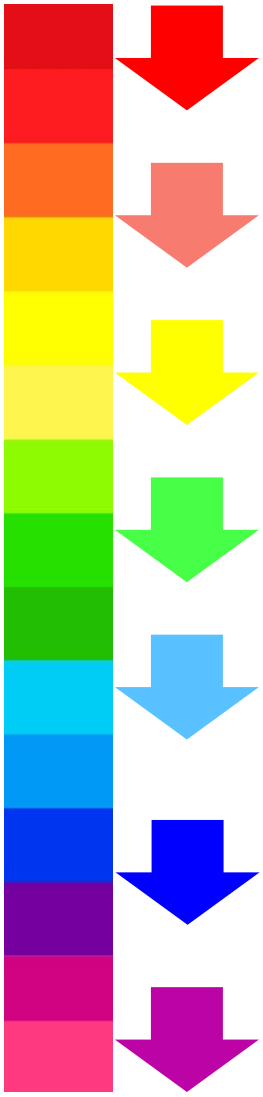
Food safety concerns may differ among High Acid vs Low Acid foods

Microorganisms	Min. Growth pH
Bacillus cereus	4.3
Clostridium botulinum Type A & Non-proteolytic types B & F	5.0
Clostridium perfringens	5.0
Pathogenic strains of *Escherichia coli*	**4.0**
Listeria monocytogenes	4.4
Salmonella spp.	**3.7**
Shigella spp.	4.8
Staphylococcus aureus growth	4.0
Staphylococcus aureus toxin formation	4.0
Vibrio cholerae	5.0
Vibrio vulnificus	5.0
Vibrio parahaemolyticus	4.8
Yersinia enterocolitica	**4.2**

Pathogens is less likely to be an issue in high-acid than in low-acid foods

Different Levels of Spoilage Organism Characterization

Spectrum of Microbial Analysis

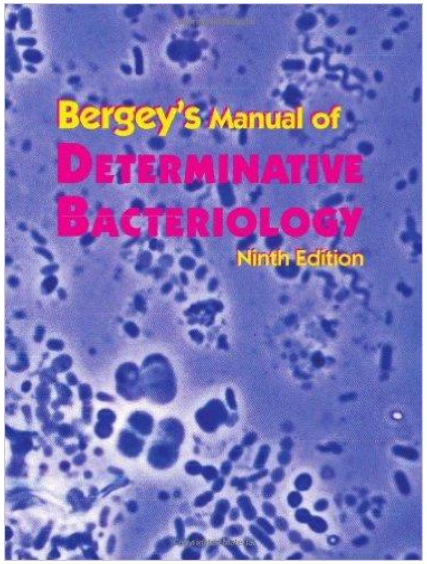


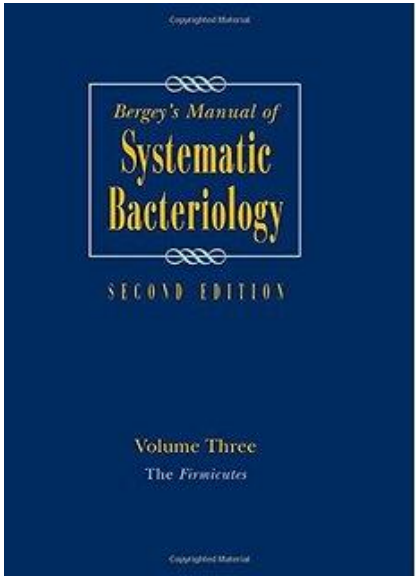
- **Direct microscopic observation**:
  - Cell morphology in sample (cocci, rods, etc.)
  - Pure or mixed cultures
- **Plating** - Colony morphology, CFU level
- Gram stain
- Type of organisms – yeast, mold, bacteria
- Sporeformers vs non-sporeformers
- Mesophilic vs thermophilic
- **Biochemical characterization** - Catalase, Oxidase, etc.
- **Genus** identification
- **Species** identification
- **Strain** identification
- **Genome**

Listeria spp.
vs
L. monocytogenes

Level of micro analysis should be adequate to answer the two objectives

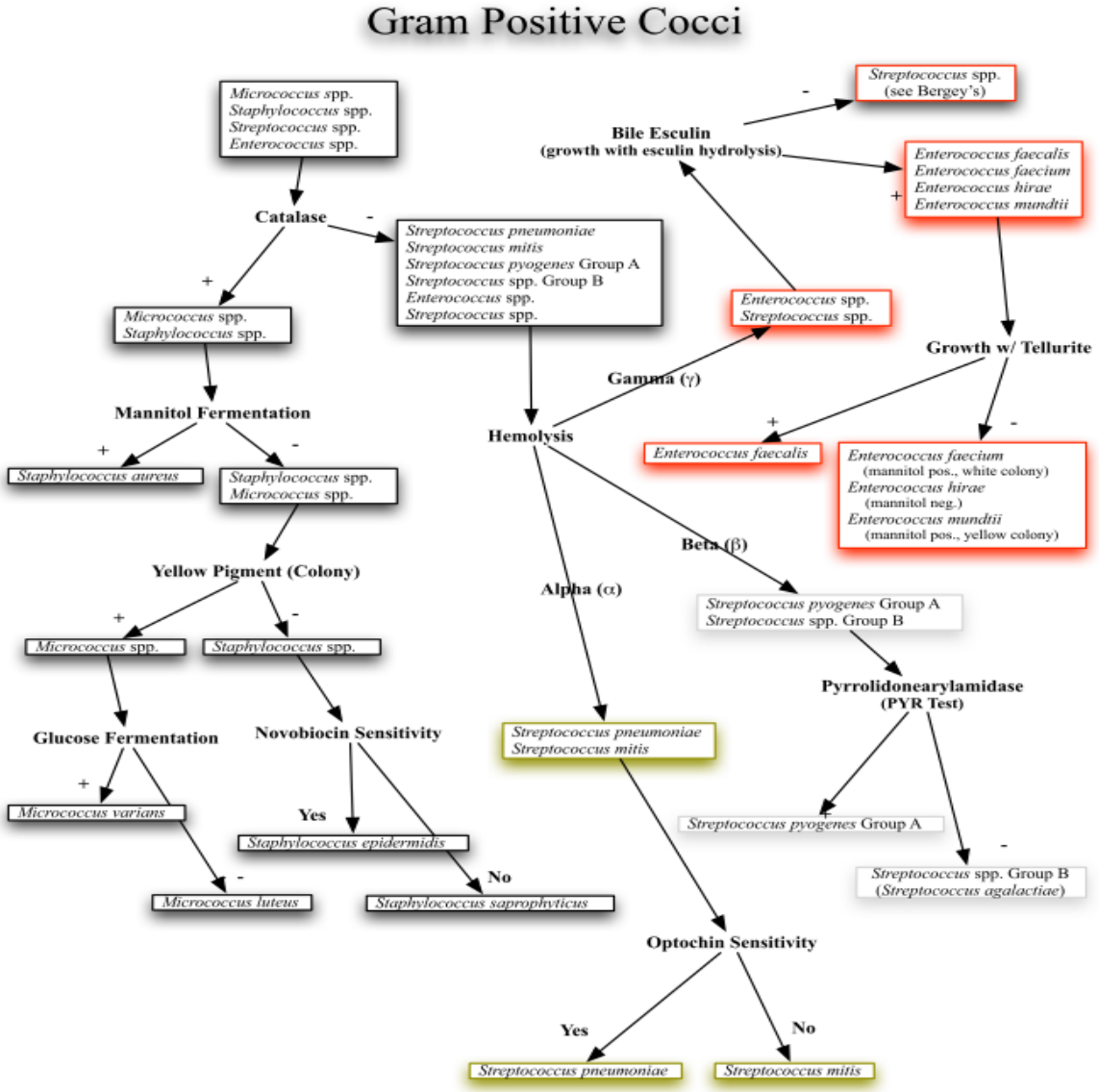
Methods of Microbiological Characterization





Identification flow charts

Gram Positive Cocci ID Flowchart



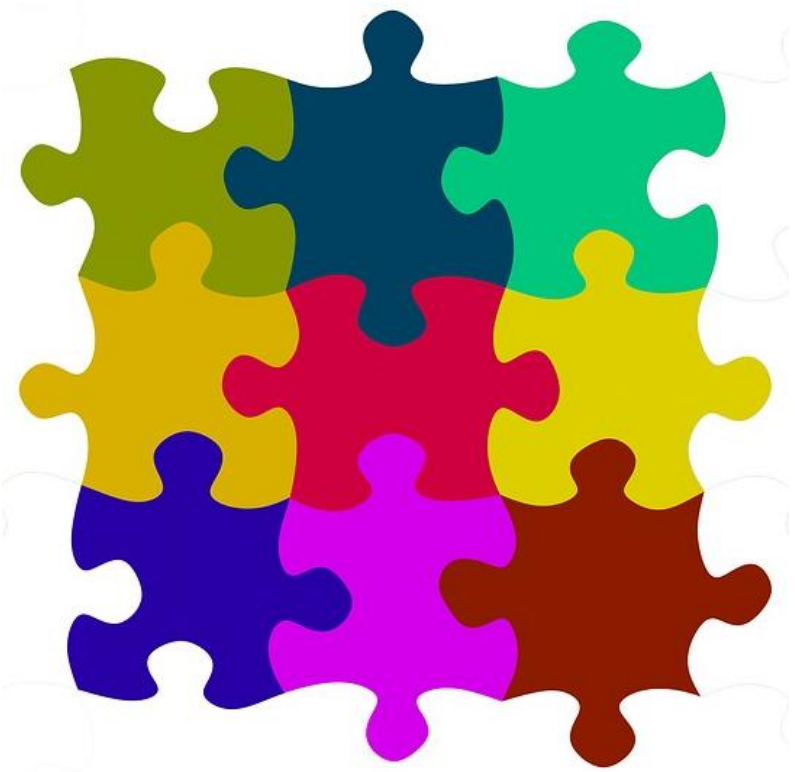
Methods of Microbiological Characterization

Technology has advanced greatly in microbial identification

- Automatic microbial identification via biochemical reactions
- DNA/RNA based methods
- Genome analysis
- etc.

Ideal Outcome of In-Container Spoilage Analysis

All puzzle pieces fall into place perfectly



Conclusions & facts match - do not contradict

Information on different aspects of an in-container spoilage, such as microbial ID, microbial growth behaviors, biochemical & physiological characteristics, product properties, spoilage symptoms, source of contamination, mode of survival, processing parameters, etc., all fit into each other perfectly like a puzzle.

Examples
of
In-Container Micro Analysis

Examples of In-Container Micro Analysis

Ex. 1 - Low-Acid Aseptic Product in bottle

Scenario:

Routine micro analysis of incubated samples:

- Found one streak plate with growth of 8 CFU

Additional information provided to TPA:

- pH still within spec.
- Bottle not bloated
- Sample discarded after plating
- Process records review - passed

Examples of In-Container Micro Analysis

Additional info gathered:

- All 8 colonies on plate were on streaking line

Was it due to lab error?

- Possible, but colonies were on streaking line?

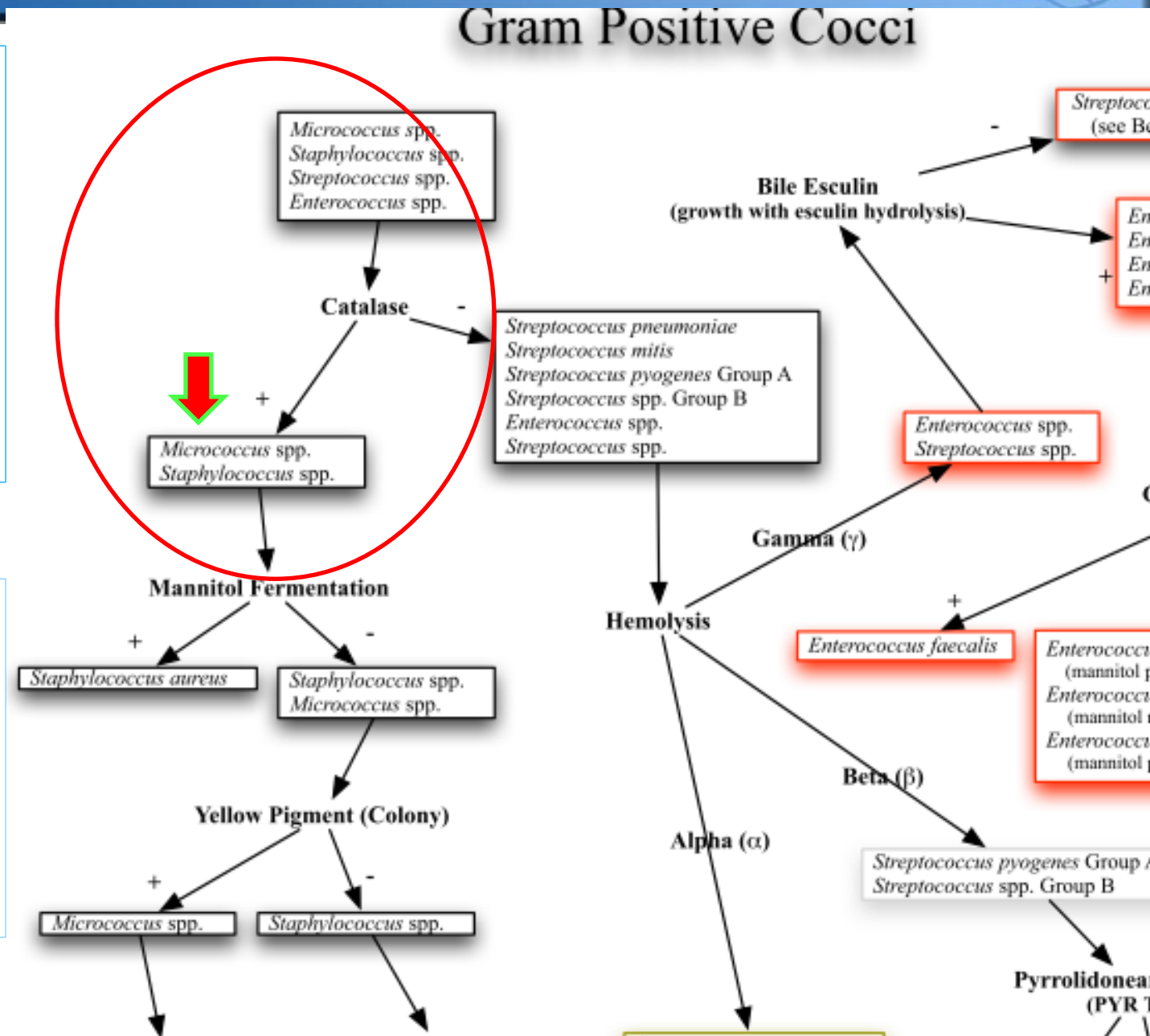
Examples of In-Container Micro Analysis

Prelim. ID:

- Gram stain +
- Cocci,
- Catalase +
- Oxidase -
- Spore stain negative

It could be:

- *Micrococcus* spp.
  – no known foodborne pathogen
- *Staphylococcus* spp.
  – *S. aureus*



Examples of In-Container Micro Analysis

- Positive release – the lot still on QA hold
- Decided to identify the isolate to species

- Accugenix result:
  - *Staphylococcus epidermidis*

Most likely source of colonies:

Examples of In-Container Micro Analysis

Ex. 2 - Low-Acid Aseptic Product in Bottle with Foil Seal:

Scenario:

- Routine incubated production samples, a plant microbiologist found **one (1) sample with pH drop**

Additional information provided to TPA:

- pH 5.49 (Spec is neutral pH)
- Bottle not bloated at time of pH testing
- No visible bottle or seal defect
- Food sample discarded, but empty bottle saved
- Process records review - passed

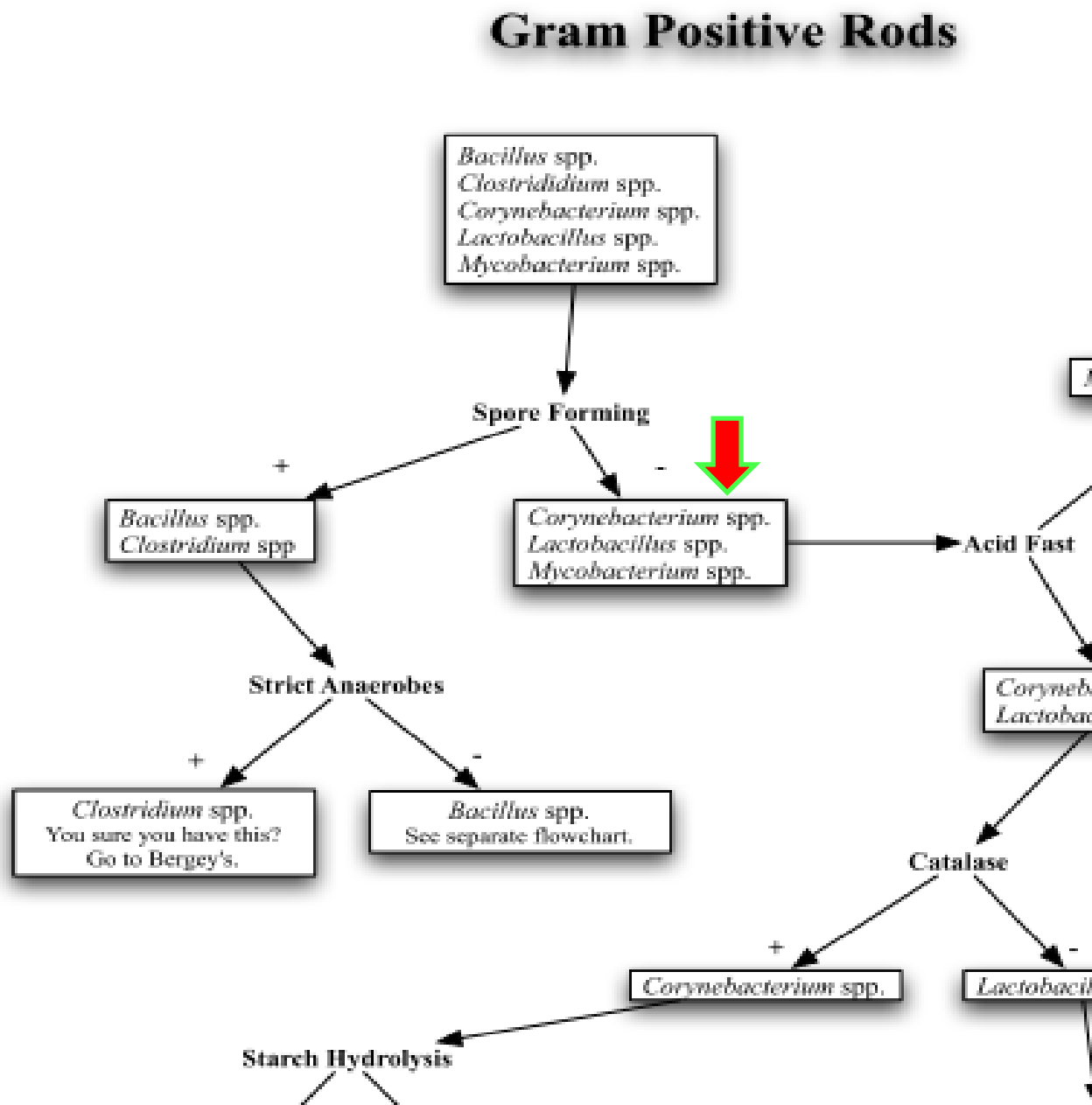
Examples of In-Container Micro Analysis

Prelim. characterization:

- Rod,
- Gram stain +
- Catalase -
- Oxidase -
- Spore stain negative

It could be **Lactic Acid Bacteria**:

- Gram-positive,
- Catalase-negative
- Oxidase-negative
- Rod-shaped



Examples of In-Container Micro Analysis

Seal Integrity analysis report:

- Foil seal "was not intact and a defect was evident."
- "The electro-conductivity test was positive"
- "dye penetration was observed."
- "Under magnification, the land surface was abnormal and the seal impression was not uniform."

For further confirmation, plant incubated additional 1250 bottles & tested – 0 defects found

Examples of In-Container Micro Analysis

Ex. 3:

Trouble-shooting

Spoilage Incident in R&D Development of An ESL Low-Acid Beverage

Background:

- This was a R&D trial – all products were not for sale.
- All products were on QA hold (never released in the end).
- PET bottle with foil seal
- Thermal process delivered to product was sufficient to destroy mesophilic spores

Background: Spoilage Incident

1st R&D plant trial:

6 spoilages found

⇩

Trouble shooting

⇩

Corrective actions

⇩

Confirm corrective actions were effective

⇩

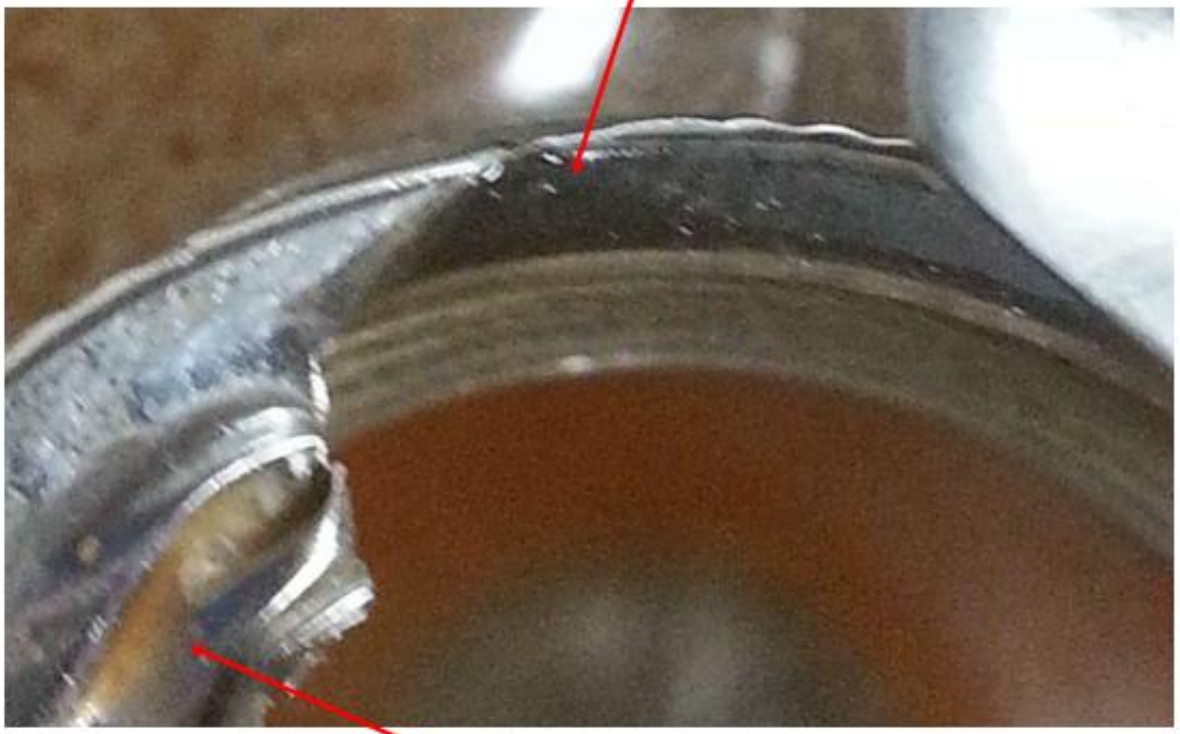
Final product qualification

A total of 6 spoilages found in 1st R&D trial

- **1 spoilage** – found by plant routine test
  - No sample was saved
    - No follow-up micro analysis or bottle integrity testing

- **5 spoilages** – found by R&D micro test
  - All 5 bottles (seal area still intact)
    - No visual seal defect
    - Sent out for seal integrity analysis
  - Isolated & identified spoilage micro

5 bottles sent out for seal integrity analysis



All 5 bottles were leakers

Microbial ID - via Accugenix

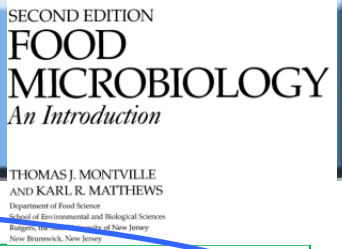
Sample	Agar	Micro ID
Beg 776	TGY	*Bacillus cereus*
	LVA	
Beg 262	TGY	*Bacillus thuringiensis*
	LVA	
Mid 252	TGY	*Bacillus thuringiensis*
	LVA	
Beg 528	TGY	No ID yet
	LVA	*Bacillus circulans*
Sample #5	TGY	Spoiled, but no micro was isolated
	LVA	

All isolates were *Bacillus* spp.

From *B. cereus* group

Systemic

B. cereus facts



- *"Bacillus anthracis, B. cereus, B. mycoides, B. thuringiensis, B. pseudomycoides,* and *B. weikenstepkanensis* are members of the ***B. cereus* group**."
- "They have diverged from a common evolutionary line."
- "B. *anthracis* is the most distinctive member of this group, both in its highly virulent pathogenicity and in its notoriety as an agent for biological warfare."
- …extensive studies of DNA from *B. cereus* and *B. thuringiensis* suggest that there is no scientific reason for them to be in separate species.
- "Indeed, *B. thuringiensis* strains used as a natural bioinsecticide have caused cases of *"B. cereus"* food poisoning, and some *"B. cereus"* strains produce anthrax toxins."
- "Most *B. cereus* strains are unable to grow below 10°C or in milk products stored between 4 and 8°C. "
- "However, psychrotolerant strains grow at temperatures as low as 4 to 6°C."

B. cereus facts

- *B. cereus* can readily form biofilm
- *Bacillus* spp. are everywhere
- Spores can survive heating, dry conditions, sanitizing, etc. better than vegetative cells

Trouble-Shooting Theory

- **Unlikely due to under-processing or filling contamination** – since all non-leaker samples were not spoiled
- **Likely due to post-filling contamination** (likely outside filler)
- ***Bacillus* spp. had established themselves somewhere after filling**
  - When leakers passed by the hot spots – got contaminated
- **Likely area – Taptone reject area**
  - Splash of leaking samples during rejection
  - Food residue seen on equipment in that area

Post-filling contamination of leaking samples - Root Cause?

Trouble-Shooting Discovery

Assessment report by corporate sanitarian:

- "Specific to answering the question of where microorganisms could be introduced to a package with a seal integrity failure, **only one area generated concern**."
- "The area surrounding the **Tap Tone rejection** bin creates **a pool of water and product**. The area includes a floor drain."
- "Signs of **splashing or splattering** are visible on two different conveyors – conveyor for bottles with foil seal exposed and conveyor for bottles with dust cover applied."
- "**With a seal integrity failure, exterior contamination could be drawn into the package** or be present for lab exposure when handling an individual package or subsequent packages."

Possible Corrective Actions

To reduce leakers:

- Control product foaming / splashing
- Correct filling valve spilling issues
- Improve leaker detection
  - Plant did not use instrument to assess seal quality
  - Relied on “hand-squeezing”

Corrective Actions before 2nd Trial

Corrective actions implemented:

- Extensive cleaning/sanitation
- Filling cleanliness improvement

Corrective actions to be implemented:

- Bottle seal integrity monitoring improvement
  - Water submersion vacuum testing instrument - but not in place yet

To confirm corrective actions were effective

R&D ran 2nd trial & collected 3000 samples:

- Two spoilages found

Only leakers were spoiled

- Both were leakers
  - Micro characterization indicated that the spoilage organisms were not *Bacillus* spp.

So:

- The contamination source (hot spot) had been eliminated
- But leaker issue had not been resolved

Corrective Actions before Final Qualification

Plant Corrective Actions before Final Qualification

- Upgraded product seal integrity testing from hand-squeezing to water submersion vacuum testing

Final qualification results after all corrective actions

- 0 spoilage out of 16,211 incubated samples from several product flavors & multiple qualification runs

In-Container Spoilage

All puzzle pieces fall into place “perfectly”

- Spoiled pack features – pH drop
- Not visual leakages
- Not leaking noticed via hand-squeezing bottles
- True leakers – revealed by dye testing, microscopy, etc.
- Sporeformers isolated
- Sporeformers can survive common sanitizing & form niches in Taptone area
- All containers passed Taptone areas
- Sporeformers from biofilm could enter leakers (but not non-leakers)
- All spoiled packs were leakers
- After elimination of harborage sites, no more spoilages due to the sporeformers.
- After implementing a better seal integrity monitoring, neither leakers nor spoilages were seen.

Final Qualification Results

Learning:

- Not all leakers are “just a spoilage issue”
  - Some could be a food safety issue
- Not all leaker spoilages are due to “mixed cultures”


THANKS