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- **Work/Professional Experience**
  - **Eurofins Microbiology, Inc.,** Senior Director, Advanced Microbiology 2018 – Present
  - **Covance Foods Solutions,** Senior Director, Food Safety and Aseptic, 2016 – 2018
  - **The National Food Laboratory, Inc.,** Chief Science Officer, 1989 – 2016
  - **National Advisory Committee for Microbiology Criteria for Foods,** Member, 2014 -2018
  - **IFSH Executive Advisory Board,** Member, 2015 – Present
  - **IAFP Beverage and Acidified Foods PDG,** Chair/Co-Chair, 2015 – 2019
- **Education**
  - **Doctorate in Food Science (Food Microbiology)**, University of Illinois, Champaign-Urbana, IL
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- **Hobbies**
  - Wine tasting (beer too), road cycling, running, following MLB

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Troubleshooting Spoilage in Aseptic Processes

March 1, 2023

Wilfredo Ocasio Ph.D.
Advanced Microbiology Group
Eurofins Microbiology Laboratories

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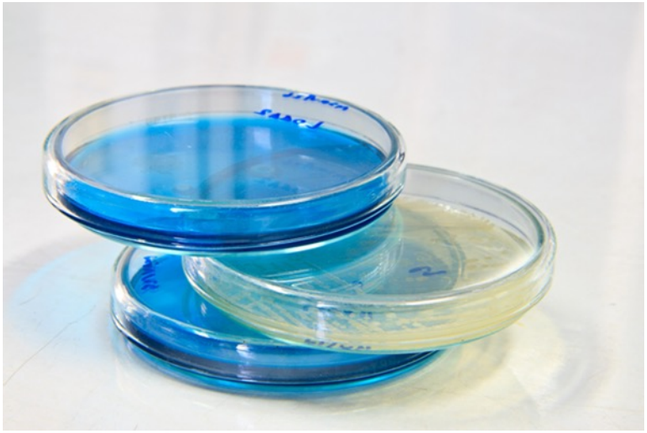
Presentation Outline

- Aseptic Processing, Definition and Facts
- Microbial Risks
- Spoilage Investigation Strategy: Plant and Lab
- Causes of Spoilage – Top 10 List



Definition

Aseptic processing and packaging: The filling of a commercially sterile and cooled product into presterilized containers, followed by aseptic hermetical sealing, with a presterilized closure, in an atmosphere free of microorganisms. (21CFR113.3a)

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Is it true aseptic?

- These are not True Aseptic:
  - Ultra clean filling
  - Hygienic filling
  - Clean filling
  - ESL filling
- Reduced robustness/redundancy on sterilization and maintenance of sterility parameters
- Relies on intrinsic parameters of product (pH, aw, natural antimicrobial properties), preservatives or refrigeration

If using a filler other than VDMA Hygienic Class IV or Class V, then you are relying on factors other than aseptic filling to protect the product.

➢**Thus, not really aseptic!!**

Aseptic Technology Facts

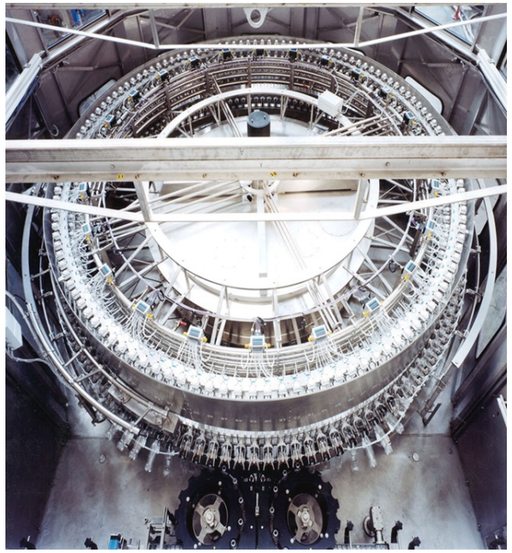
Advantages

- Effective and Robust Technology
- Microbiological safe and stable products
  - Very few recalls, illness or outbreaks
- Energy savings
- Lower shipping costs (lighter packaging)
- Allow production of shelf-stable, heat sensitive products
- Higher quality and nutritional value
- Clean label
- Expanded package design options

Challenges

- High initial capital cost
- Use of chemical sterilants
- Complex technology
  - Multiple components
  - Numerous critical factors
  - Large aseptic zones
  - Difficult to diagnose problems

Microbiologically safe and stable technology



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Sometimes Things Do Not Go as Planned


U.S. FOOD & DRUG ADMINISTRATION

Home / Safety / Recalls, Market Withdrawals, & Safety Alerts / Lyons Magnus Voluntarily Recalls 53 Nutritional and Beverage Products Due To The Potential For Microbial Contamination

COMPANY ANNOUNCEMENT

Lyons Magnus Voluntarily Recalls 53 Nutritional and Beverage Products Due To The Potential For Microbial Contamination

Most of the time spoilage problems are contained within production facility but…..

The recalled almond milk

Botulism case linked to almond milk in Australia

Ripple Foods Original Pea Milk Recall

November 23, 2021

Ripple Foods has issued a voluntary recall of its original 48 oz. milk due to possible contamination with Bacillus cereus, a bacteria that can cause digestive issues. The illness is often mild and there is only a remote possibility of serious adverse health effects. No illnesses have been linked to this recall.


FSN | Food Safety News

Breaking news for everyone's consumption

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Slim Fast Was Recalled for Bacillus Cereus

By Dan Flynn on March 6, 2010

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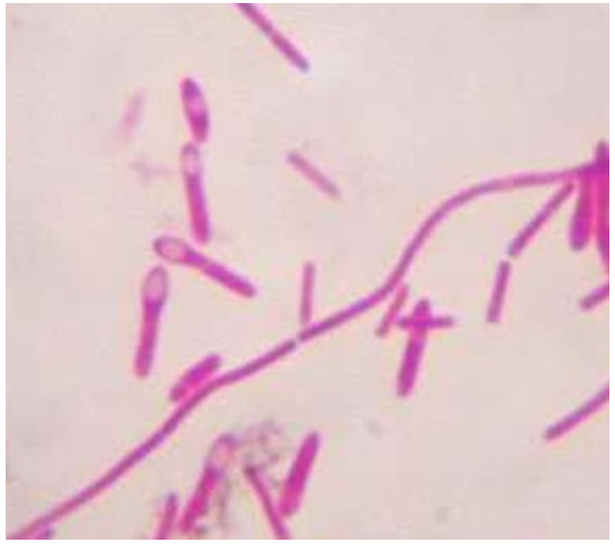
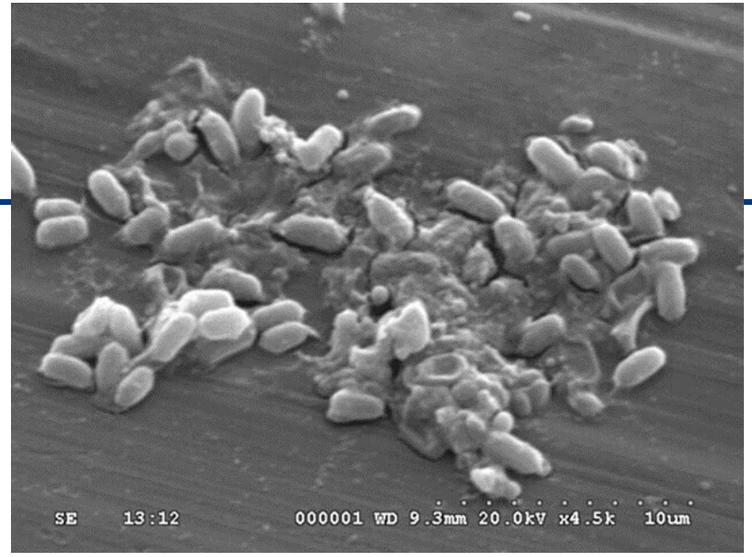
Are we seeing more spoilage issues? Why?

1. Market growth and technology maturity
2. Increasingly complex installations
3. Longer runs between full CIP/SIP
4. Use of novel and exotic ingredients
5. More intense and sensitive microbiological testing

Source: https://scitechdaily.com/food-waste-study-reveals-much-fridge-food-goes-there-to-die/



Microbial Risks



Aseptically Processed Foods

Microbial Risks: Bacterial Pathogens

Sources of contamination: soil, water, dust, air, raw ingredients, packaging material, production environment

Spore-Forming Bacteria

- Highly heat resistant spores
- Require germination and growth to cause illness (except for infant botulism)
- Examples:
  - *Clostridium botulinum*
  - *Bacillus cereus*
  - *C. perfringens*

Nonspore-forming Bacteria

- Heat labile
- Low-infectious dose, may not require active growth to cause illness
- Examples:
  - *Salmonella* spp. (Nontyphoidal)
  - Shiga-toxin-producing *E. coli*
  - *Listeria monocytogenes*
  - *Campylobacter spp. (chicken and poultry)*
  - *Cronobacter sakazakii*

Microbial Risks: Spoilage Organisms

Spore-Forming Bacteria

- Most common problem
- Mesophilic and thermophilic
- *Bacillus* spp.
  - *B. licheniformis, B. subtilis, B. pumilus, B. thuringensis, B. coagulans, B. sporothermodurans**
- *Clostridium* spp. (anaerobic)
  - *C. tyrobutyricum, C. halophilum, C. sporogenes, C. pasteurianum, C. butyricum*
- *Geobacillus stearothermophilus** (thermophile)

*Produces highly resistant spores capable of surviving conventional aseptic processes

Non-Spore-Forming Bacteria

- Heat labile
- Lactic acid bacteria (LAB)
  - *Lactobacillus, Streptococcus, Pediococcus, Leuconostoc,* etc.
- Acetic Acid Bacteria
  - *Acetobacter, Acidomonas, Ameyamaea, Asaia, Gluconacetobacter*, etc.
- Coliforms
  - *Citrobacter, Enterobacter, Escherichia, Klebsiella,* etc.

Spoilage Fungi

- Molds (airborne spores)
  - *Aspergillus, Penicillum, Rhizopus, Mucor, Geotrichum, Fusarium, Byssochlamys*, Neosartorya*, Talaromyces**
- Yeast (osmotolerant, preservative resistant)
  - *Zygosacharomyces, Saccharomyces, Debaryomyces*, etc.

*Heat resistant mold, some yeast produce heat resistant ascospores

Spoilage Investigation

Why is my aseptically packaged product blowing up in the warehouse?

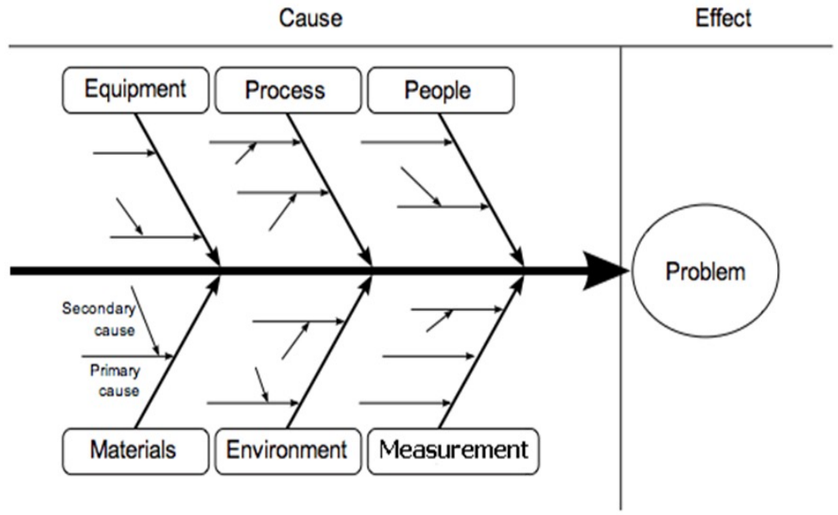
SPOILAGE INVESTIGATION STRATEGY

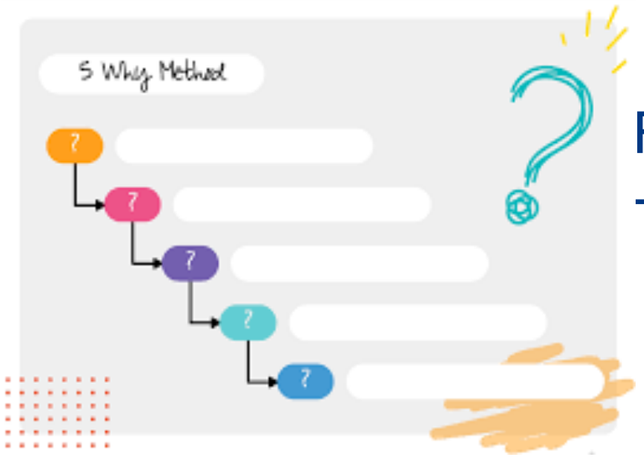
Starting point:

- Assemble investigation teams
  - Microbiologist, Process Engineers, Process Authority, Operators, QA Personnel, etc.
- Assign each aspect of the investigation to expert team
- Designate team leaders
- Define timeframe to report back to management

ROOT CAUSE INVESTIGATION STRATEGY

1. Map chronology and process steps/events surrounding event
2. Evaluate forensic microbiology results (pure culture? organism type(s)? heat-resistant forms? chemical resistance? etc.)
3. Examine thermal process design and execution
4. Examine processing equipment: Cleaning and pre-sterilization (CIP, SIP)
5. Examine processing equipment: Maintenance of sterility
6. Filler enclosure: Examine sterilization cycles (bottles, closures, filling/capping area)
7. Filler enclosure: Examine maintenance sterility (aseptic zone)
8. Examine the closure process: Hermetic seal integrity
9. Examine critical ingredient specifications and bioloads
10. Examine batching and blending procedures





Fish-bone
+ 5 WHYs

Processing System – CIP/SIP

Was CIP/SIP adequately designed and executed?

- ✓Proper CIP chemicals for food type
- ✓CIP volumes, flow rates, temperatures, etc.
- ✓CIP cycle working properly (visual inspection)
- ✓Pre-sterilization temperatures are adequate
- ✓Sensors appropriately located and calibrated
- ✓No dead ends or lack of valve seat cycling
- ✓Processing records (critical factors) reviewed
- ✓All the above also reviewed for aseptic surge tank, aseptic homogenizer and connecting pipes/valves

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Thermal Process

Was the thermal process appropriately design and executed?

- ✓Process source?
- ✓Hold tube properly measured
- ✓Sensors appropriately located and calibrated
- ✓Processing records (critical factors) reviewed
- ✓Process temperatures adequately calculated (flow rate, hold tube dimensions and product viscosity)
- ✓Keep your P&ID up-to-date and your line components adequately identified and labeled

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Processing Equipment: Maintenance of Sterility

- ✓ Aseptic valve integrity (diaphragms, etc.)
- ✓ Steam traces appropriately designed and monitored in aseptic surge tanks, homogenizers and valves?
- ✓ Differential pressure in coolers
- ✓ Wall integrity (aseptic tanks, coolers, etc.)
- ✓ Aseptic product leaks
- ✓ For surge tanks, check sterile air provision
  - ✓ Air filter function and maintenance

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Aseptic Filler: Sterilization Cycles

- ✓ Steam sterilized product contact areas (i.e., product line, filling nozzles, etc.)
  - No dead ends preventing proper steam flow
  - Location and function of temperature sensors
  - Efficacy of CIP Cycles
- ✓ Chemically sterilized: aseptic zone, bottles and closures
  - Validated critical limits match operation
  - Confirm concentration of sterilant
  - Proper flow of sterilant (flow rate, spray time, plugged spray nozzles)
  - Location and function of temperature of sensors

Aseptic Filler: Maintenance of Sterility

- Review all critical factors
- Sterile air flow: Sources of turbulence, smoke test results
- Proper seal on windows
- Function and maintenance of sterile air filters (aseptic chambers)
- Sterile air provision to filler bowl
- Function of chemical/steam barriers at entry points to sterile zone

Ingredient Specs, Mixing and Blending

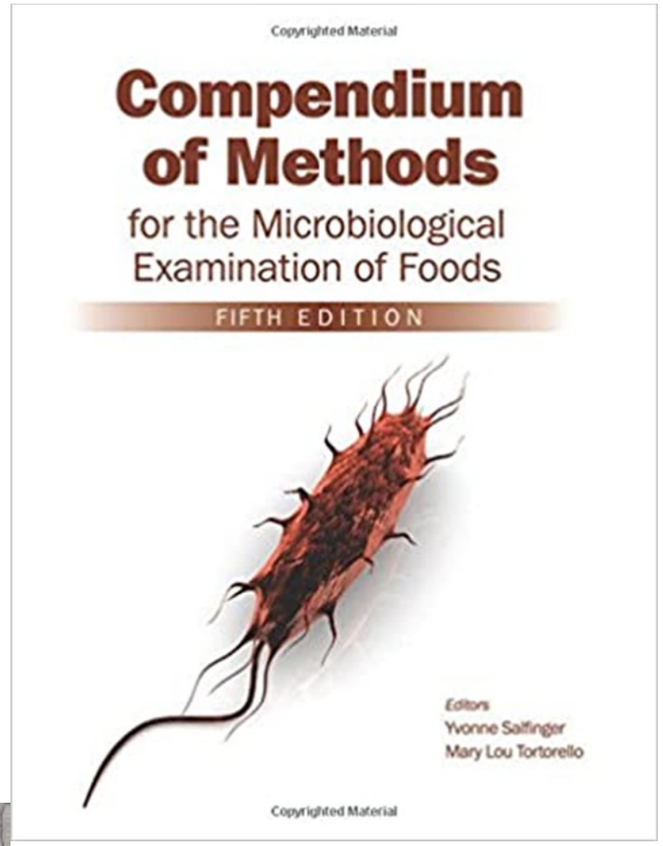
- Were micro specifications for ingredients met?
- Were there recent changes to formulation?
- Were there any recent changes to mixing and blending procedures?
- Were there recent changes on ingredient suppliers?
- Were mixing and blending procedures appropriate to assure hydration?

At the Microbiology Lab

Cause of Spoilage Investigation Procedure

At the Micro Lab: Cause of Spoilage Exam

- Get samples to the lab as soon as possible
  - Keep them cool unless thermophilic spoilage is suspected
- Find competent lab with experience in commercially sterile products
- Follow proper COS procedures
  - Compendium of Methods for Microbiological Examination of Foods
    - Chapter 62. Canned Foods – Tests for Cause Spoilage
    - Chapter 61. Canned Foods – Tests for Commercial Sterility
- Microbiology is only one piece of puzzle
  - Results must be kept in context of other findings



*Compendium of Methods for the Microbiological Examination of Foods 5th Ed. 2015, Chapter 62 Canned Foods – Test for Cause of Spoilage

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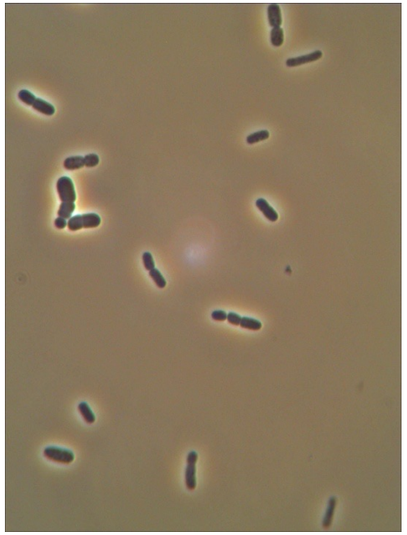
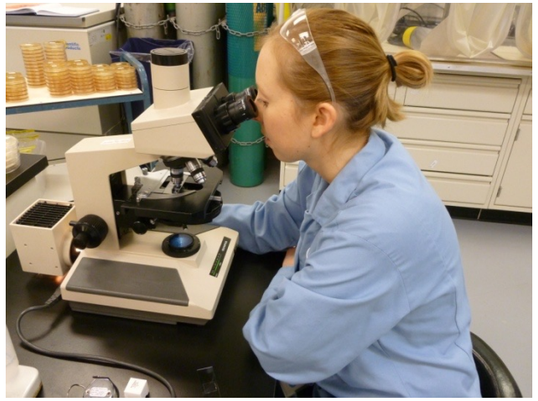
At the Micro Lab: Cause of Spoilage Exam* cont.

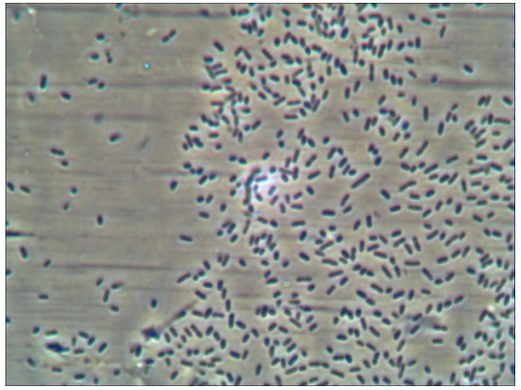
- Open container as aseptically as possible
  - Clean, sanitize, keep in laminar flow hood, open with sterile utensil
  - If possible, preserve seal area for later evaluation
- Conduct a microscopic exam (wet mount) directly from product
  - Are there microorganisms present? Do they appear viable?
  - Record morphology and motility (cocci, rods, yeast cells, mold)
  - Are there spores present?
- Check pH, record product appearance and odor (never taste!)
- Save container for seal integrity exams

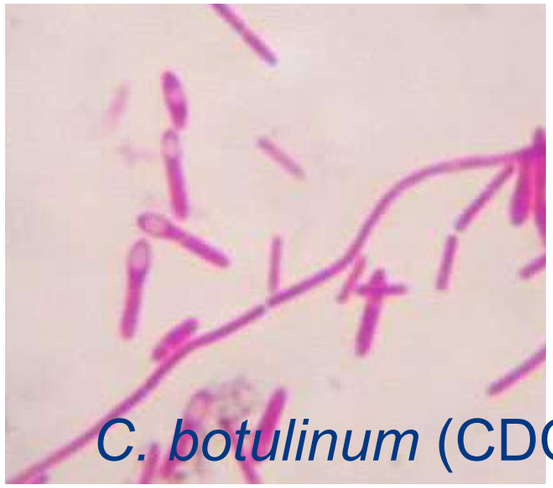
*Compendium of Methods for the Microbiological Examination of Foods 5th Ed. 2015, Chapter 62 Canned Foods – Test for Cause of Spoilage

Microscopic examination of sample

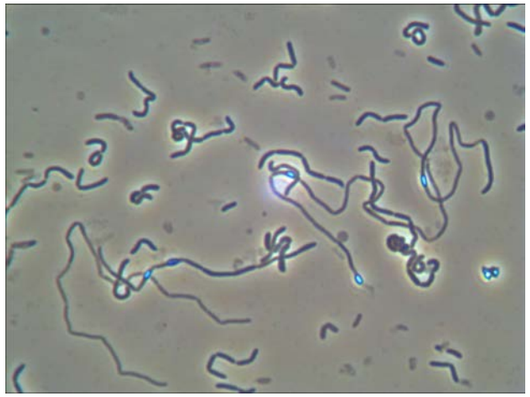


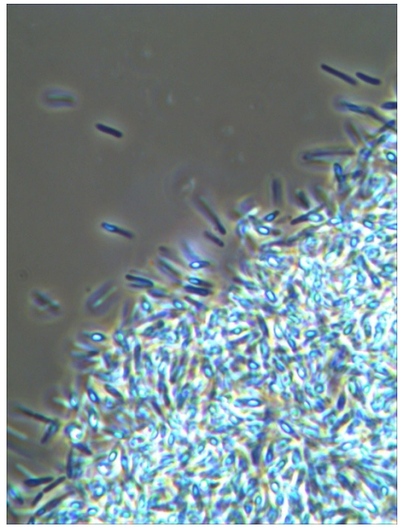


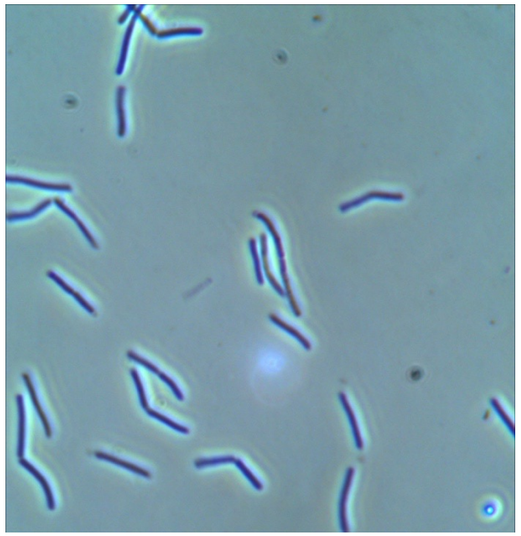


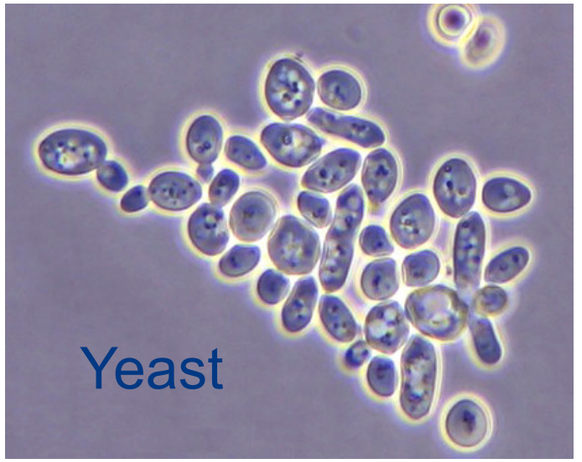


C. botulinum (CDC)





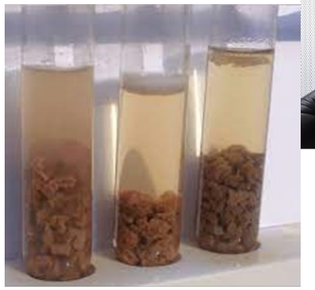




Yeast

At the Micro Lab: Cause of Spoilage Exam cont.

- Culture Procedure (low-acid, pH>4.6)
  - Dextrose tryptone broth (aerobes)
    - 30-35°C (mesophiles)
    - 55°C (thermophiles)
  - Deareated Cooked meat medium (anaerobes)
    - Heat-shocked (detect spores) and non heat-shocked
    - 30-35°C (mesophiles)
    - 55°C (thermophiles)



CMM

DTB

Media/Incubation Temperature	Sample No. Visual Observation (Date Noted) Microexam (Date Examined)
DTB 30°C–35°C	cocci + mixed rods (no spores), acid
CMM or PE-2 (no HS) 30°C–35°C	mixed rods, gas
CMM or PE-2 (HS) 30°C–35°C	no growth
DTB 55°C	no growth
CMM or PE-2 (no HS) 55°C	no growth
CMM or PE-2 (HS) 55°C	no growth

Media/Incubation Temperature	Sample No. Visual Observation (Date Noted) Microexam (Date Examined)
DTB 30°C–35°C	medium rods, terminal spores
CMM or PE-2 (no HS) 30°C–35°C	medium rods, terminal spores
CMM or PE-2 (HS) 30°C–35°C	medium rods, terminal spores
DTB 55°C	no growth
CMM or PE-2 (no HS) 55°C	no growth
CMM or PE-2 (HS) 55°C	no growth

*Compendium of Methods for the Microbiological Examination of Foods 5th Ed. 2015, Chapter 62 Canned Foods – Test for Cause of Spoilage

Microbiology Results

Media/Incubation Temperature	Sample No. Visual Observation (Date Noted) Microexam (Date Examined)
DTB 30°C–35°C	cocci + mixed rods (no spores), acid
CMM or PE-2 (no HS) 30°C–35°C	mixed rods, gas
CMM or PE-2 (HS) 30°C–35°C	no growth
DTB 55°C	no growth
CMM or PE-2 (no HS) 55°C	no growth
CMM or PE-2 (HS) 55°C	no growth

- **Mixed microflora of non-spore forming bacteria**
- Indicative of contamination on “cool” side
  - After product is cooled down to near room temperature
  - At the filler
  - At surge tank
  - Hermetic seal failure

Media/Incubation Temperature	Sample No. Visual Observation (Date Noted) Microexam (Date Examined)
DTB 30°C–35°C	medium rods, terminal spores
CMM or PE-2 (no HS) 30°C–35°C	medium rods, terminal spores
CMM or PE-2 (HS) 30°C–35°C	medium rods, terminal spores
DTB 55°C	no growth
CMM or PE-2 (no HS) 55°C	no growth
CMM or PE-2 (HS) 55°C	no growth

- **Single heat resistant, spore-forming bacteria**
- Indicative of contamination at a point where “selective pressure” applied to eliminate sensitive microorganisms
  - Poor hydration
  - At cooling HE while product is still hot
  - Faulty thermal sterilization (**UNDERPROCESSING**)
  - Faulty chemical sterilization

Microbiology: Additional Testing

- Other tests:
  - Identification and characterization of isolates
    - Some value in finding root cause, important if assessing a recall situation
  - Genomics (16sRNA, WGS)
  - Confirmation that isolated MOs is/are truly the spoilage MOs
    - Reintroducing isolate to product / Reproduce spoilage characteristics
    - Consider age of samples and microbial succession
  - Aseptic zone swabbing (filler, product contact, valves, tank, etc.)
    - Big challenges: preventing of contamination during sampling, misinterpretation of results

Microbiological Results

Are the recovered spoilage organisms growing in product?

Are the recovered organisms heat/chemical resistant?

YES

- Thermal process
- Package sterilization
- Equipment pre-sterilization
- Sterility maintenance – “Hot”
- Ingredient specs
- Preparation procedures

NO

- Hermetic seal
- Gross process failure
- Sterility maintenance – “Cold”
- Pitting on coolers or tanks

Microbiology Results

FDA U.S. FOOD & DRUG ADMINISTRATION

← Home / Inspections, Compliance, Enforcement, and Criminal Investigations / Compliance Actions and Activities / Warning Letters / Lyons Magnus, LLC - 645766 - 01/30/2023

WARNING LETTER

Lyons Magnus, LLC

MARCS-CMS 645766 — JANUARY 30, 2023

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Specifically, on July 26, 2022, you notified FDA that you initiated a voluntary recall of all products aseptically processed and packaged between **(b)(4)**, due to potential microbiological contamination in finished products, including by *Bacillus subtilis* and *Cronobacter sakazakii*. We note that *Bacillus subtilis* indicates a potential under-processing that could lead to contamination with *Clostridium botulinum*. Your notification to FDA occurred **(b)(4)** after your positive lab tests for gram-positive rods on **(b)(4)**, and **(b)(4)** after lab testing confirmed the *Bacillus subtilis* result. Moreover, our review of

Causes of Spoilage

Top 10 List!

WHY DOES “TRUE” ASEPTIC FAIL?

Reason 1
Hermetic seal failure

- Maintenance is key
- Adequate testing
- More pressure seals (plastic on plastic)
- Sporadic failure may be difficult to detect
- More information needed on defining a proper hermetic seal and evaluating leak detection methods

Reason 2
Valve design or function

- Need true aseptic valves
  - ✓ Can be sterilized
  - ✓ Moving parts protected from environment
  - ✓ Leak detection
- Valve not actuated CIP/SIP
  - ✓ Poor valve logic or malfunction
  - ✓ Actual position not detected by PLC
  - ✓ Heavy reliance on automation
- **Proper Maintenance is key**

Reason 3
Poor design or SIP/CIP practices in product lines

- Dead ends
- Improper joints or welding
- Inadequate drainage
- Pin holes or gasket failures in cooler plates or tubes
- Pin holes in product floaters
- 3A Sanitary Standards, European Hygienic Equipment Design Group (EHEDG)
- **Too long production runs**

WHY DOES “TRUE” ASEPTIC FAIL?

Reason 4

Physical (bellows) or chemical barrier malfunction

- Critical for protecting reciprocating movement:
  - ✓ Agitator shaft in sterile surge tanks
  - ✓ Aseptic homogenizer pistons
  - ✓ Rotary fillers
- Proper function (temperature, chemical conc.) must be properly monitored

Reason 5

Overly complex design

Keep it simple – Keep it linear!

- Avoid complex valve clusters
- Avoid multiple options: filler, surge tanks, hold tube configurations
  - IDEAL: One processor feeds one surge tank, and one filler

Reason 6

Ineffective sterile air overpressure in filler

- Must validate efficacy
- Account for turbulence due to large moving parts
- Changes in pressure within room (opening closing of doors)

WHY DOES “TRUE” ASEPTIC FAIL?

Reason 7

Plugged sterilization nozzles

- Filler pre-sterilization
- Bottle/closure sterilization
- Must use atomizing quality liquid sterilant
  - Presence of preservatives (solids) in H_2O_2 may cause plugging

Reason 8

Heat resistant microbes

- Heat resistant molds
  - *Byssochlamys fulva, B. nivea, Neosartorya fischeri, Talaromyces macrosporus*
  - D_{90C} = 10-20 min; z values 5–12^{o}C
  - Survive typical high-acid process
- *Alicyclobacillus* spp.
  - *A. acidoterrestris* most common
  - Apple juice often implicated
  - Extremely resistant (D_{90C} ~ 30 min)
- Thermophilic bacteria
  - *G. stearothermophilus*.
  - Proper GMP’s: flume water, evaporators, etc

Reason 9

Overreliance in automation

- Lack of properly validated alarms and interlocks
- 100% reliance on automation without human confirmation is cause of problems
  - Lack of understanding of critical factors
  - Inability to understand complex production charts

WHY DOES “TRUE” ASEPTIC FAIL?

- **Reason #10: Poor hydration of ingredients = dry heat process**

Organism	D_{250} (min.)	D_{285}	F_{285}
B. subtilis	0.3 min	0.07 sec	0.35 sec. (5D)
C. botulinum	0.22 min	0.15 sec	1.8 sec. (12D)
Reduced a_W Heat Resistance			
C. botulinum – dried on metal surface	70 min	5.2 min	62.4 min (12D)
B. subtilis @ a_W = 0.60	330 min.	38 min	192 min (5D)

  - **Cocoa hydrated to a minimum of 190°F for 30-45 min with high shearing**
  - **Sequence of ingredient addition may also be important**
  - **Plant proteins difficult to hydrate**

WHY DOES “TRUE” ASEPTIC FAIL?

- Poor ingredient quality
- Prerequisite program failure
  - Training**, maintenance, plant sanitation, CIP’s**
- Sterilization failures
  - Product, package, closure, equipment surfaces, air
- Failure to maintain sterile environment
  - Aseptic filler (aseptic zone), sterile product lines, aseptic surge tanks, aseptic homogenizers, etc.
- Hermetic seal failures
- Lack of or inadequate “**Change Control Program**”

Spoilage Investigation - Summary

- ✓ Complexity of aseptic systems makes investigation very difficult
- ✓ Most investigations reveal many “smoking guns”
- ✓ Investigation requires multidisciplinary team
- ✓ Investigation requires time and resource commitment
- ✓ Micro testing is critical - but only one piece of puzzle

Thank You!

Questions?

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