Microbiology 101
for Small Meat Processors

NICHE MEAT PROCESSOR
ASSISTANCE NETWORK

February 1, 2012 Webinar

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MICROBIOLOGY 101

Microbiology 101

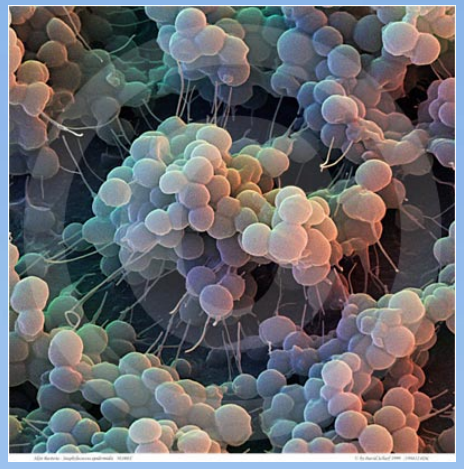
- **Microorganisms in Food**
- **Pathogens**
- **Spoilage Organisms**
- **Microbial Analysis**

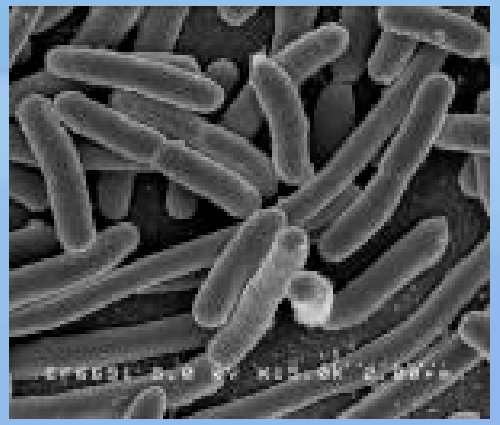
Microorganisms in Food

- What are microorganisms?
- Types of microorganisms
- Microorganisms in food
- Factors affecting microbial growth

What are microorganisms?

- Organisms that are too small to be seen clearly with the naked eye. (micro meaning small, and organism meaning living being).

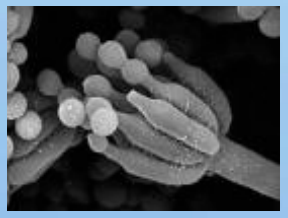




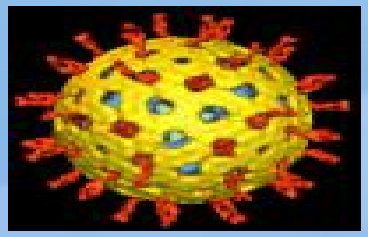
Types of microorganisms

- Bacteria

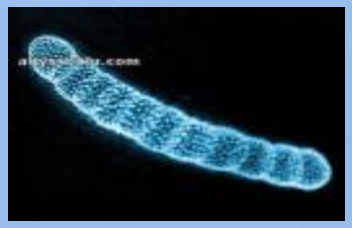
- Fungi (Molds and Yeasts)



- Viruses



- Protozoa



Bacteria

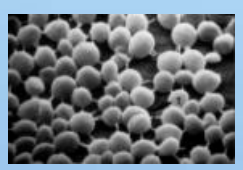
- They are the largest group of microorganisms.
- They are prokaryotic cells
- They are the most important to the food processor.
- Bacteria vary in size:

  Most bacteria range from 0.5 um to 2.0 um in diameter

  (25,000 cells side by side to equal one inch)

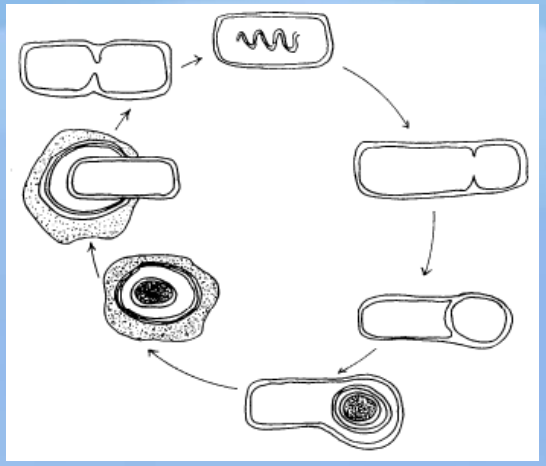
Bacterial cell shapes

- **Bacillus** (Rod)
- **Coccus** (Spherical)
- **Vibrio**
- **Spirillum**
- **Spirochete**



Bacterial spores: Endospores

- Some bacteria produce spores to survive adverse conditions.
- The main bacterial genera that are of significance in foods are *Bacillus* and *Clostridium*.
- Endospores are resistant to heat, drying and chemicals.
- When the conditions are favorable, these spores can germinate and grow into vegetative cells.

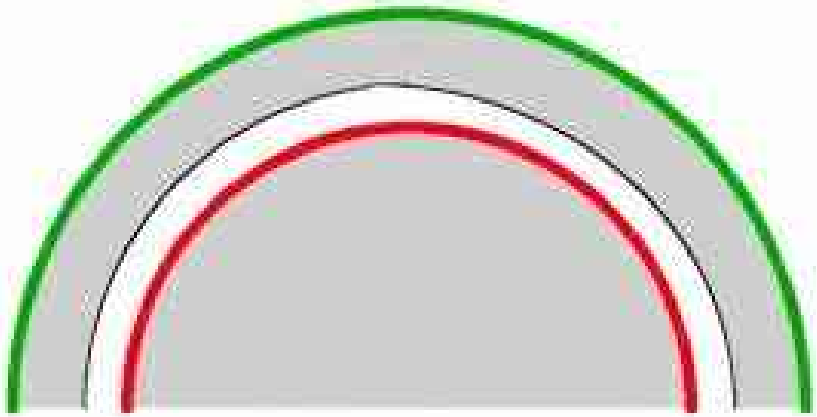


Differentiating Bacteria by Cell Wall

- **Gram-Positive Bacteria**: Cell wall in Gram-positive bacteria has a thick layer of peptidoglycan.
- **Gram-Negative Bacteria**: Cell wall is more complex, it has thin layer of peptidoglycan, polysaccharides, proteins and lipids.

Gram positive

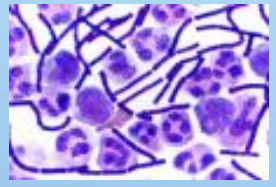
Gram negative



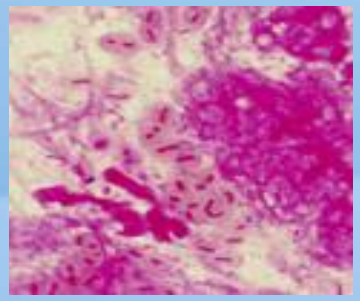
Red: cell membrane
Black: peptidoglycan
Green: Outer membrane

Bacterial Staining: Gram stain

- Based on the ability of bacteria to retain specific dyes:
  - Gram Positive: purple with crystal violet

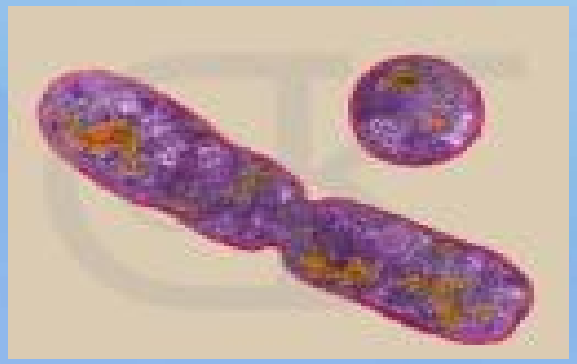


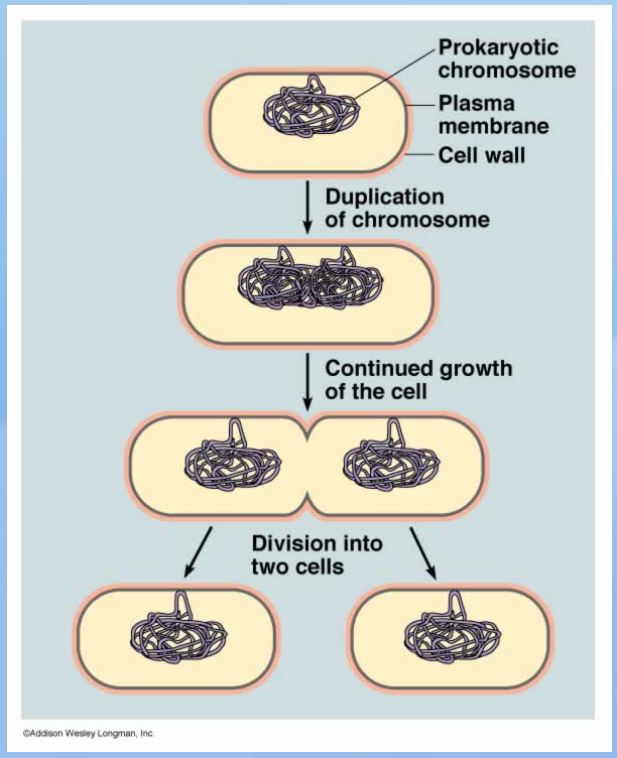
  - Gram Negative: red with safranin (counter-stain)



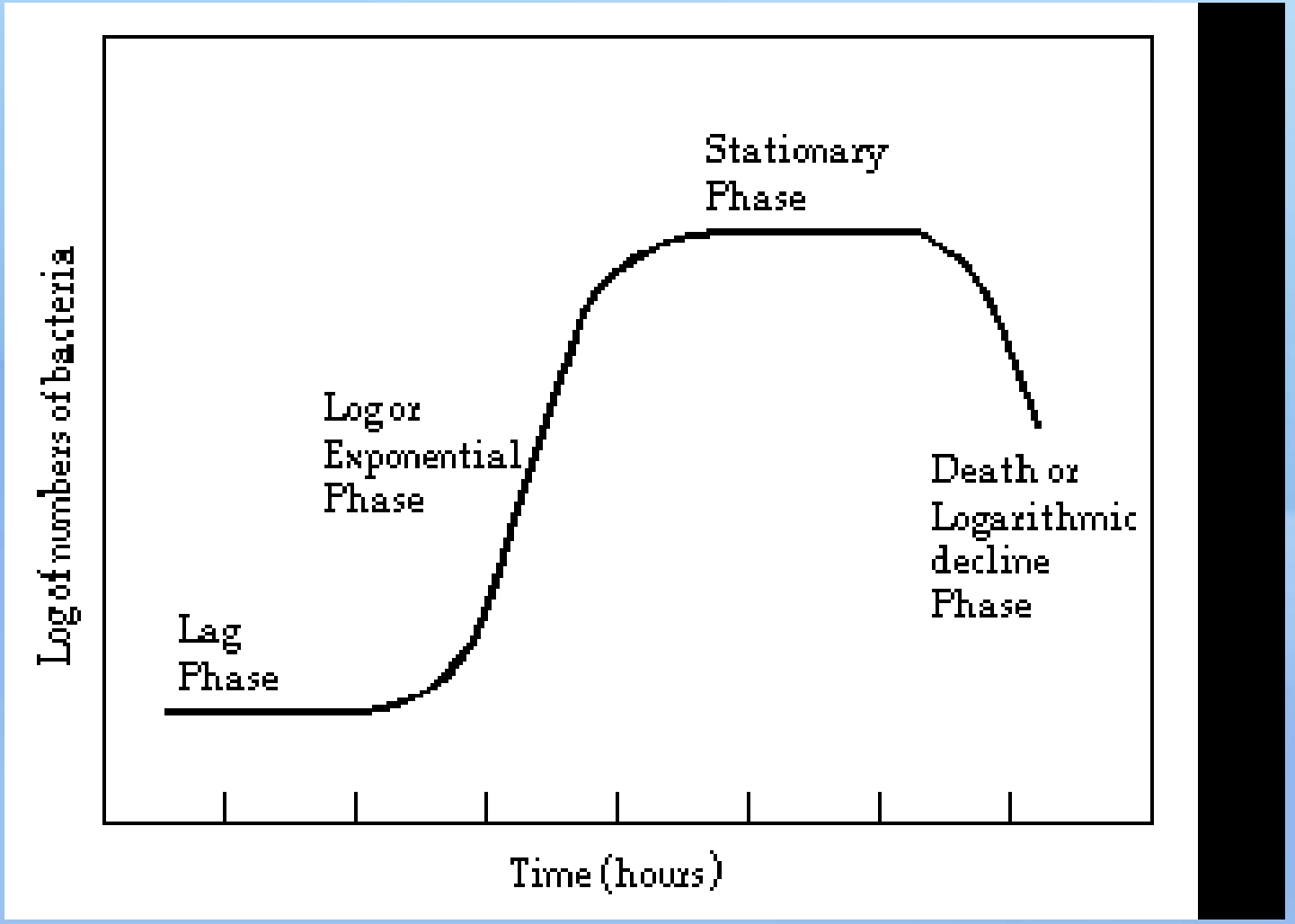
Bacterial growth

- Bacterial growth is defined as the increase in the number of cells, which occur by cell division: binary fission





Bacterial growth curve



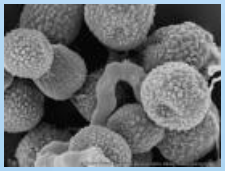
Molds

Multicellular microorganisms with microscopic filaments called *hyphae*.

The mass of intermeshed hyphae, as seen macroscopically, is a *mycelium*.

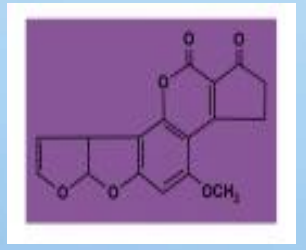
Molds

- Molds propagate by producing spores.
- These spores are the primary agents for the dispersal of molds.
- Mold spores are transported by air, insects, animals...



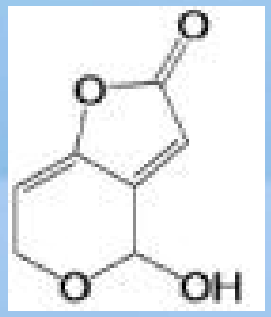
Concerns of molds in foods

- Spoilage



- Mycotoxins

  (Aflatoxins, Patulin…)



- Allergies

- Infections

Yeasts

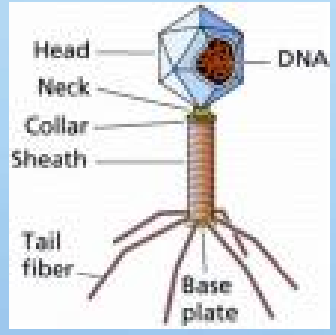
- Non-filamentous fungi, unicellular
- Cells that are ovoid or spheroid
- Reproduce mainly by budding

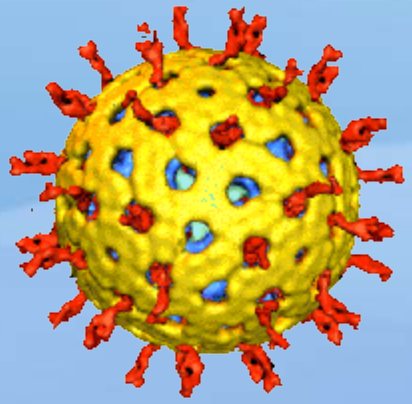
Food spoilage by yeasts

- Yeasts cause spoilage of various foods:
  - Frankfurters, ham
  - Honey, jam, jellies, syrups, candy…

Viruses

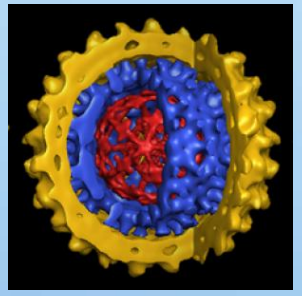
- Submicroscopic, parasitic, acellular microorganisms composed of a nucleic acid (DNA or RNA) core inside a protein coat.

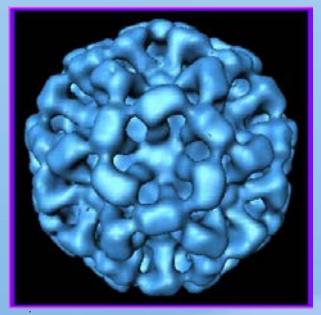


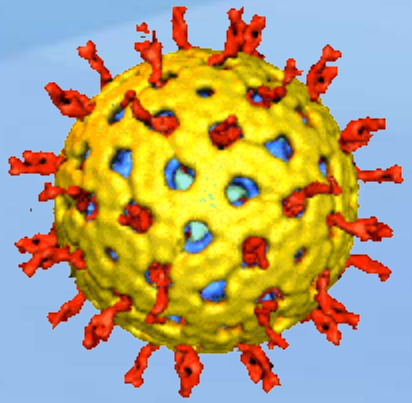


Enteric viruses

- Hepatitis A virus
- Norwalk and related viruses
- Rotavirus

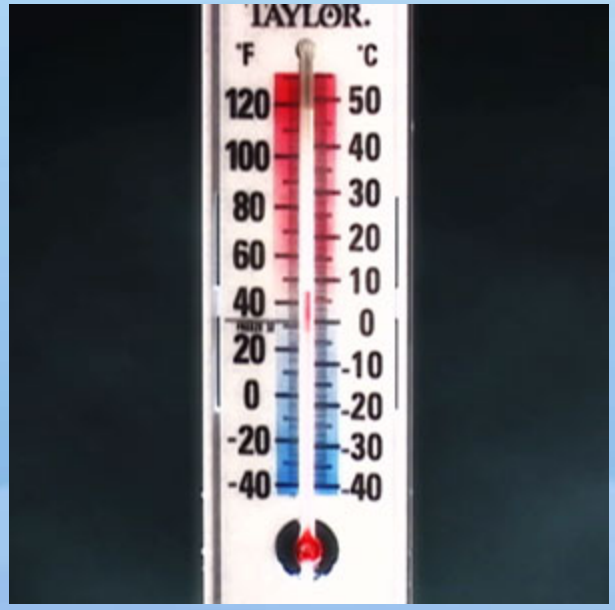






Factors affecting microbial growth

- **Intrinsic Factors**
  - Water Activity (A_w)
  - pH
  - Oxidation-Reduction Potential
  - Nutrients
  - Preservatives
  - Inhibitory substances
- **Extrinsic Factors**
  - Temperature
  - Availability of oxygen
  - Relative Humidity



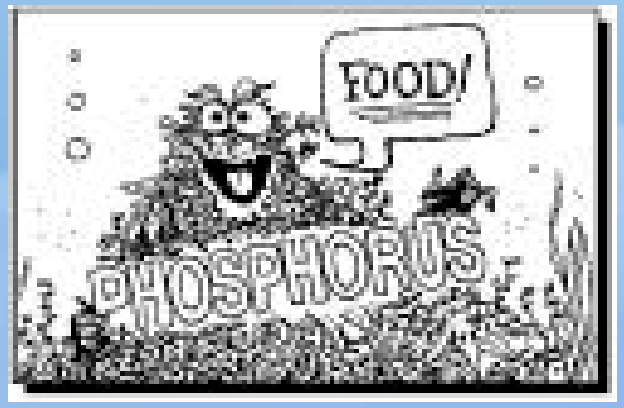
Nutrients

- Energy sources

  Carbohydrates, proteins, lipids
- Nitrogen sources

  Amino acids, peptides, proteins
- Mineral sources

  Sulfur, phosphorus…
- Vitamins



pH range for microbial growth

The optimal growth of most microorganisms is near neutrality (pH 7). However, pH range for growth for some foodborne pathogens:

- Bacteria: 4-8
- Yeast: 2-8
- Molds: 1-11

Temperature

- **Psychrophiles**

  Grow well at 0^0C (32^0F), and have an optimum growth temperature of 15^0C (59^0F) or lower.
- **Psychrotrophs**

  Can grow at 0–7^0C (32–45^0F) even though their optimal temperature is between 20–30^0C (68-86^0F). They are major factors in the spoilage of refrigerated foods.
- **Mesophiles**

  Grow very well at or near human body temperature. The optimal temperature is between 20-45 ^{0}C (68-113^0F). Almost all human pathogens are mesophiles.
- **Thermophiles**

  High temperature loving microorganisms. They often have optima between 55–65^0C (131-149^0F).

Oxygen availability

- **Aerobic microorganisms**

  Require free oxygen

  i.e. *Pseudomonas* species
- **Anaerobic microorganisms**

  Grow well in the absence of oxygen

  i.e. *Clostridium* species
- **Facultative microorganisms**

  Grow with or without the presence of free oxygen

  i.e. *Lactobacillus* species

Water Activity (Aw)

- Vapor pressure of the water in food (p) divided by the vapor pressure of pure water (p_0) at same temperature. Amount of free water available for microbial growth.

$$\mathbf{A = p/p_0 = ERH/100}$$

ERH – Equilibrium Relative (in %) Humidity

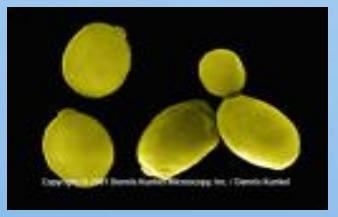
Scale: 0 to 1

Food	**A_w**
Dried milk	0.2
Cocoa powder	0.4
Raw chicken, tomatoes	0.95-1.0
Water	1.0

Minimum A_w for microbial growth

- Bacteria: 0.90

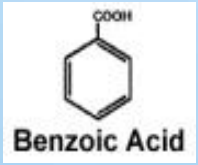
- Yeasts: 0.87



- Molds: 0.70

Chemical preservatives and other inhibitory substances

Use of chemical preservatives to inhibit growth of microorganisms



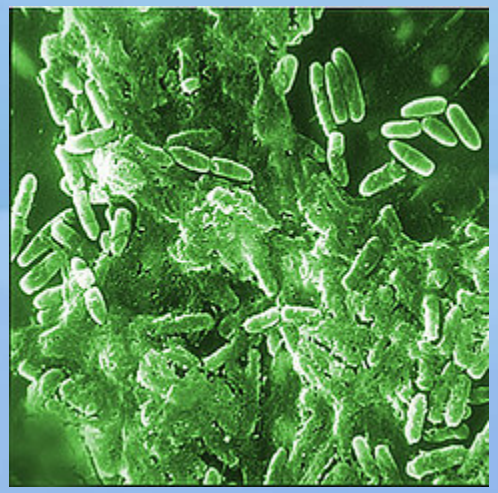
Example: Sodium benzoate

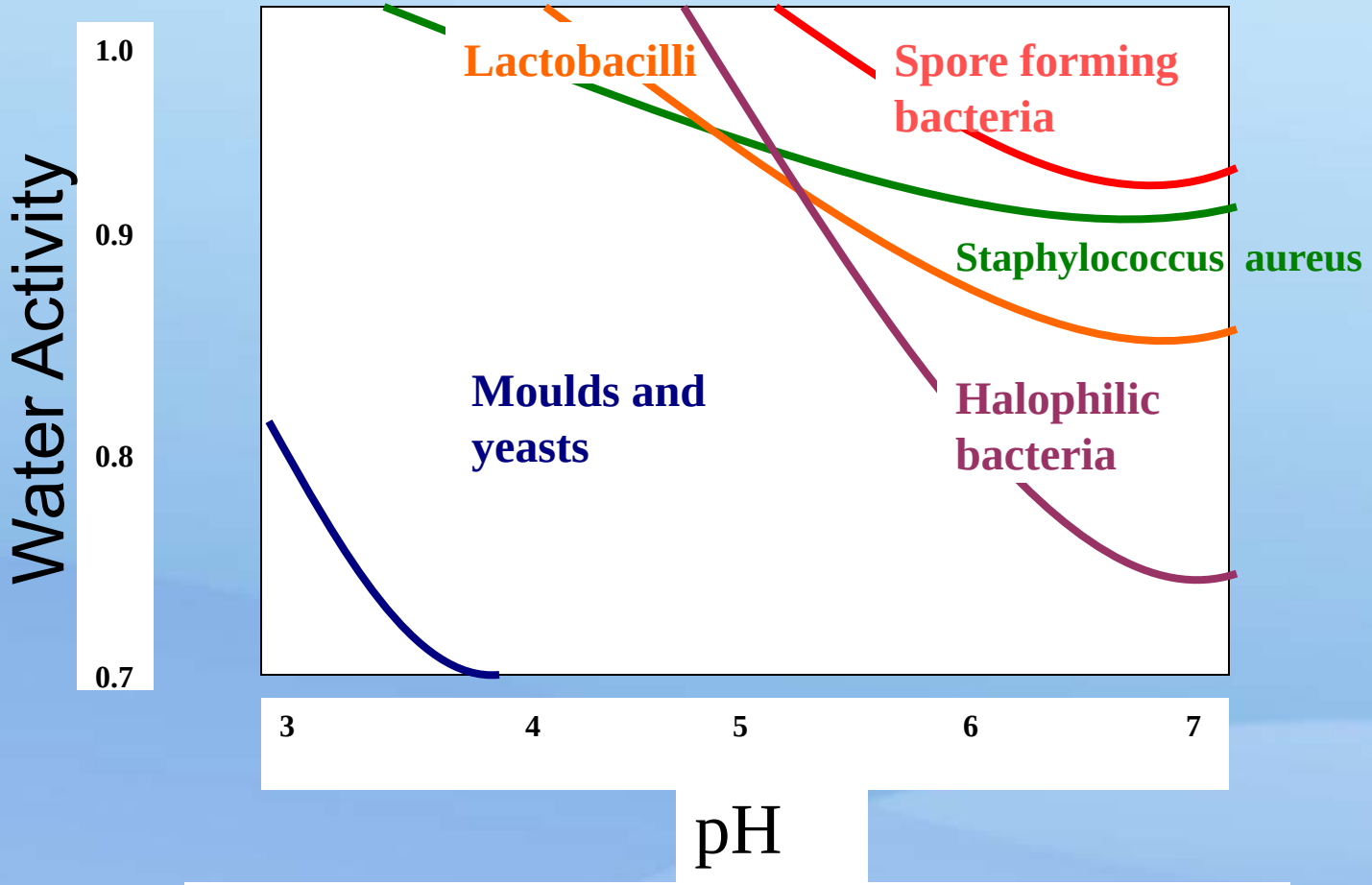
Use of chemicals to kill microorganisms

Example: Sanitizers

Other factors

- Interaction between growth factors: temperature, pH, and A_w
- Relationship between microbial load, temperature, and time to microbial growth
- Effect of biofilms





A schematic diagram of the combined influence of pH and Aw on microbial growth.

(Pitt and Hocking, 1999)

Questions

Microorganisms in Food

- **Microorganisms in Food**
- **Pathogens**
- **Spoilage Organisms**
- **Microbial Analysis**

Salmonella species

- Mode: Infection
- Causes severe diarrhea with fever, vomiting, and dehydration
- Infective dose – as few as 20 cells to 10,000
- Onset usually 12 to 14 hours
- Symptoms last 2 to 3 days (can get reactive arthritis 2 to 3 months later)
- Cases of illness associated with undercooked meat and poultry, eggs, cereal, pet food, peanut butter, produce (tomatoes, peppers)

Salmonella species

- Facultative anaerobe, Gram-negative rods
- Optimal growth 37ºC and pH 6.5 to 7.8, but can grow as low as 2 to 4ºC and up to 54ºC, and pH from 4.5 to 9.5*
- Primary reservoir – Intestinal tract of animals
- Incoming animals and animal products including eggs, contaminated produce, nuts, and grains, insects, birds
- Can survive in dry foods and refrigerated foods for prolonged periods of time

*D'Aoust etal. (2001)

Salmonella – Multi-drug resistant (MDR)

- Salmonella with resistance to 2 to 10 antibiotics.
- Overall prevalence of Salmonella strains was 4.2 percent, while the prevalence of MDR salmonella was 0.6 percent
- Research shows decontamination treatments that reduce *Escherichia coli* O157:H7 contamination on beef also reduce non-O157 Shiga toxin-producing *E. coli* (STEC), plus MDR *Salmonella*
- Greater attention to removal of lymph nodes and hides of cattle, especially culled dairy cows, important to combat Salmonella prevalence

Salmonella species

- Control
  - Heat treatment
    - Concern with low Aw products at minimally process temperatures
  - Prevention of cross contamination
    - Air flow / dust control
    - Residue on equipment
  - Reduction of incoming raw materials

Escherichia coli O157:H7

- Mode: Toxin mediated infection
- Causes severe bloody diarrhea, cramping, fever, and can lead to HUS
- Infective dose – as few as 10 cells
- Onset usually 4 to 10 days
- Symptoms last 4 to 14 days (6% lead to HUS with half of that requiring dialysis, fatality ~1%)
- Cases of illness associated with undercooked meat, produce, raw milk, cookie dough

Escherichia coli O157:H7

- Facultatively anaerobic, Gram-negative rods
- Acid tolerant – growth at pH 4.0 to 4.5
- No unusual heat resistance (D value of 9.6s at 64.3ºC)
- Primary reservoir – Intestinal tract of cattle, as well as sheep and goats.
- Seasonal pattern of outbreaks during warmer months
- Sources of outbreaks include undercooked beef, raw milk, and produce contaminated via manure.

E. coli Non-O157 STECS

- USDA extended a zero-tolerance policy for *E. coli* O157:H7 in raw beef products by declaring six additional strains of *E. coli*, known as non-O157 Shiga-toxin producing *E. coli* (STECs)
- Beginning in March 2012, any raw ground beef, its components, and tenderized steaks that test positive for non-O157:H7 Shiga-toxin producing *E.coli* strains O26, O103, O45, O111, O121 and O145 will be considered adulterated

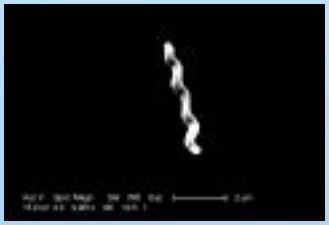
E. coli Non-O157 STECS

- An outbreak of *E. coli* O26 –associated with ground beef produced by Cargill's Wyalusing, Pa. was the first non-O157 STEC outbreak linked to beef.
- USDA's Food Safety and Inspection Service (FSIS) will begin testing for the six serogroups of STEC and enforcing the new policy on March 5, 2012.
- USDA Procedure - the confirmatory PCR shall include the *stx* and *eae* multiplex PCR assay and the serogroup (*wzx*) specific multiplex PCR assay.

Escherichia coli O157:H7

- Control
  - Heat treatment
  - Prevention of cross contamination
    - Manure
    - Raw beef
  - Intervention strategies to eliminate from raw meat

Campylobacter



- Mode: Infection
- Causes diarrhea (sometimes bloody), cramping, fever, can result in Guillain-Barre syndrome
- Infective dose – less than 1,000 organisms
- Symptoms last several days to a week
- Most common cause of sporadic bacterial gastroenteritis
- Cases of illness associated with undercooked poultry and meat, unpasteurized milk, and contaminated water

Campylobacter

- Microaerophilic (low oxygen) Gram-negative curved rods; motile
- Does not grow well below 30ºC; sensitive to drying
- No unusual heat resistance
- Primary reservoir – Many animals including poultry, cows, pigs, sheep, and wild birds. Also contaminated water and flies.

Campylobacter

- Control
  - Heat treatment
  - Intervention strategies to reduce prevalence in raw poultryn (ex. antimicrobials, air chilling)

Listeria monocytogenes

- Mode: Infection
- Causes diarrhea, meningitis, encephalitis, septicemia, miscarriages, stillbirths
- Affects high risk groups – Pregnant women, neonates, and immunocompromised adults
- Symptoms develop within 3 to 70 days
- Transmitted to foods via post process handling, or food is inadequately processed.
- Recent issues – Sliced deli meat and other RTE meats, hot dogs, cheese, ice cream, coleslaw

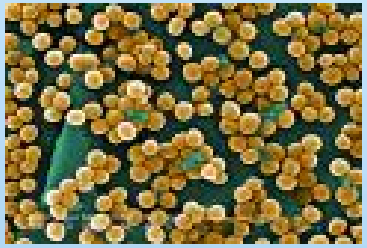
Listeria monocytogenes

- Gram-positive, non-spore forming, facultative, motile rod
- Associated with animals, soil, water, food plants (cold and moist areas)
- Growth and survival
  - Low temperature - growth as low as 0ºC, and can survive freezing
  - pH – growth down to 5.6*, and may survive below 4.3
  - Aw – growth down to 0.93, can survive to 0.83
  - Salt – growth up to 10 to 12% NaCl, can survive in higher concentrations for extended periods of time
  - High temperature – inactivated at temperatures above 50ºC* (D-values at 160ºF – up to 5 seconds)

Listeria monocytogenes

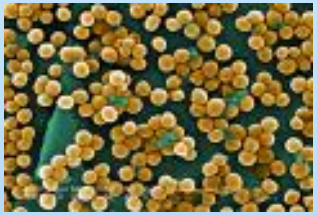
- Control
  - Plant sanitation to prevent harborage sites and points for cross contamination with specific attention to biofilms
  - Prevent entry through people, materials, and equipment
  - Monitoring of environment
  - Proper temperature control of foods
  - Temperature and moisture control in environment

Staphylococcus aureus



- Mode: Intoxication
- Causes vomiting, nausea, and abdominal cramps
- Symptoms develop within 4 hours of ingestion and last 24 to 48 hours
- Transmitted to foods via post process handling - organism grows on a temperature abused food item and produces a heat-stable toxin. The ingested toxin causes the illness.
- Recent issues – batter, cooked chicken, canned mushrooms, cream filled pastries

Staphylococcus aureus



- Gram-positive cocci that grow under aerobic and anaerobic conditions
- While organism can grow as low as 6.7ºC, optimal growth and toxin production occurs between 40 and 45ºC
- Toxin is heat stable
- Salt tolerant (up to 20%) and can grow at Aw as low as 0.84
- Primary reservoir – Animals including humans (skin, hair, nose, open sores, boils, saliva)

Staphylococcus aureus

- Control
  - Good personal hygiene practices
    - Washing hands
    - Covering wounds
  - Proper temperature control of foods
    - Keep hot foods hot and cold foods cold

Clostridium perfringens

- Mode: Toxin-mediated infection
- Causes diarrhea, severe dehydration, cramps
- Symptoms develop within 8 to 22 hours
- Organism grows to large numbers in food (10^8), organisms are ingested, and then produce toxin in intestine
- Associated foods: temperature abused foods, roast beef, stews, meat gravy, poultry

Clostridium perfringens

- Gram positive, spore forming, anaerobic rod
- Heat resistance – spores can survive boiling
- Found in soil, intestinal tracts of animals
- Control – Cool foods rapidly after heating

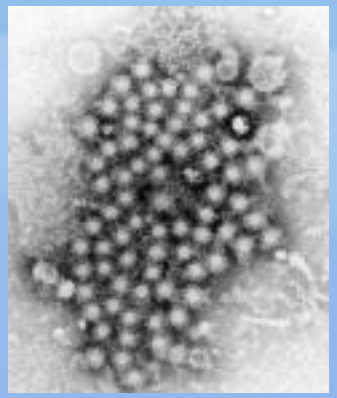
Other Bacterial Pathogens

- *Clostridium botulinum*
- *Bacillus cereus*
- *Vibrio* spp.
- *Yersinia enterocolitica*
- *Shigella* ssp.
- *Aeromonas hydrophila*

Viruses

Hepatitis A

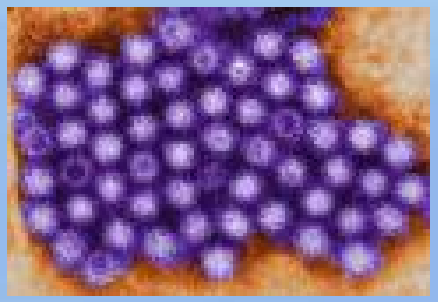
- Low infective dose (10 to 100 virus particles)
- Fever, malaise, nausea, abdominal discomfort, jaundice
- Spread through personal contact to food or food contact surface
- Associated with ready-to-eat foods
- Control – good personal hygiene



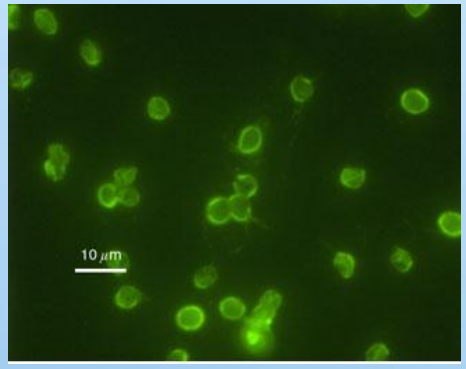
Viruses

Norwalk virus

- Low infective dose (10 to 100 virus particles)
- Vomiting, diarrhea, and dehydration
- Spread through personal contact to food or food contact surface
- Associated with ready-to-eat foods, shellfish, and contaminated water
- Control – good personal hygiene



Parasites



- Giardia
- Cryptosporidium parvum
- Cyclospora
- **Sources** – contaminated water or foods that have come in contact with contaminated water, employees with organism
- **Control** – use of potable water, good personal hygiene

Questions

Microorganisms in Food

- **Microorganisms in Food**
- **Pathogens**
- **Spoilage Organisms**
- **Microbial Analysis**

Spoilage Organisms

- Lactic Acid Bacteria
- Gram negative psychrotrophic organisms
- Psychrophilic *Clostridium*
- Yeast and Mold

Lactic Acid Bacteria Spoilage

- 13 genera of gram positive bacteria which include *Lactococcus*, *Lactobacillus*, *Leuconostoc*, *Pediococcus*, and *Streptococcus*
- Produce lactic acid in either homofermentive or heterofermentive pathways
- Fastidious – require certain nutrients for growth (such as preformed amino acids)
- Can grow below 5ºC and as high 45ºC, from pH 3.2 to as high as 9.6
- Will be predominate on vacuum packed meat where aerobic gram negative organisms are limited

Gram-negative Psychrotrophic Spoilage Organisms

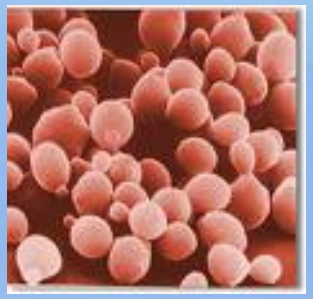
- Numerous genera of gram negative bacteria which include *Acinetobacter*, *Pseudomonas*, *Flavobacterium*, *Alcaligenes, Psychrobacter, and Moraxella*
- Many species capable of growth down to refrigeration temperatures
- Aerobic organisms such as *Pseudomonas* will grow rapidly on air chilled meat products producing off odors from the breakdown of proteins.
- In dairy products, species such as *Pseudomonas* and *Flavobacterium* produce the fruity, rancid, and bitter flavors

Psychrophilic *Clostridium*

- Species of gram positive sporeforming *Clostridium* bacteria which include *Cl. esterheticum*
- Production of carbon dioxide, hydrogen causing pack distention and butonal, butanoic acid, ethanol, acetic acid, and sulfur containing compounds
- Fastidious, do not compete as well against other microorganisms, and can be sensitive to antimicrobials

Yeast and Mold Spoilage

- Grow over a wide range of acid pH and Aw compared to bacteria
- Yeast can form slime on wieners and other meat products
- Some molds can produce mycotoxins
- Spoil fruits, vegetables, grains, nuts, meat
  - *Geotrichum* referred to as dairy mold or machinery mold
  - *Mucor* – forms whiskers on beef



Mold

- Multicellular fungi – form mycelium
- Grow over a wide pH range (below 1 to 11)
- Grow at low Aw (as low as 0.6)
- Some species produce mycotoxins

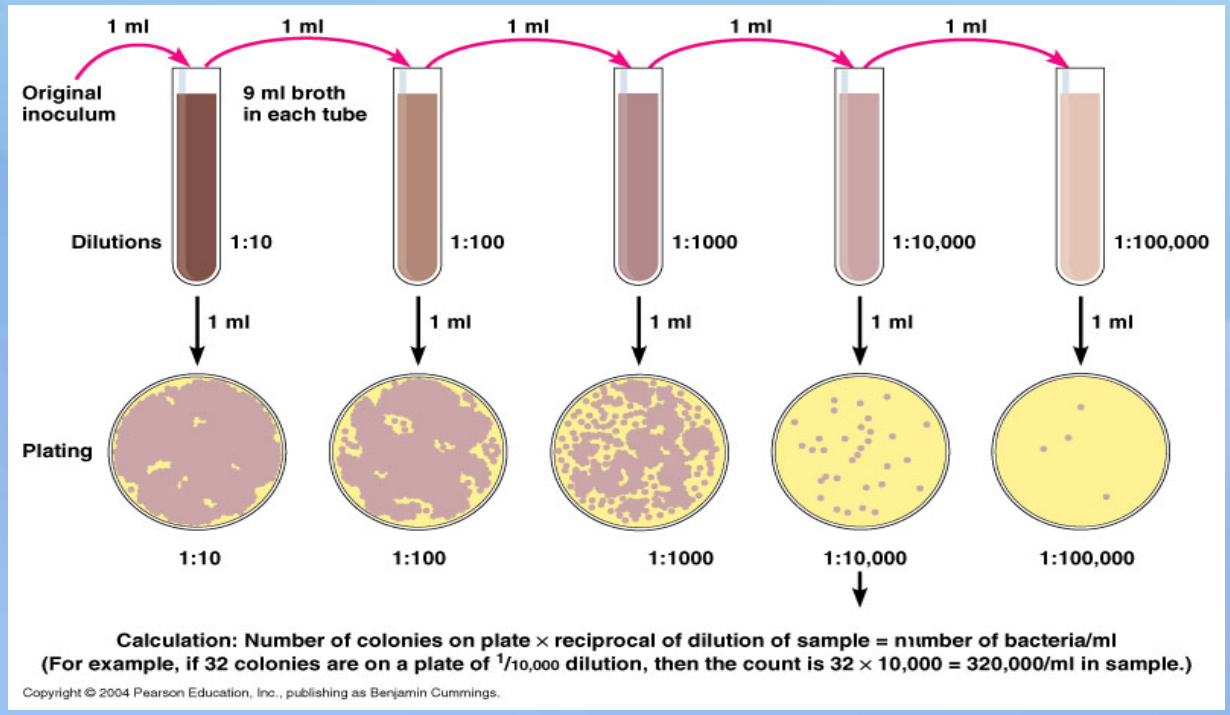
Questions

Microorganisms in Food

- **Microorganisms in Food**
- **Pathogens**
- **Spoilage Organisms**
- **Microbial Analysis**

Microbial Analysis

- Want Count (versus Presence/Absence)
- Counts by plating (CFU – colony forming unit)



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Microbial Analysis – APC / SPC

- Aerobic Plate Count / Standard Plate Count
  - Uses Standard Methods Agar incubated at 35 or 37ºC for 48 hours
  - Advantage - standardized methodology producing comparable results, easy analysis to conduct
  - Disadvantages
    - will not get good growth of fastidious organisms,
    - will not get much, if any, growth of anaerobic organism,
    - will not get good growth of psychrophilic organisms

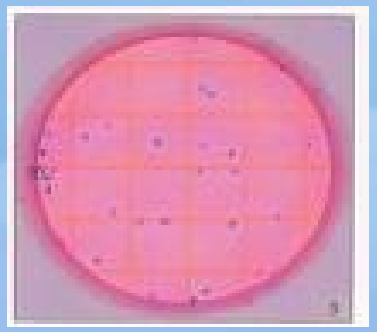
Microbial Analysis - SPC

Considerations

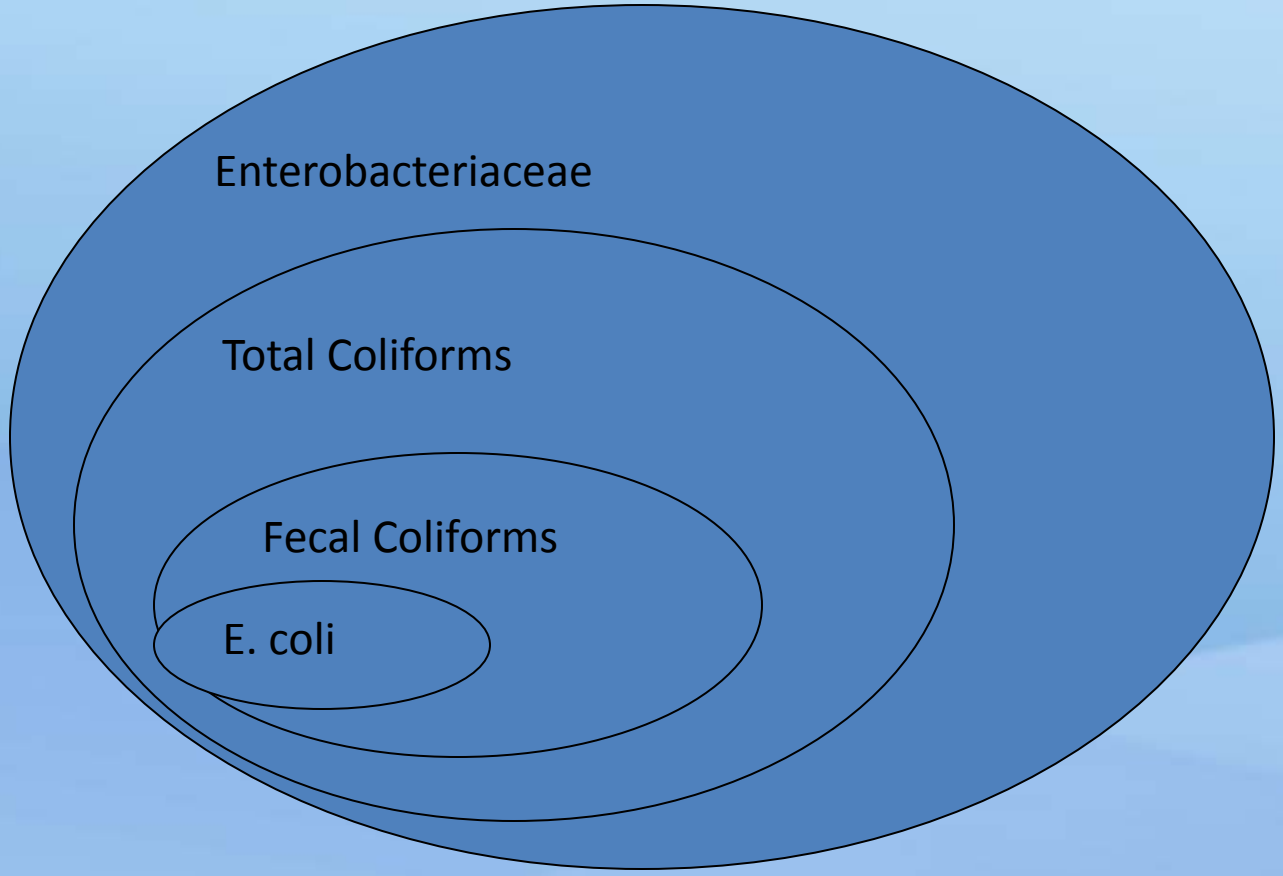
- Use more nutritious media – such as MRS, to capture lactic acid bacteria
- Incubate plates anaerobically to capture levels of anaerobes
- Incubate plates at low temperature / longer time to capture psychrotrophic bacterial counts
- Use media designed for yeast and molds if those types of organisms are considered an issue

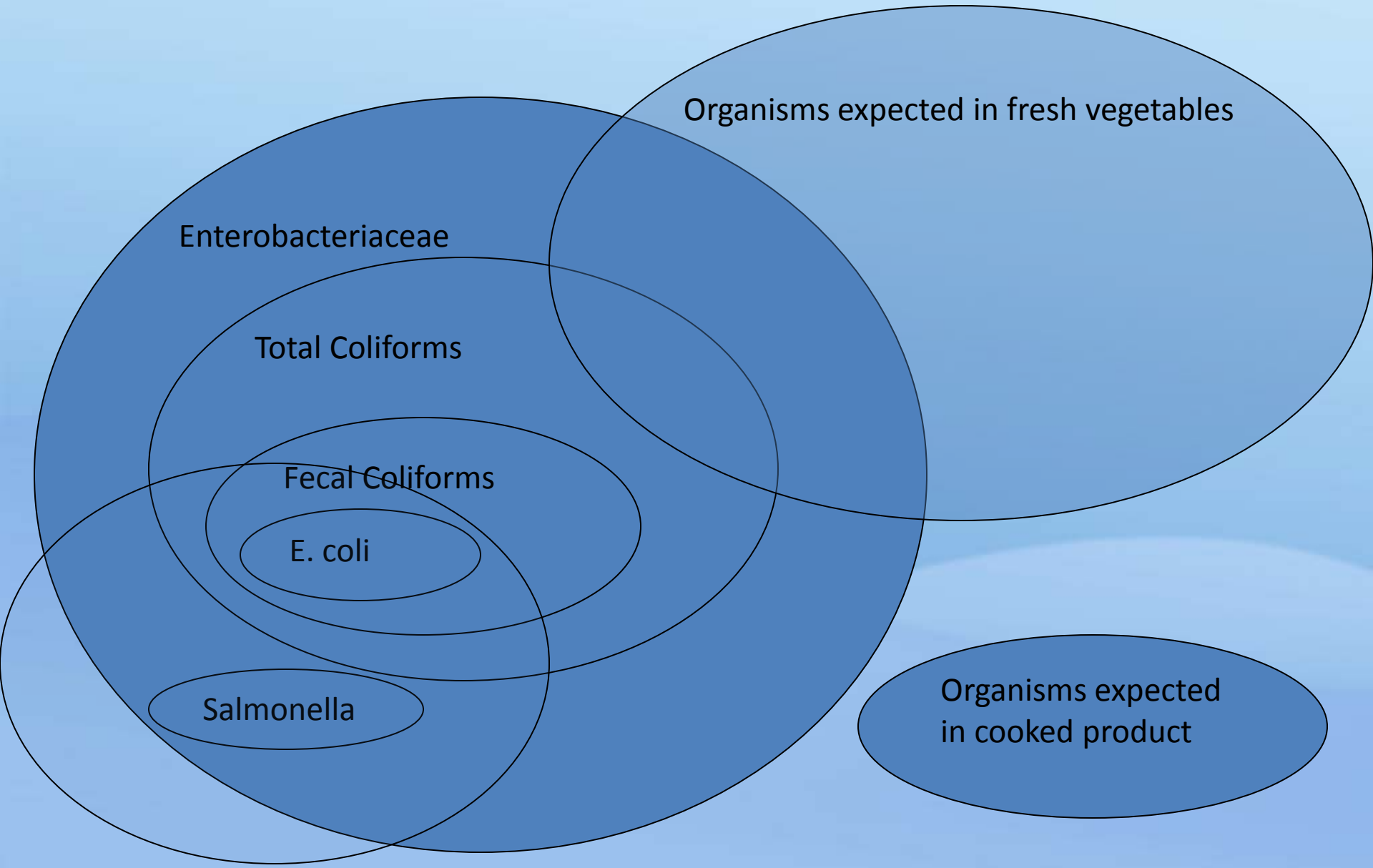
Microbial Analysis - Indicators

- Coliform / Fecal Coliform / generic *E. coli*
  - Methods include MPN, 3M Petrifilm, VRB, etc
  - Progressing from coliforms to *E. coli*, the correlation to fecal contamination increases



Total Coliforms and generic *E. coli*




Organisms expected in fresh vegetables
Enterobacteriaceae
Total Coliforms
Fecal Coliforms
E. coli
Salmonella
Organisms expected
in cooked product

Microbial Analysis - Indicators

- Coliform / Fecal Coliform / generic *E. coli*
  - Advantages - standardized methodology producing comparable results,
  - easy analysis to perform
  - Can give an indication of insanitary conditions

Microbial Analysis - Indicators

- Coliform / Fecal Coliform / *E. coli*
  - Disadvantages – coliforms counts may not be applicable on some products,
  - Not always a good assessment for determining shelf-life or spoilage,
  - Coliform counts for a product may meet standard even though enteric pathogens such as salmonella may be present in low numbers,
  - Not a good indicator of viruses

Analysis for Indicator Organisms

Why?

- Assess food safety and sanitation
  - Correlate to the presence of pathogenic organisms
  - Can be useful to assess sanitary conditions under which food was processed
- Reflect the microbiological quality of foods
  - Relative to shelf life

Pathogen Analysis

Presence/ Absence (versus count)

- Sample size
  - 375 grams versus 25 gram sampling
  - N=60 sampling
- Environmental sampling
  - Use sponge versus a swab
  - Zone sampling

Sampling

- Determination of lot
- Location of samples
- Number of samples
- Size of samples
- Will samples be composited?

Sampling

Lot Definition

- A lot is a quantity of food or food units produced and handled under uniform conditions*
- Should be composed of food produced with as little variation as possible for a given process or commodity*
- From clean-up to clean-up

*ICMSF (2002)

Sampling

A ***representative sample*** reflects as far as possible the composition of the lot from which it is drawn.

- Important to avoid bias and draw sufficient sample units to confidently make a judgment about the given lot.
- Must be able to traceback all product

Sampling

Number of samples

- When testing for food pathogens (absence/presence), normally using 15, 30, and 60 sample units
- As units increase, probability of accepting a defective lot decreases.

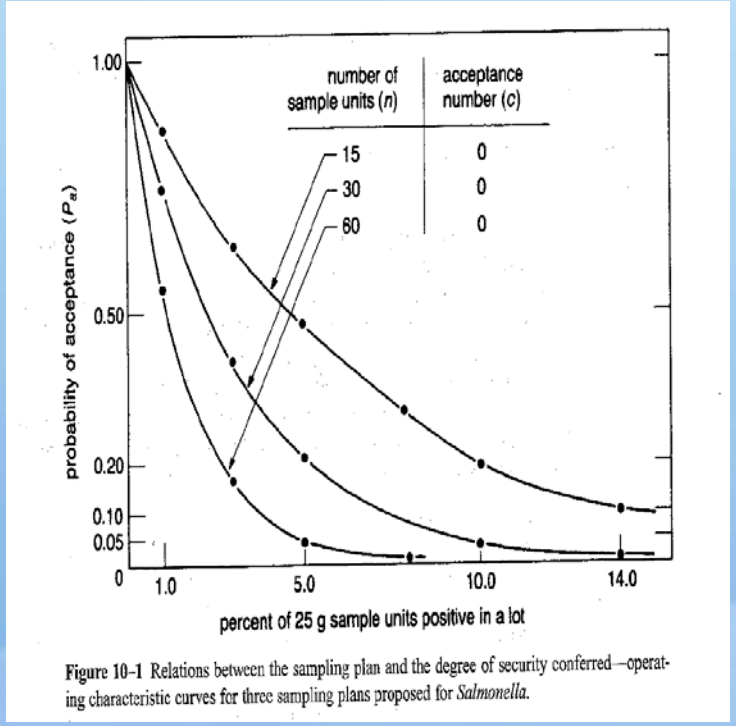


Figure 10-1 Relations between the sampling plan and the degree of security conferred—operating characteristic curves for three sampling plans proposed for *Salmonella*.

Source: ICMSF (2001) Microorganisms in Food 7

Sampling

Number of samples

- N = 60 - For 5% defective rate, the probability of accepting a defective lot is 0.05
- N = 15 samples, P is 0.46 – thus this would be missed approximately 50% of the time).

Sampling

Pooling samples – combining of stomached samples or pre-enriched samples to decrease the number of tests.

- Difficult to determine which sample was positive, especially a problem if pooling different lots
- Risk of diluting out a positive sample
- Need to validate pooling procedure

Testing

Choosing the testing methodology

- Traditional methodology using plating and biochemical reactions
- Immunological based assays
- DNA based assays

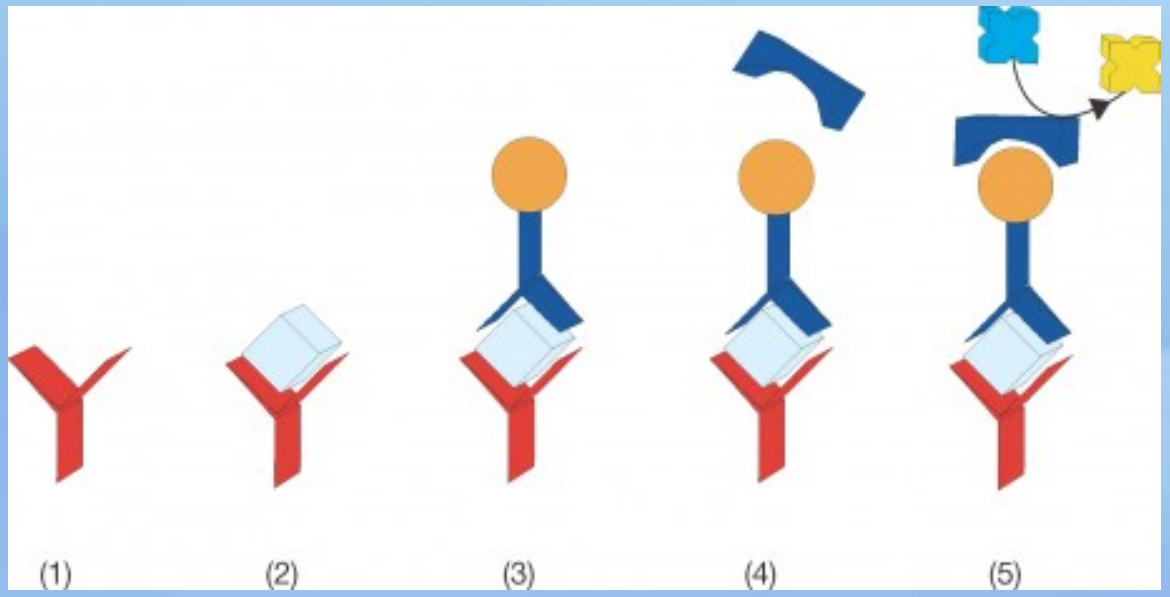
Analysis for Pathogens

Focus on Detection (Presence/Absence)

- Pre-Enrichment / Enrichment
  - Increase the number of target organisms
  - Control or reduce the number of competing organisms
  - Required time usually 8 to 24 hours
- Detection method
  - Cultural
  - ELISA (Enzyme-linked Immuno Sorbent Assay)
  - PCR (Polymerase Chain Reaction)

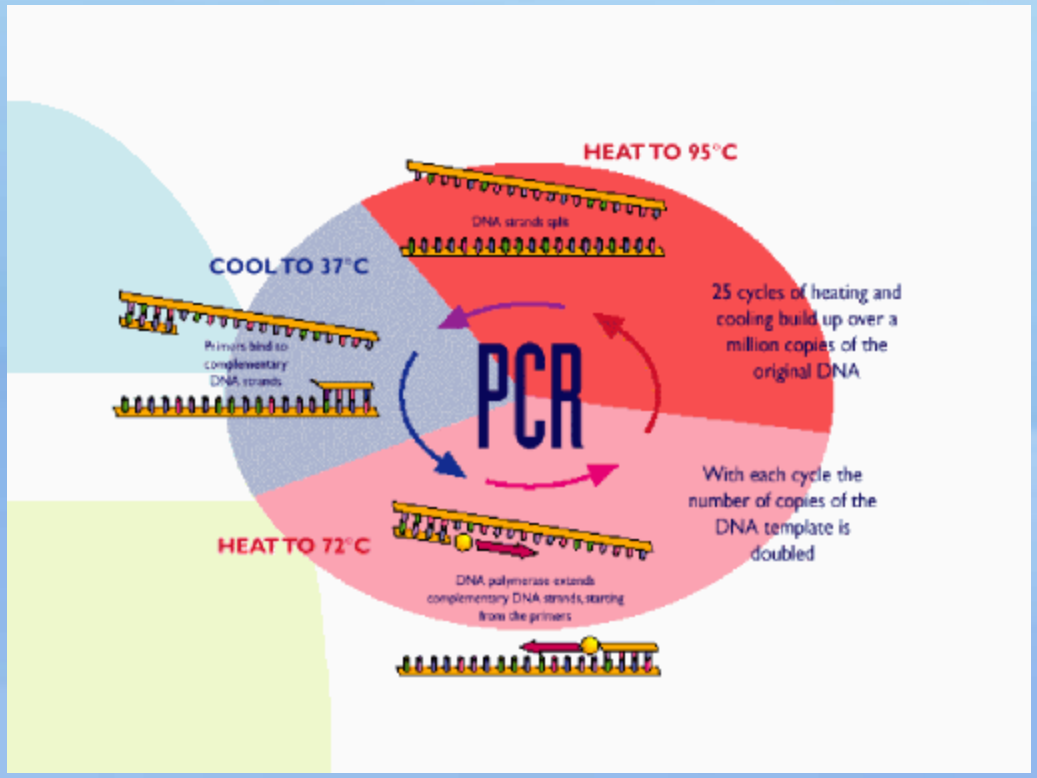
Analysis for Pathogens

- Detection Methods - ELISA



Analysis for Pathogens

Polymerase chain reaction (PCR) enables researchers to produce millions of copies of a specific DNA sequence in approximately two hours



"DNA-Based Methods for Identifying and Subtyping Foodborne Pathogens" Bhushan Jayarao, Penn State University

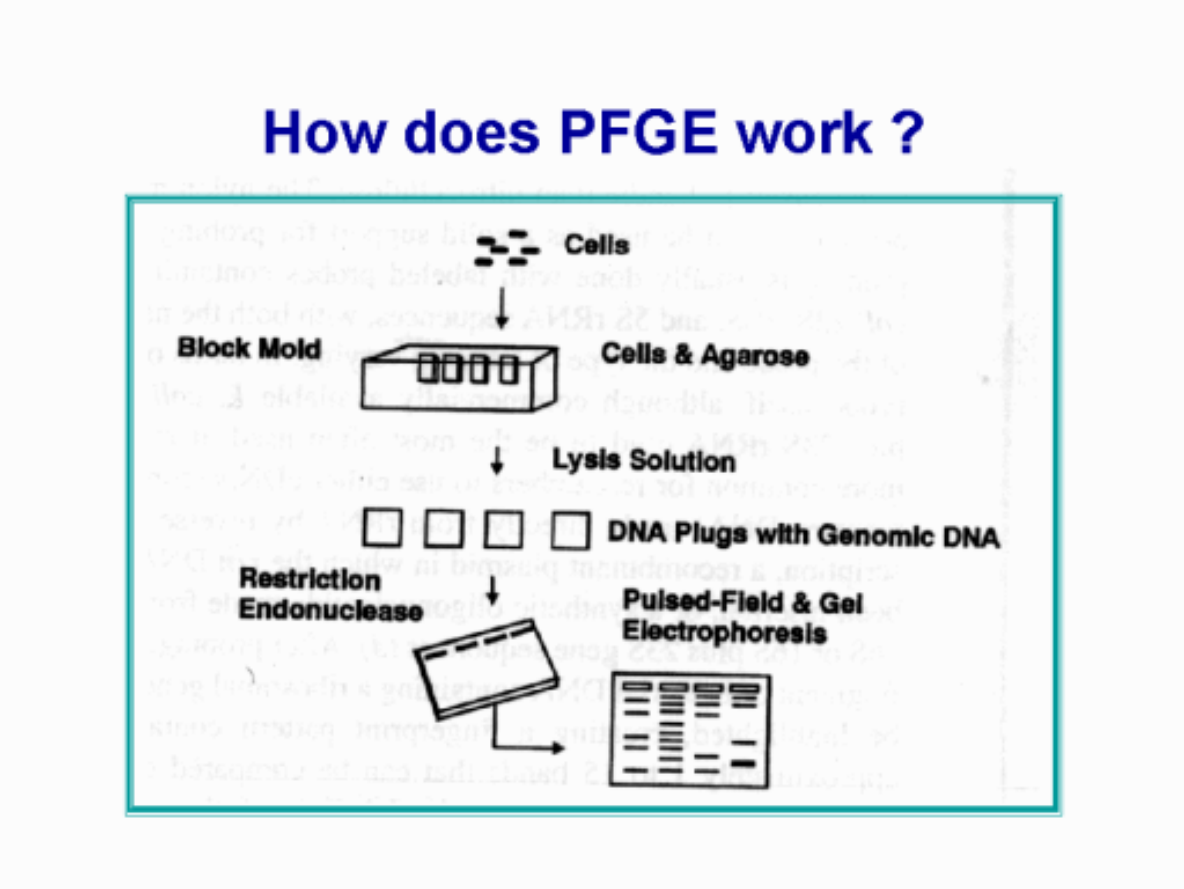
Analysis for Pathogens

Pulsed-Field Gel Electrophoresis

- Scientists find bacterial fingerprints by cutting the bacteria's DNA into tiny pieces and then placing them on a gel, which is a flat slab of gelatin.
- Electricity is sent through the gel, causing the DNA pieces separate.
- Small pieces of DNA get carried farther down the gel than bigger pieces. This process creates a banding pattern or "fingerprint"

Analysis for Pathogens

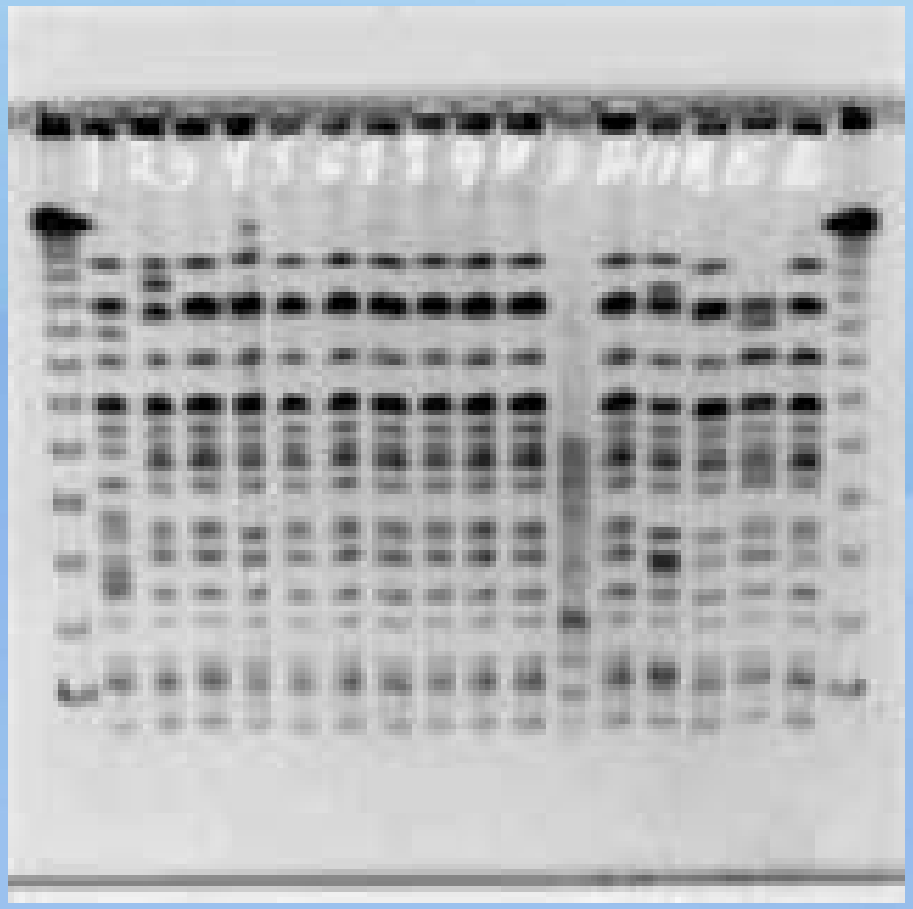
Pulsed-Field Gel Electrophoresis



"DNA-Based Methods for Identifying and Subtyping Foodborne Pathogens" Bhushan Jayarao, Penn State University

Analysis for Pathogens

- PFGE Pattern



Testing Errors

Testing errors can occur

- Type 1 error – test result is positive when the sample is actually negative
- Type 2 error – test result is negative when the product lot tested is actually positive

Microbiological Analysis

Discussion of different analyses

- *E. coli* O157:H7
- *Listeria monocytogenes*
- Verification of Sanitation Program

Analysis for *E. coli* O157:H7

- Raw ground beef and raw ground beef products
- Organism may be at low levels
- Issue with own slaughter as well as incoming whole meat
- FSIS has protocols for working with small establishments handling raw ground beef

Analysis for *E. coli* O157:H7

USDA Sampling and Analysis

Number of samples - *E. coli* O157:H7

- n = 60 is used by USDA for trim that is used in ground meat products - get a total of 325g

  (Sample 12 trim pieces from 5 combos.)

- For ground meat, take five, 65g for 325g total
- Can use n = 60 for produce

Number of Sample Pieces to Collect Per Container	
# of containers in each specific production	*# of sample pieces to select from **each** container*
5	12 pieces
4	15 pieces
3	20 pieces
2	30 pieces
1	60 pieces

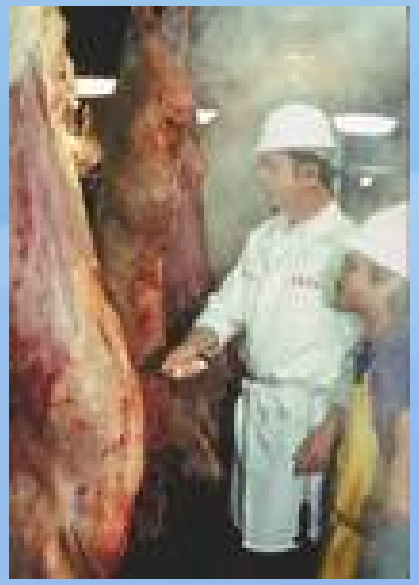
Analysis for *E. coli* O157:H7

Detection Method

- Presumptive: For FSIS testing, a presumptive positive for *E. coli O157 is that reacting to the O157 somatic antiserum. This test and reaction indicates a "strong possibility that E. coli O157:H7 is present."*
- For industry testing, test results that indicate a strong possibility that *E. coli O157:H7 is present are considered presumptive positive results. Positive results of industry testing for E. coli O157:H7 using the BAX (trademark name) methodology would be considered presumptive positive results.*

Analysis for *E. coli* O157:H7

- Request for Certificate of Analysis (COA)
- Use an intervention method
- Composite sample and test



Microbiological Analysis

- *E. coli* O157:H7
- *Listeria monocytogenes*
- Verification of Sanitation Program
- Premature spoilage

Analysis for *Listeria monocytogenes*

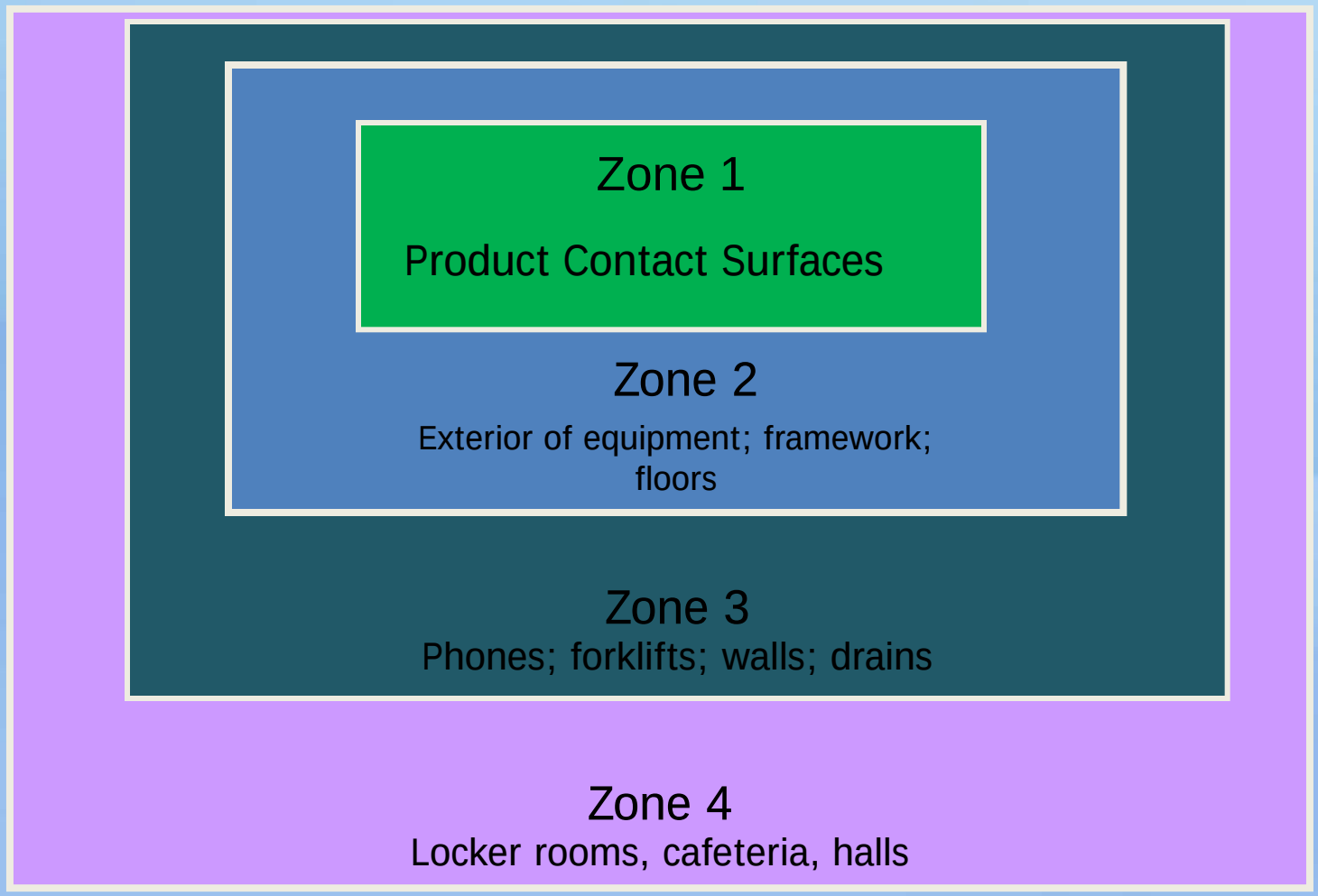
- Ready-to-Eat Meat Products (or products that are heat processed)
- Need to assume that the organism may be present

Analysis for *Listeria monocytogenes*

- Product sampling
  - May not detect low level contamination
  - Test and hold until results complete
  - Best as a verification of LM control system
- Environmental sampling – Zones
  - Zone 1 – Product contact
  - Zone 2 – Adjacent to product contact
  - Zone 3 – Areas in process not Zone 1 or 2
- Waste / scrap sampling

Environmental Sampling

Sanitary Zones Concept



Analysis for *Listeria monocytogenes*

- Environmental sampling – Zones
  - Use moistened sponges
  - Prepare a map so that you can identify each area for easy recording; record legibly
  - Standardize testing protocol
  - Maximize swabbed area
  - Sanitize swabbed areas afterward (Zone 1)
  - Pre-op and occasional operational

Microbiological Analysis

- *E. coli* O157:H7
- *Listeria monocytogenes*
- Verification of Sanitation Program

Microbiological Analysis

- Verification of Sanitation – Why?
  - Best way to verify effectiveness of cleaning and sanitizing
  - Establishes a record for review
  - May help identify trends and thus prevent potential issues

Microbiological Analysis

- Verification of Sanitation – Types of analysis
  - Microbiological testing
    - Swabs
    - Sponges
    - APC, Listeria
  - ATP Testing
  - Food residuals

Microbiological Analysis

- Verification of Sanitation – Surfaces
  - Food contact
  - Non-contact
  - Personnel
  - Air sampling

Microbiological Analysis

- Verification of Sanitation
  - Establish a program
    - Standardized methodology
    - Establish frequency
    - Indentify areas for sampling
    - Random sampling of areas, but resampling trouble areas, areas not appearing clean
  - Go off the program occasionally to test new items (maintenance tools, etc)

Microbiological Analysis

- Verification of Sanitation – Program
  - Analyze data for trends
  - Ensure follow-up on high count areas, including resampling
  - Standards for APC
    - <100 cfu /50 sq cm (<100 cfu /~8 sq in) USPHS*
    - <5 cfu / sq cm (USDA 1994)
    - <1.3 log cfu / sq cm (<20 cfu / sq cm)
    - <100 cfu / 4 sq in

*Compendium of Methods for the Microbiological Examination of Foods 2001

Microorganisms in Food

- **Microorganisms in Food**
- **Pathogens**
- **Spoilage Organisms**
- **Microbial Analysis**

Questions

THANK YOU!

PENNSTATE

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Microbiology 101
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