Shelf Life & Stability Testing

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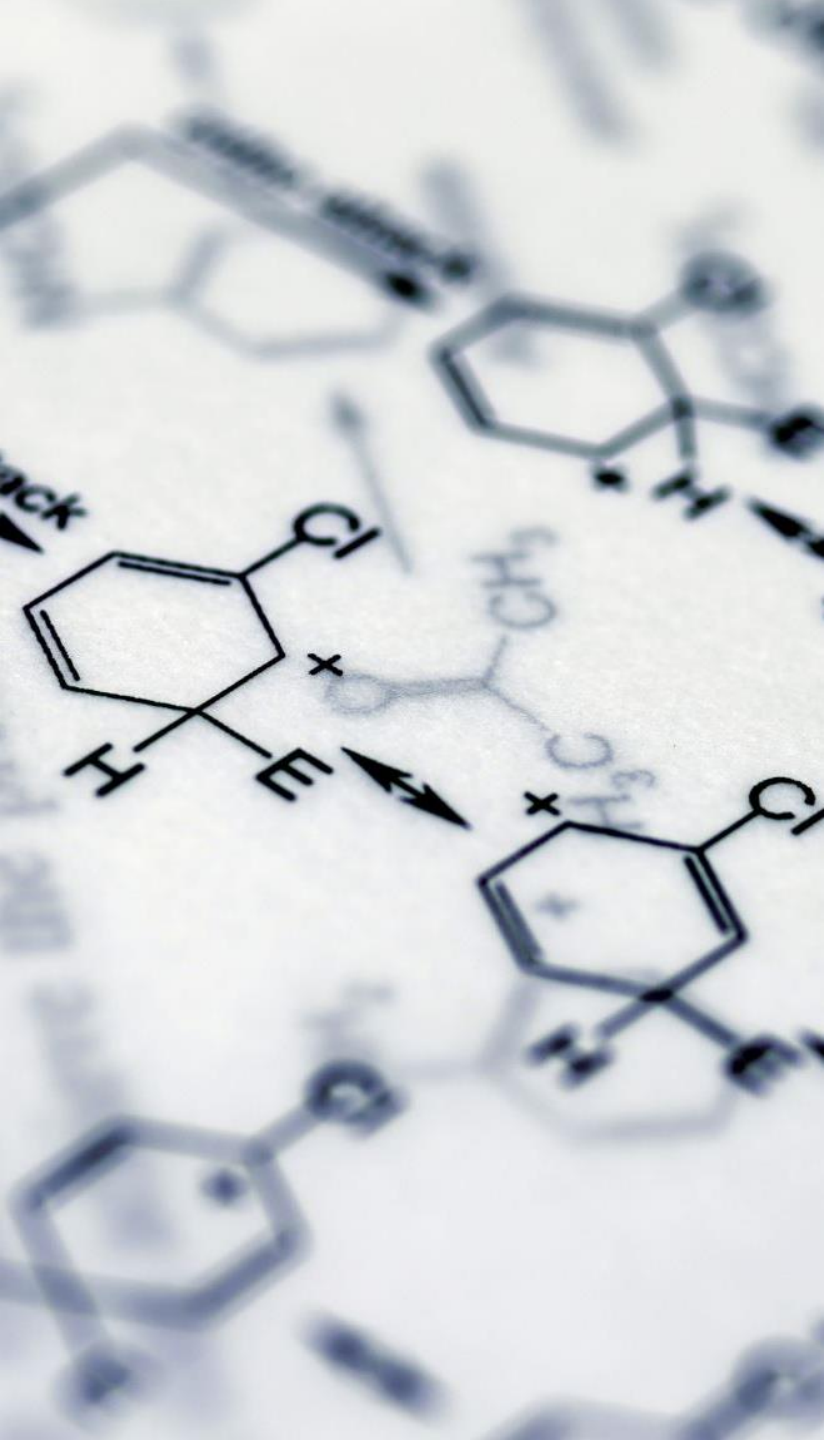
Boswell Ag Tech Center for Food Safety



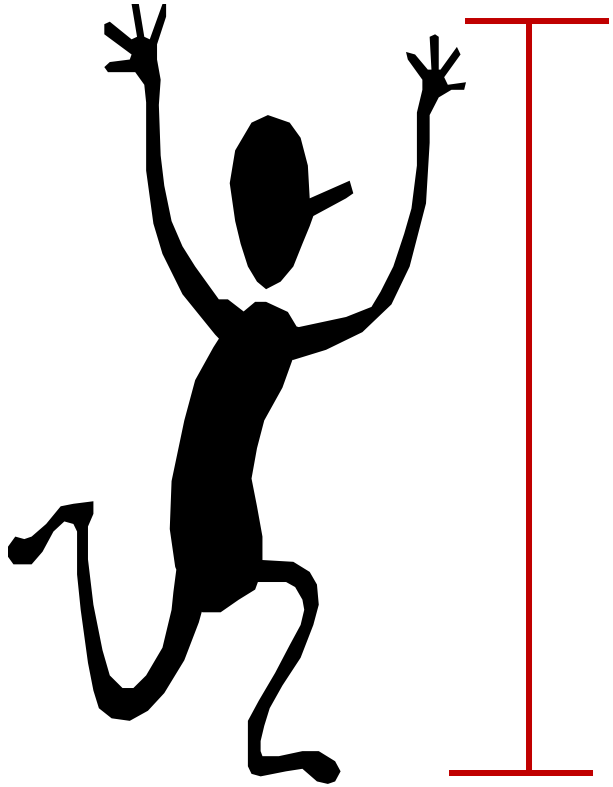
Outline

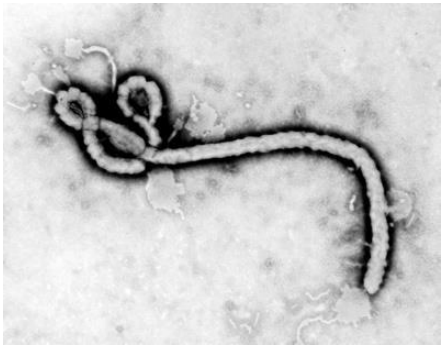
1) What are microorganisms?
   - Types of microorganisms
   - Classifying bacteria
   - Bacterial growth conditions
2) What issues do they cause?
   - Spoilage
   - Illness
3) How do we quantify them?
4) Milk Packaging
   - Shelf life

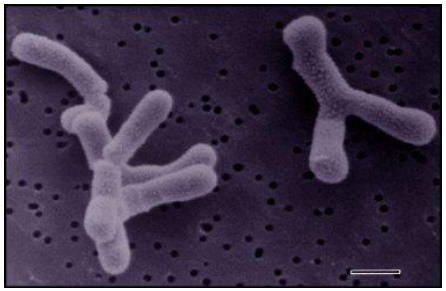
How big are microorganisms?

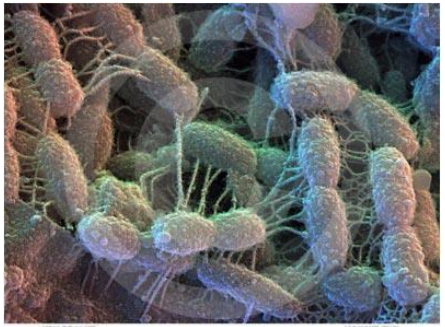
If bacteria were one cm long

A man would be 10.5 miles (17 km) tall









Types of Microorganisms

Protozoa - **Eukaryotic, single-celled organisms, generally motile**

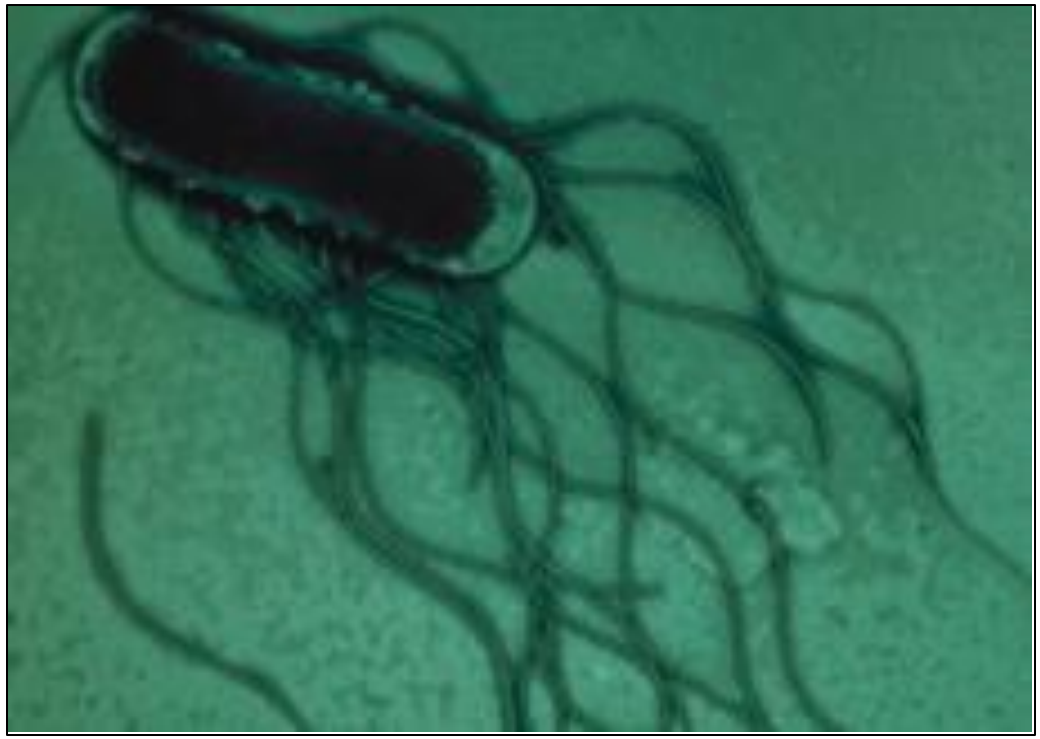
Viruses - **non-living, non-cellular organisms that require a host to grow**

Fungi - **single or multicell organisms such as mold or yeast**

Bacteria - **living, single-celled organisms**

Characteristics of Bacteria

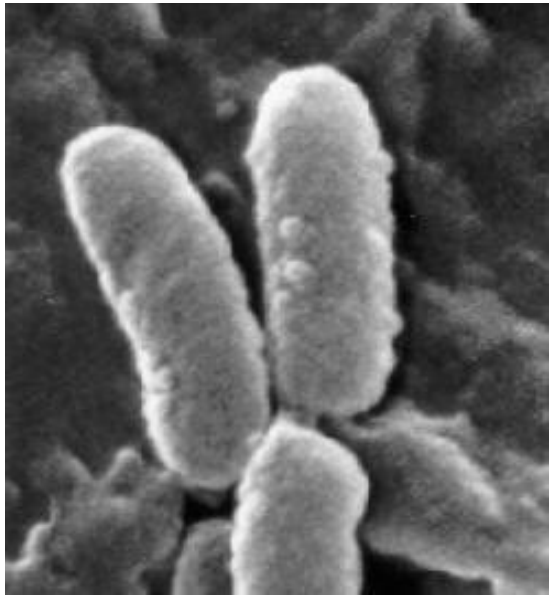
- Largest group of microorganisms
- Thousands of species
- Some are pathogenic, others are harmless



www.usda.gov

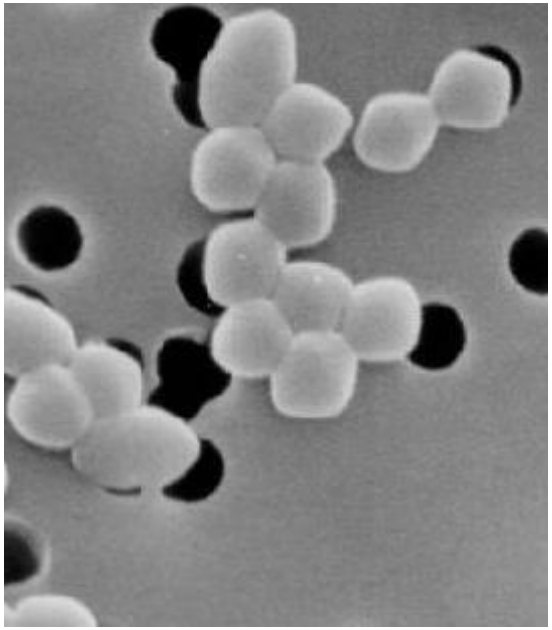
Classifying Bacteria

According to cell shape



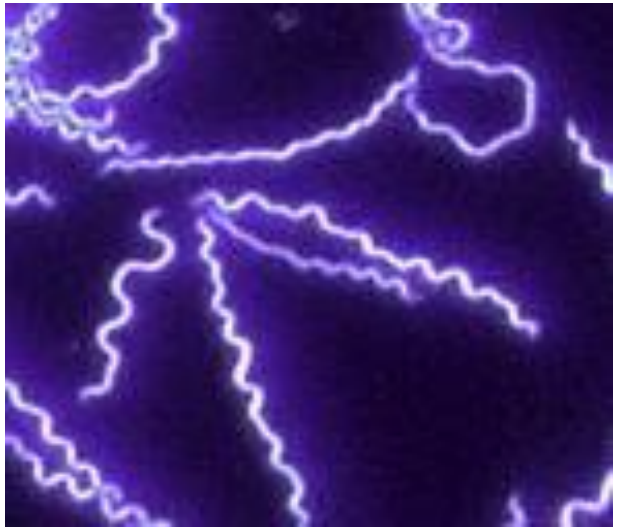
Rods

CDC/Janice Carr



Cocci

CDC/Janice Carr

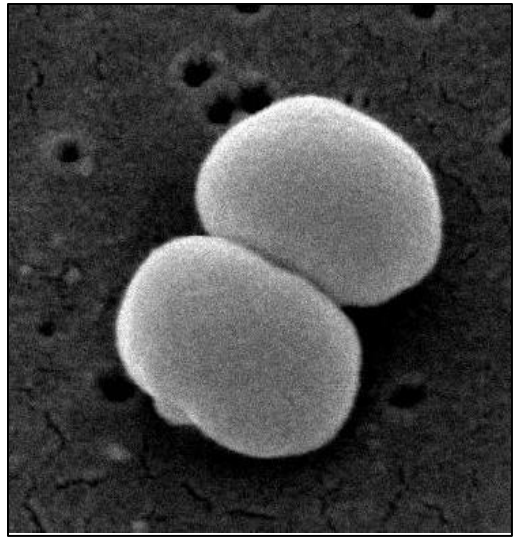


Spiral

www.asm.org

Classifying Bacteria

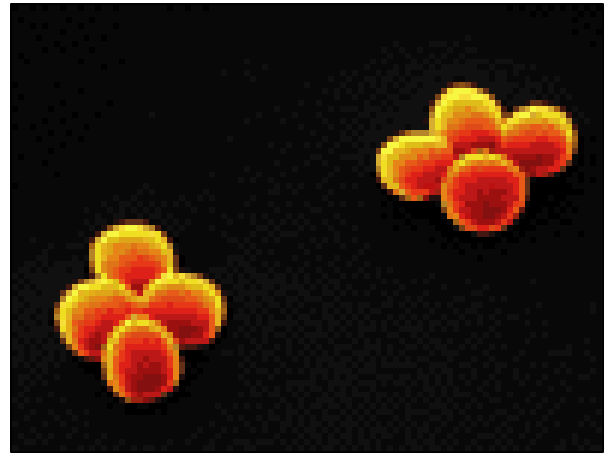
According to cell grouping



Pairs

Enterococcus

CDC/Janice Carr



Clusters

Staphylococcus aureus

www.cellsalive.net

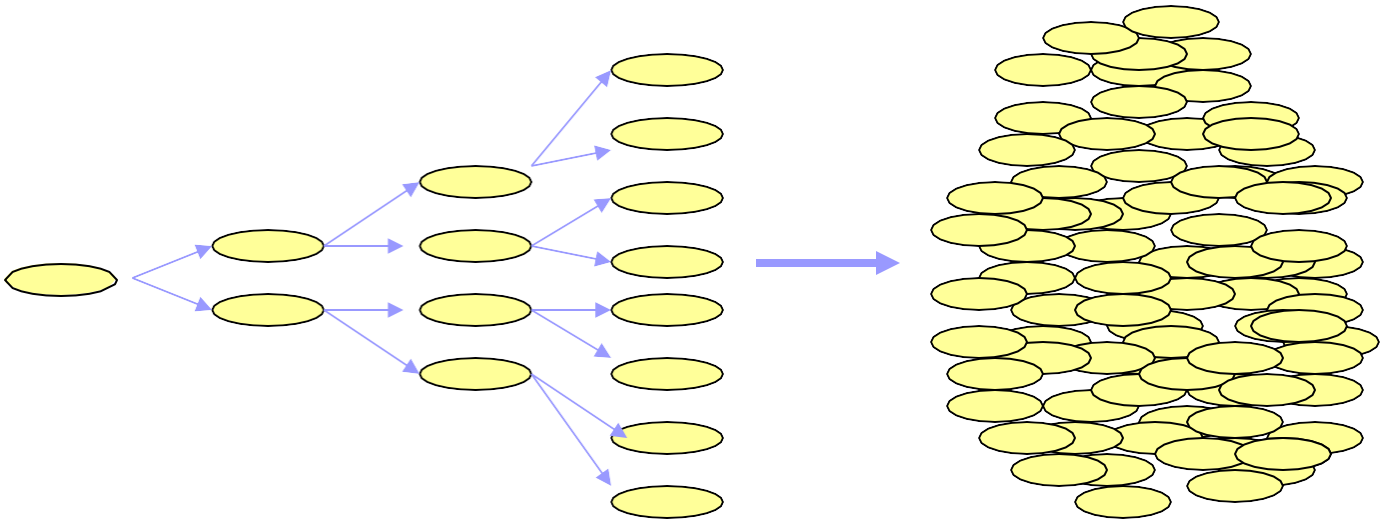
Chains

Lactococcus lactis

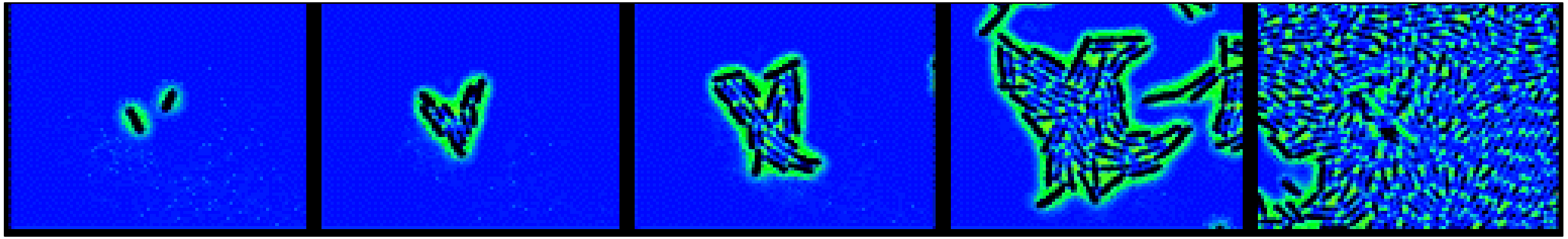
www.asm.gov

How Do Bacteria Grow?

Bacteria reproduce through binary fission:



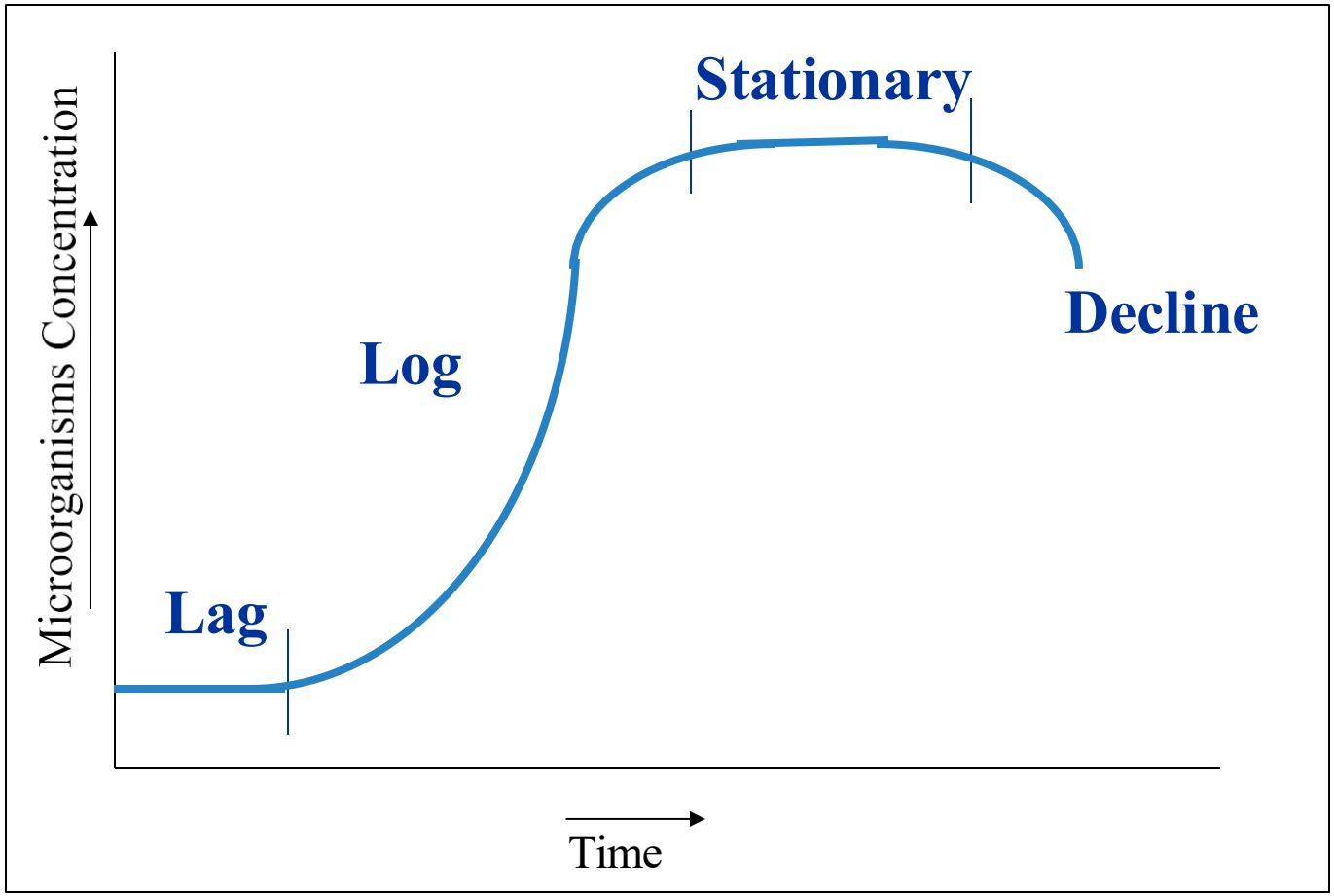
Bacteria Grow Rapidly



www.cellsalive.net

If bacteria divide every 20 minutes, it would take only **6 hours** for 1 bacteria cell to become 1 million bacteria

Bacterial Growth Curve



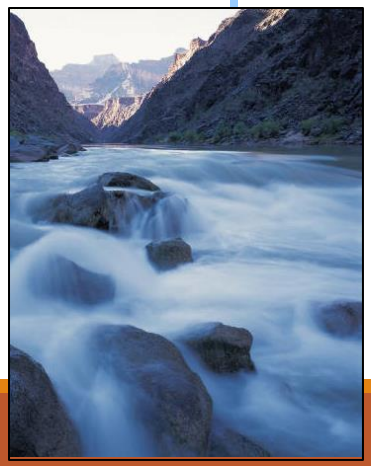
What Affects Bacterial Growth?

Intrinsic Factors

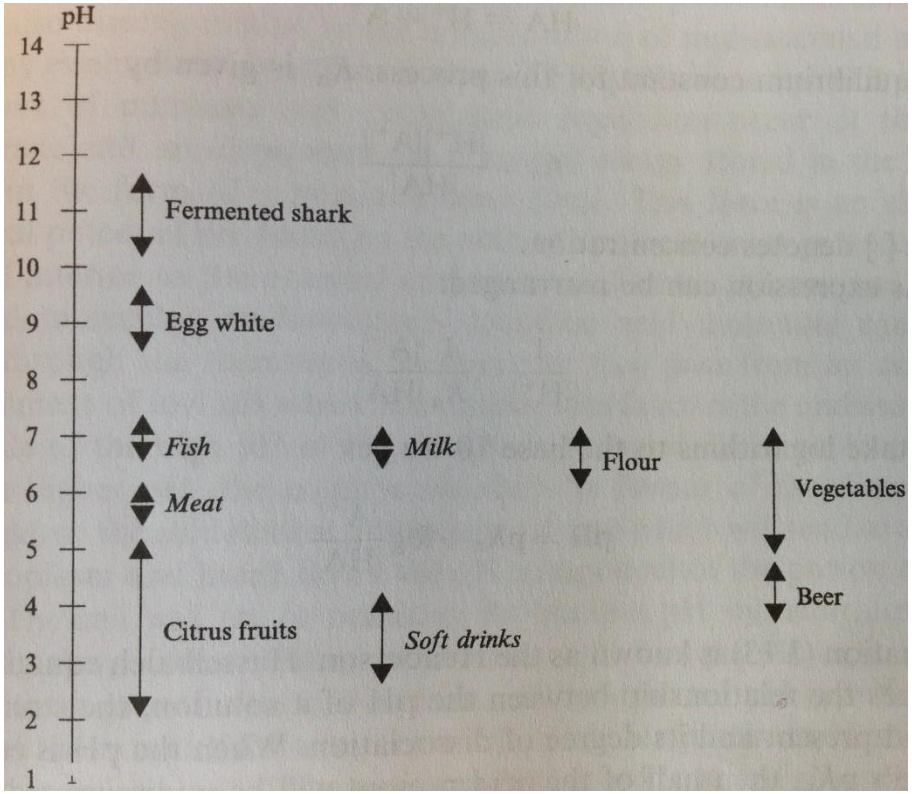
- Those that are integral to the food
  - Acidity (pH or TA)
  - Water Activity (a_w)
  - Oxidative State (Eh)
  - Nutrient Content
  - Naturally-occurring Inhibitors
  - Biological Structure

Measure of Acidity or pH

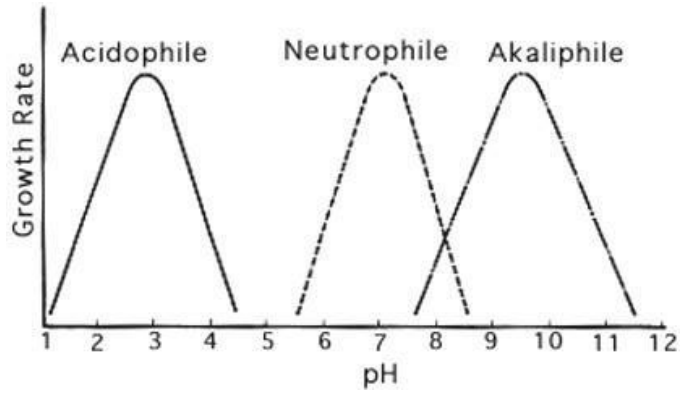
1 4 7 10 14



pH of various foods



Adams and Moss, Food Microbiology Table 3.2



Textbookofbacteriology.net

General pH values for microbial groups

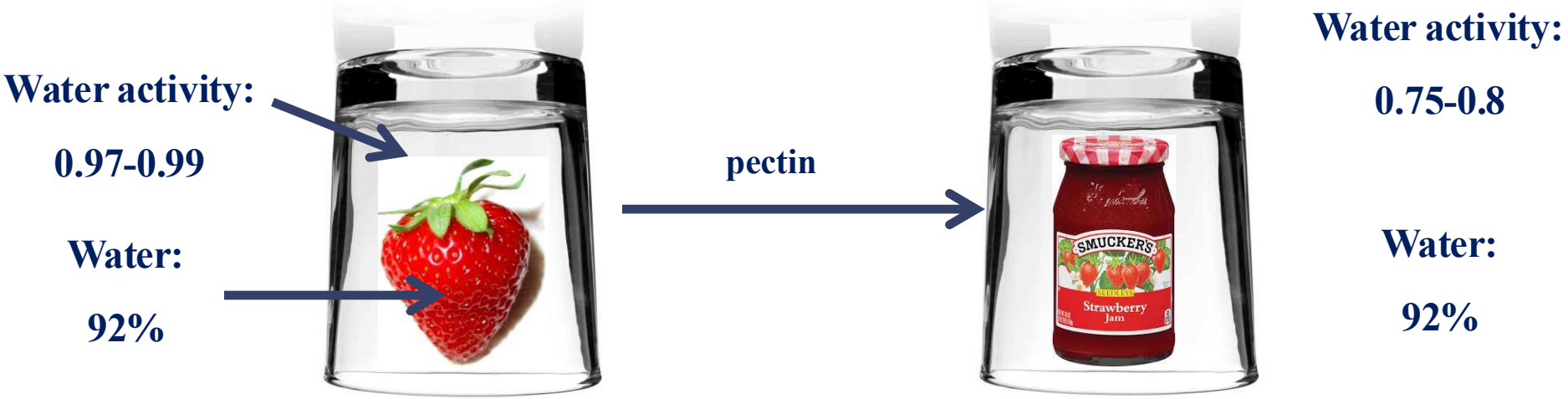
Category	Minimum	Optimum	Maximum
Bacteria	4.5	6.5 - 7.5	8.5 - 9.0
Yeasts	1.5 - 3.5	4.0 - 6.5	8.0 - 8.5
Molds	1.5 - 3.5	4.5 - 6.8	8.0 - 11.0

Water activity

"available water"; $a_w = P/P_o$; R.H. = a_w x 100

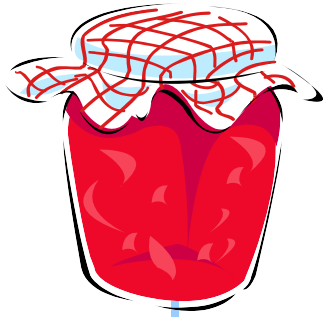
Reduce by:

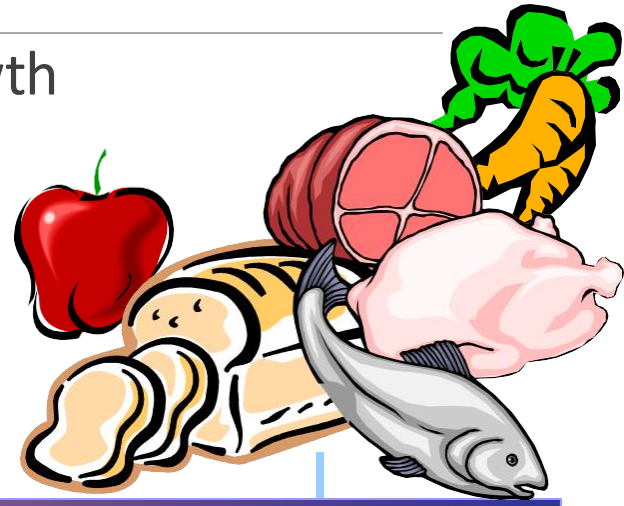
- Dehydration, evaporation, freeze drying
- Water-binding polymers (pectin, gelatin, gums, agar)
- Solutes such as salts and sugars



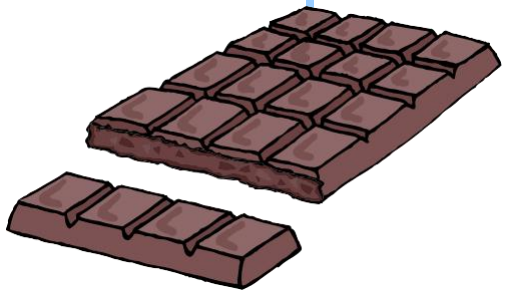
Water Activity (a_w)

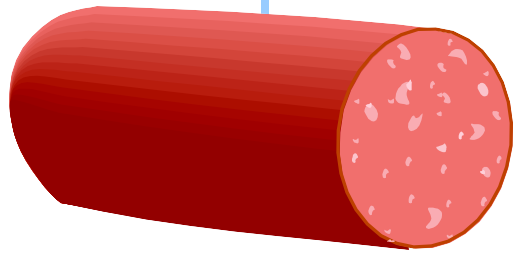
Measure of the “free” water available for growth





0.5 .75 1.0





Water Activity (a_w)

Measure of the “free” water available for growth

	Minimum a_w for growth
Bacteria	0.90
Yeasts	0.87
Molds	0.70
Staphylococcus aureus	0.83

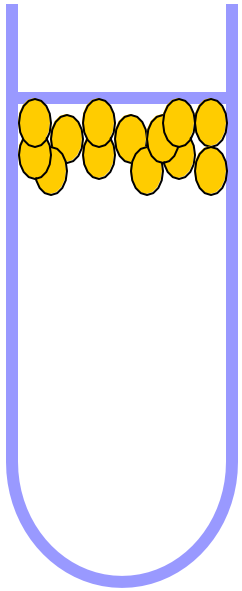
What Affects Bacterial Growth?

Extrinsic Factors

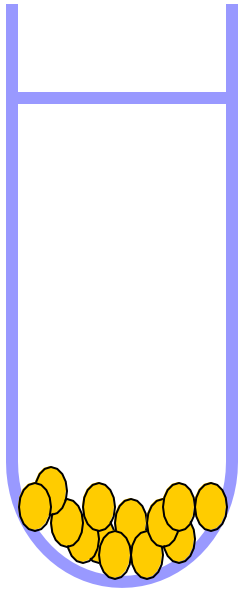
- Those that are external to the food (environmental)
  - Level of Oxygen
  - Temperature
  - Relative Humidity

Tolerance to Oxygen

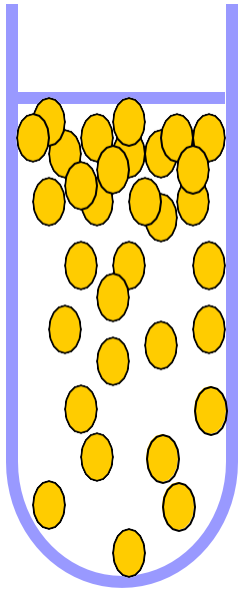
Aerobic



Anaerobic



Facultatively Anaerobic



Temperature

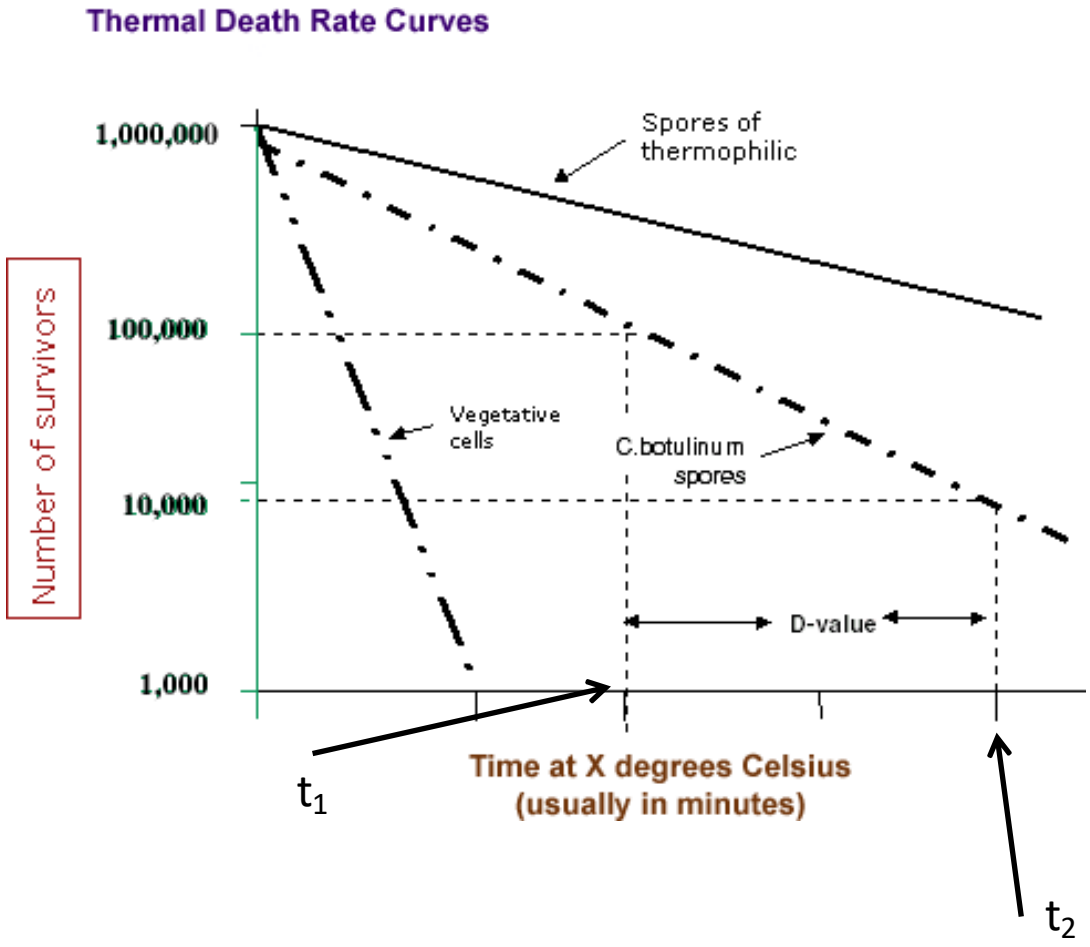
Optimum Growth Temperature

- Psychrotrophs 0 - 7°C (32 - 45°F)
- Mesophiles 15 - 43°C (60 - 110°F)
- Thermophiles 43 - 54°C (110 - 130°F)

D Value (Decimal reduction time)

- Time of heating at a particular temperature to cause a 1 log or 90% reduction in the number of microorganisms
- $D_{121°C}$ = X min (or sec)

Calculating the D-value



Suppose t = 121 °C

$$D_{121}{}^{o}C = \frac{t_2 - t_1}{\log_{10}a - \log_{10}b}$$

http://wiki.ubc.ca/Course:FNH200/Lesson_06

Organism	Medium	Temperature (°C)	D-value (min)
Salmonella enterica	Milk	65.6	0.018-0.56
Salmonella Senftenberg	Various	65.5	0.56-1.11
Escherichia coli	Milk	57.2	1.3-5.1
Yersinia enterocolitica	Milk	60	0.067-0.51
Listeria monocytogenes	Milk	63.3	0.22-0.58
Staphylococcus aureus	Milk	60	0.9
Clostridium botulinum	Vegetables	110	0.49-12.42
Bacillus cereus	Various	95	1.5-36.2
Geobacillus stearothermophilus	Unknown	100	100-1,600

http://www.fda.gov/Food/FoodScienceResearch/SafePracticesforFoodProcesses/ucm100198.htm

Summary

- Microorganisms may be grouped as bacteria, protozoa, fungi, and viruses.
- Microorganisms may be classified according to their physical characteristics or their growth conditions.
- By changing growth conditions, bacteria may be controlled.
- Temperature is an easy parameter to control
- High temperatures can lead to death of organisms in a predictable manner

Microorganisms in Raw Milk

Milk is sterile when secreted from healthy cows

Contamination occurs during milking:

1) within the udder
2) exterior of the teats and udder
3) milking, transport, and storage equipment

www.usda.gov

Sources of Microorganisms (raw milk)

- Animal
- Environment
- Milker
- Dairy Utensils
- Tank trucks
- Transfer lines
- Processing Equipment

Milk as a Substrate for Microbes

Nutrient rich

- Vitamins, protein, carbohydrates
- Lacks free nitrogen

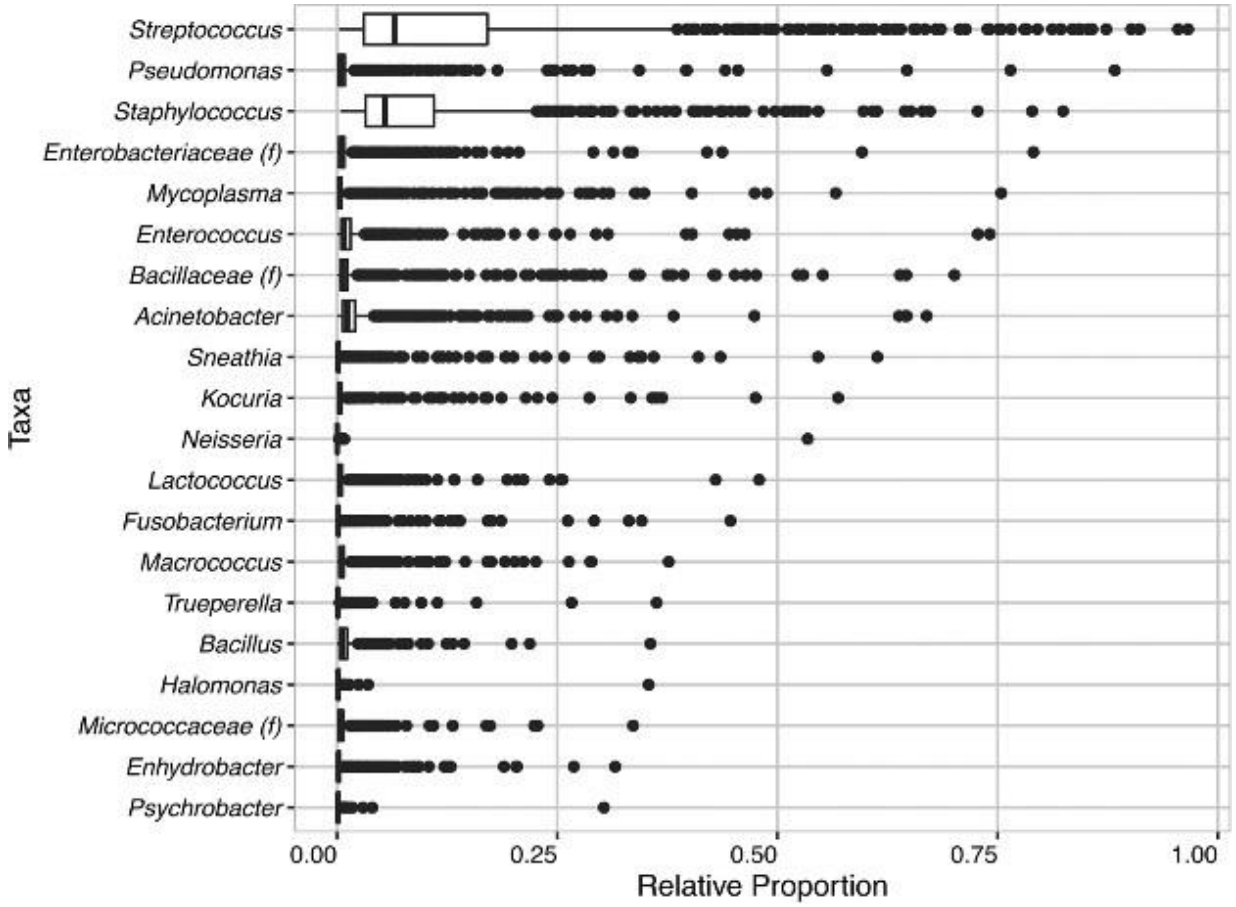
Oxygen

- In general not suitable for strict anaerobes

Natural Inhibitors

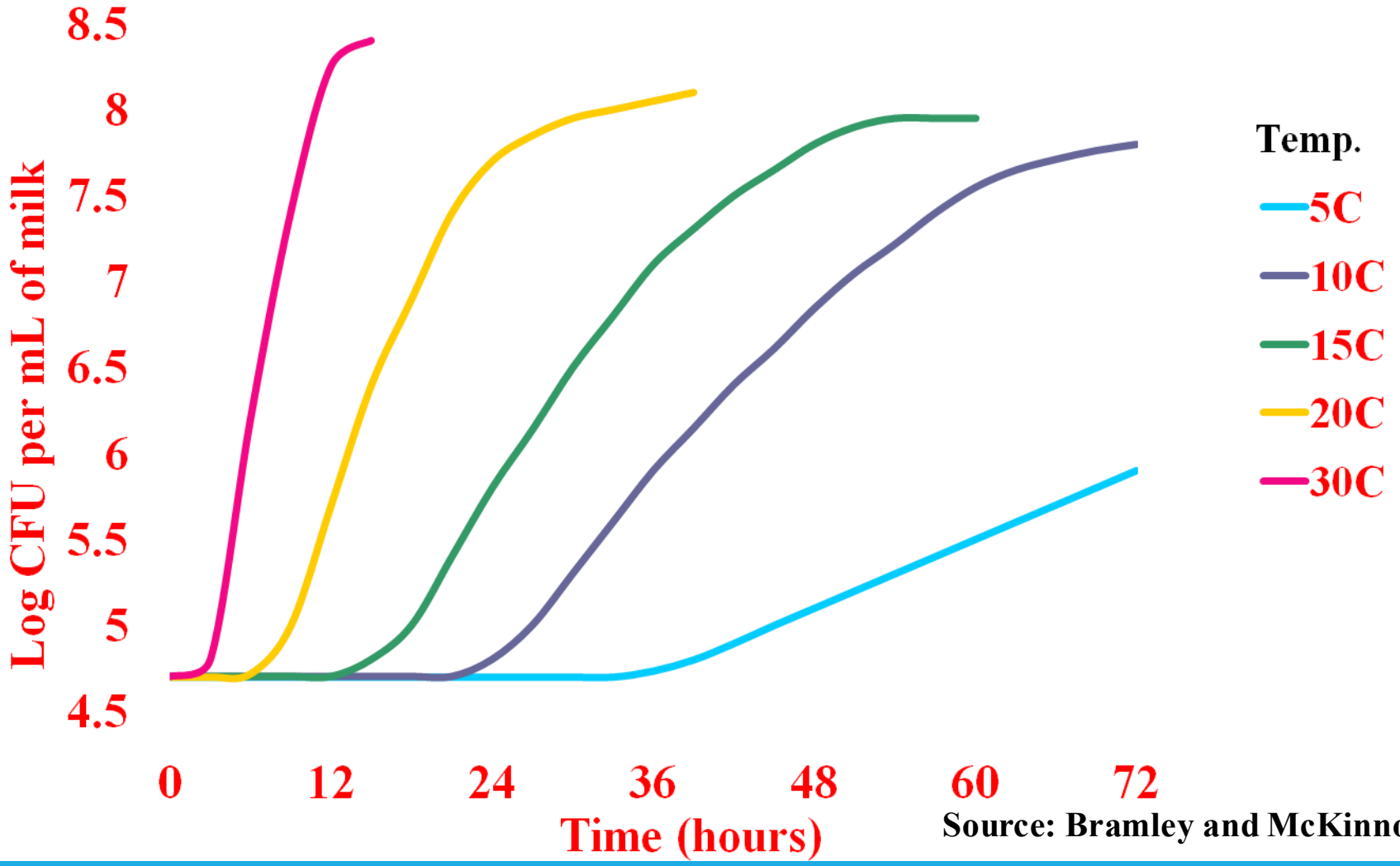
- Immunoglobulins
- Lysozyme
- Lactoferrin
- Lactoperoxidase isothiocyanate system

Bacteria in raw milk



Kabel *et al.*, 2016. mBio 7:e00836-16

Bacterial Growth in Raw Milk



Source: Bramley and McKinnon, 1990

Microorganisms in Pasteurized Milk

Post-pasteurization contaminants

- Mostly Gram-negative psychrotrophic rods
- Primary reason for spoilage of pasteurized milk

Bacteria that survive pasteurization

- Mostly Gram-positive
- Often are spore-forming
- Thermoduric

Psychrotrophs in Raw Milk

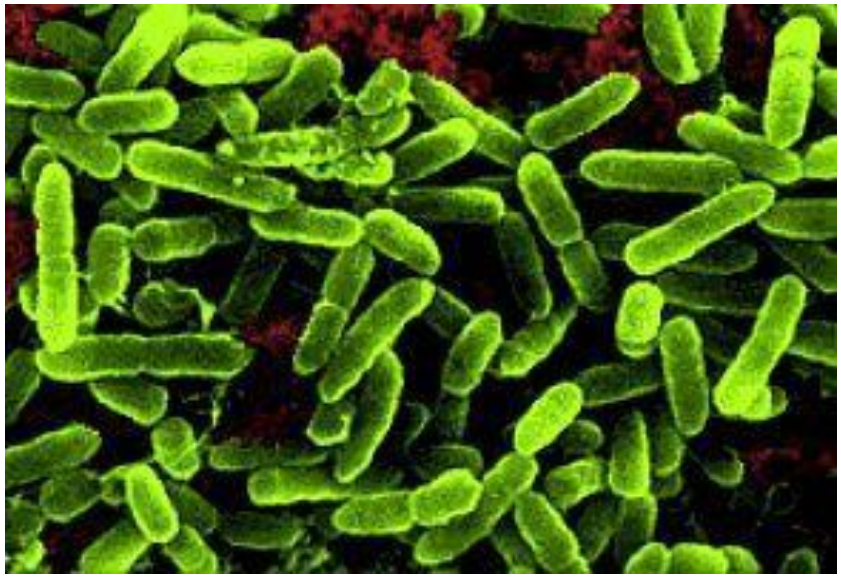
Mostly Gram-negative rods

Mainly spoilage genera

- *Pseudomonas* (~ 50%)
- *Alcaligenes*
- *Flavobacterium*
- coliforms

Some pathogens

- *Yersinia*
- *Listeria* (Gram-positive)



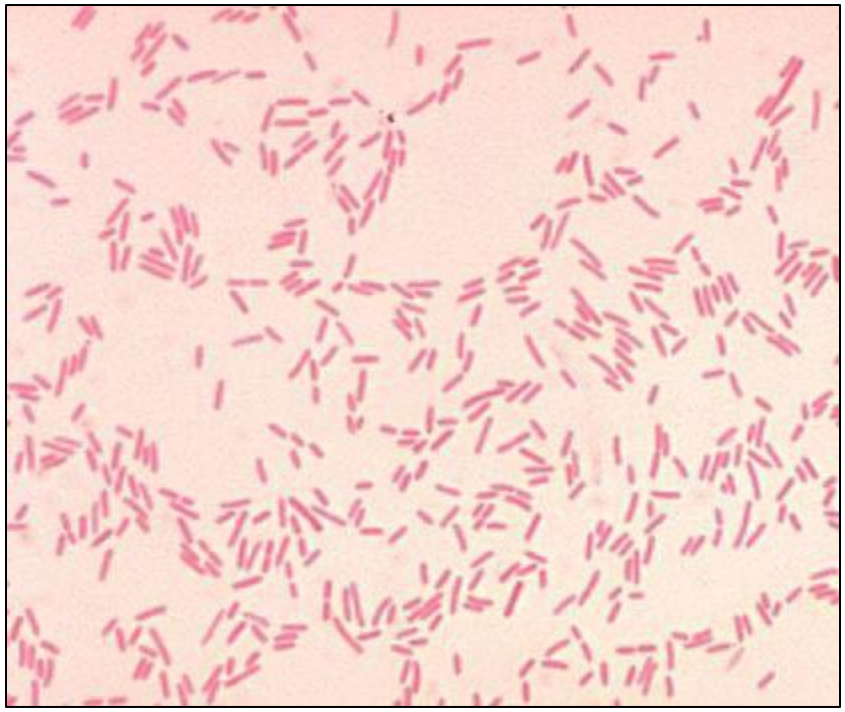
Pseudomonas

www.cellsalive.net

Thermodurics in Pasteurized Milk

Thermoduric bacteria

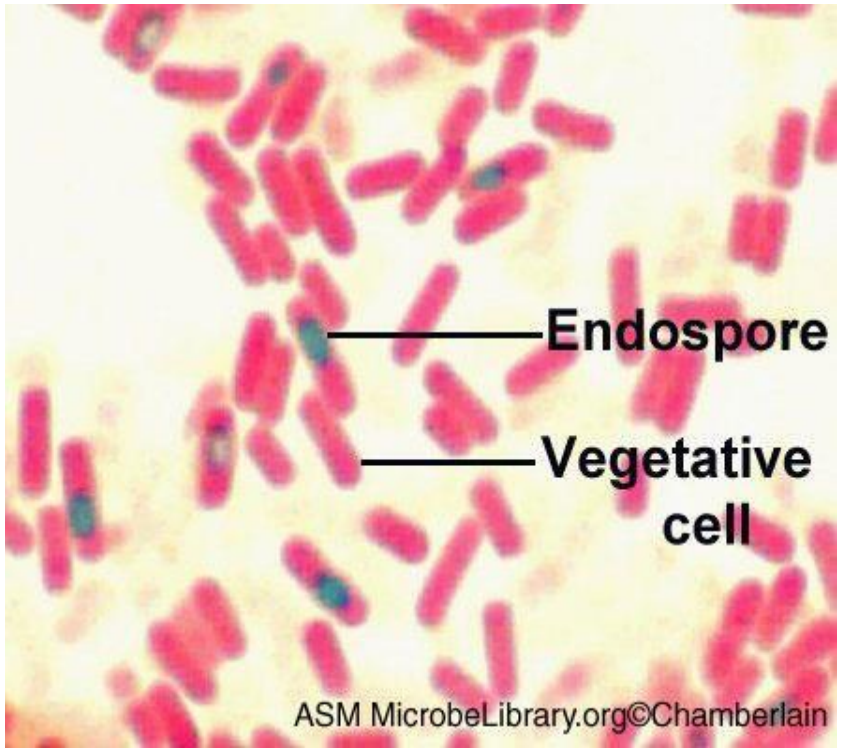
- *Bacillus*
- *Clostridium*
- *Micrococcus*
- *Streptococcus*
- *Brevibacterium*



Bacillus circulans

CDC/Dr. William A. Clark

Bacillus and *Clostridium* form spores



- Some may produce spores to survive adverse conditions
- Spores are particularly resistant to heat, drying, and chemicals
- Spores can later germinate and grow into active cells

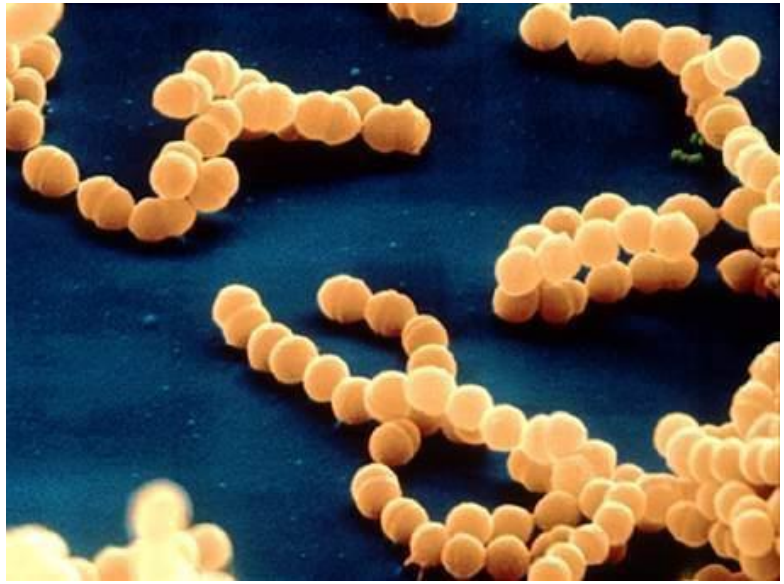
Spoilage Activities of Bacteria in Milk

- Undesirable fermentation and acid production
- Protein and lipid hydrolysis
- Slime formation

Undesirable Fermentation of Milk

Bacteria may ferment lactose to produce acids and/or gases

- Coliforms
  - Enterobacteriaceae
- Lactic acid bacteria
  - *Lactococcus*, *Streptococcus*, *Enterococcus*
- *Clostridium*



Streptococcus

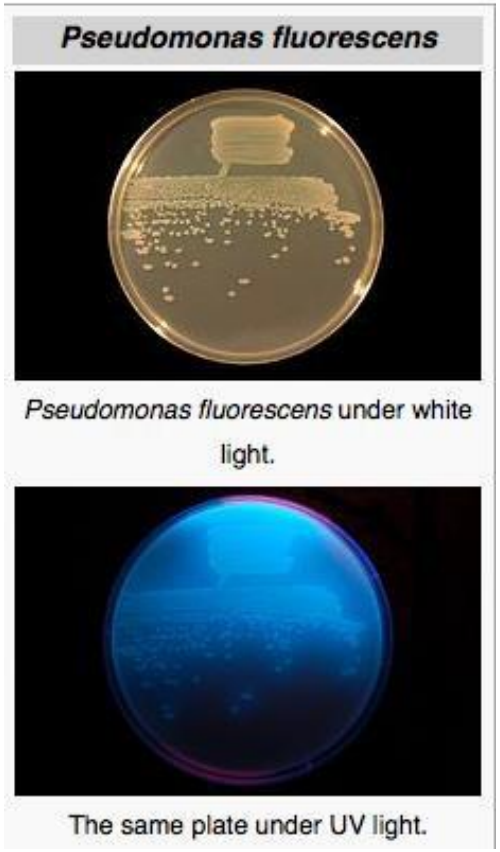
microbeonline.com

Protein and Lipid Hydrolysis of Milk

Natural milk lipases are inactivated by pasteurization.

Bacteria can produce proteases and lipases that are heat-stable.

- *Pseudomonas*
- *Flavobacterium*
- *Bacillus cereus* (bitty cream, sweet curdling)
- *Clostridium* ("late blowing" in cheese)



Pseudomonas fluorescens under white light.

The same plate under UV light.

Slime Formation

Some bacteria produce a “slimy” layer of polysaccharides

Spoilage by these bacteria results in “ropy” milk

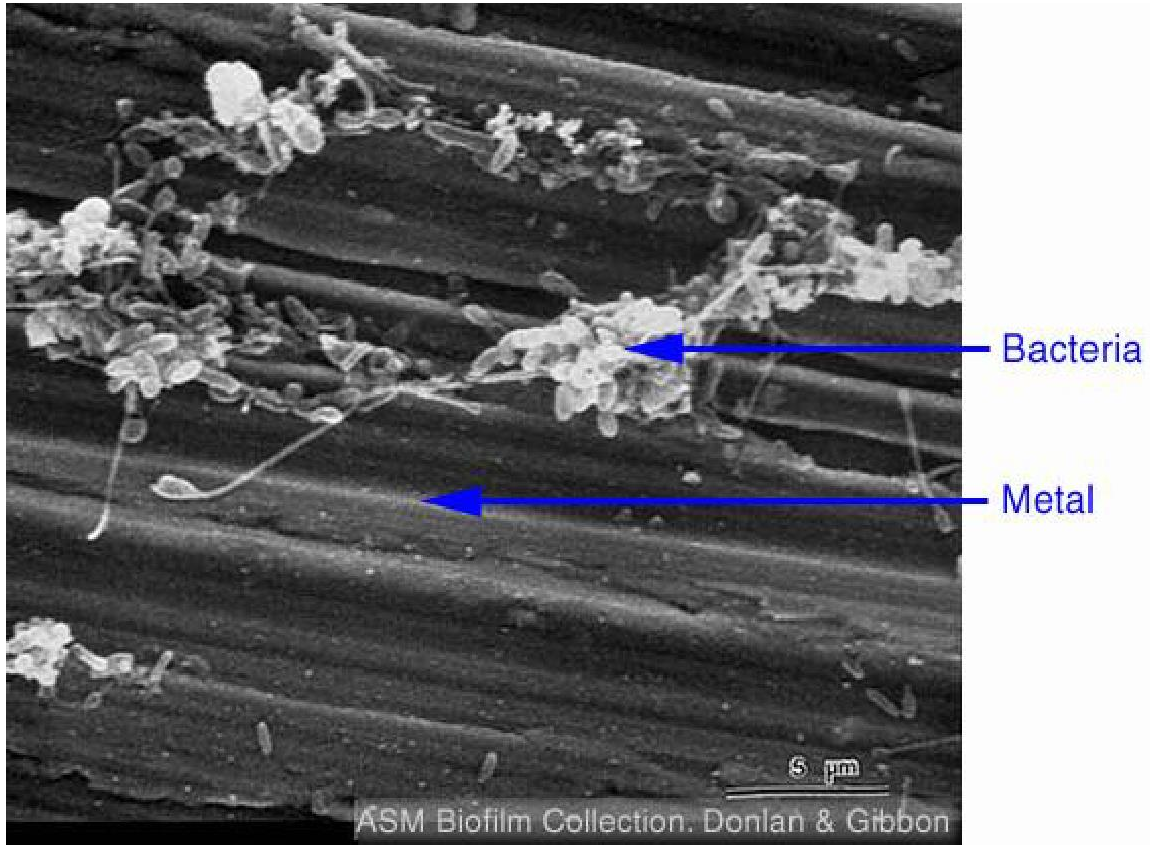
- *Alcaligenes*
- *Acinetobacter*
- *Escherichia coli*
- *Bacillus*

http://nordicfoodlab.org/

Slime Formation

May result in the formation of a biofilm, or a sticky matrix of "slime" on a surface.

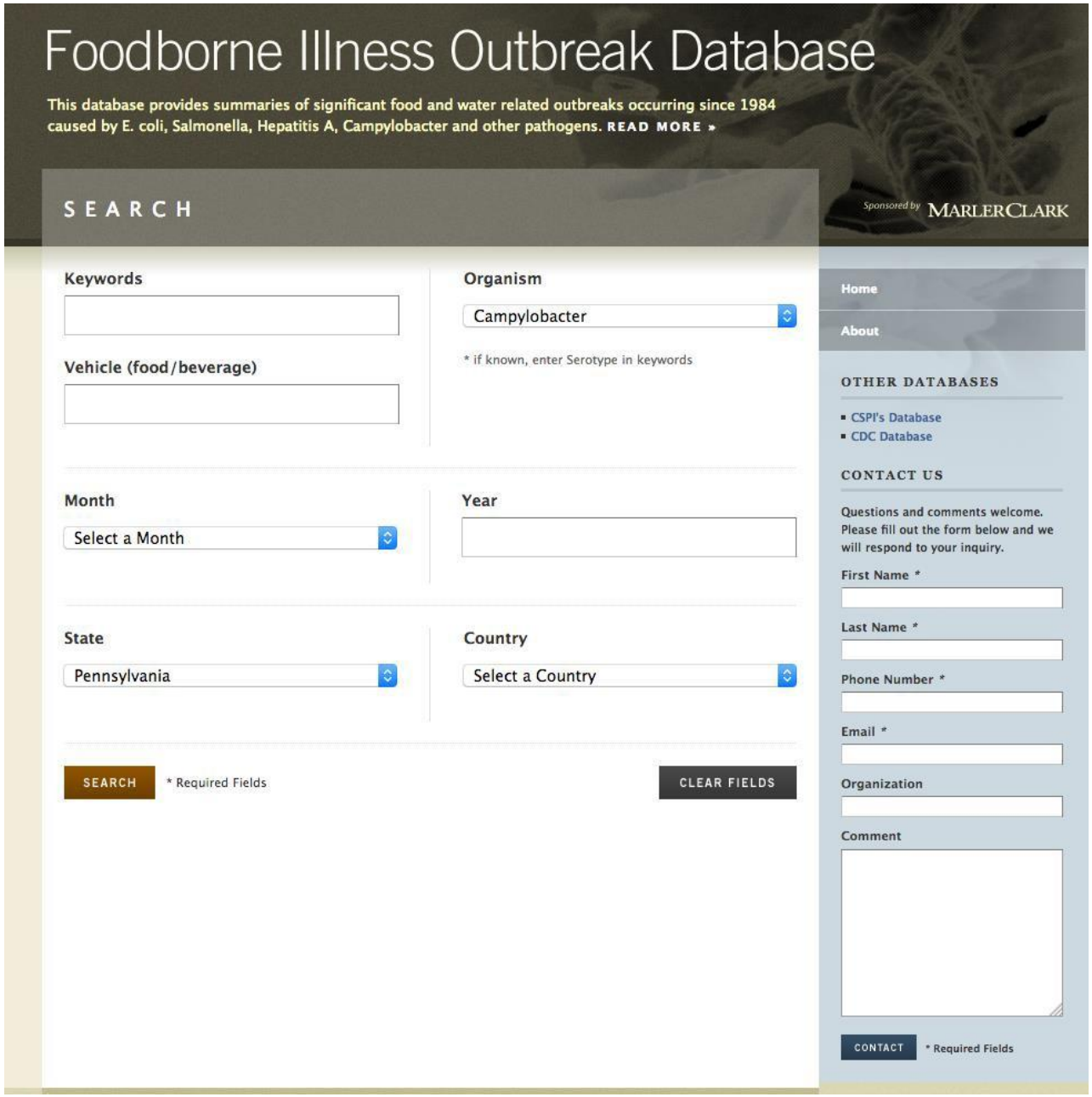
Biofilms represent opportunities for cross-contamination.



Pathogenic Bacteria Found in Raw Milk

Organism	Refrigeration growth	Survives Pasteurization
Staphylococcus aureus	No	No
Salmonella	No	No
Escherichia	No	No
Yersina enterocolitica	Yes	No
Campylobacter	No	No
Clostridium ssp.	No	Yes
Bacillus cereus	No	Yes
Listeria monocytogenes	Yes	No

After Griffiths and Phillips (1989)



http://outbreakdatabase.com/

Control of Microbes in Milk

Keep the microbes out

Clean and sanitize the udder

Keep equipment cleaned and sanitized

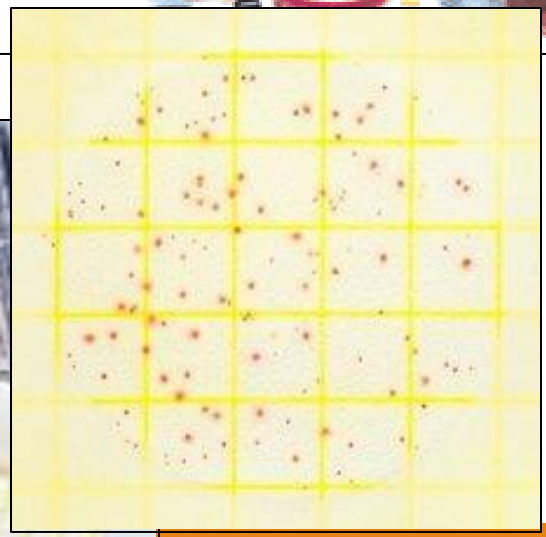
Control temperature

Minimize storage time

Pasteurize ASAP

Microbial Enumeration of Milk and Dairy Products

There are various techniques, media, and rapid methods that may be used to enumerate bacteria.



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Enumeration in Milk and Dairy Products

Bacterial Count	Growth Conditions
"Total" Bacterial Count	32° C, 2-3 days
Psychrotrophic Count	5° C, 10 days
Thermoduric Count -aka Lab Pasteurized Count	32° C, 2-3 days after heating sample to 63° C for 30 minutes
Preliminary Incubation	Incubate at 55° F then TBC
Direct Microscopic Count	

Microbial Enumeration of Milk and Dairy Products

- "Total" Bacteria Count (Standard Plate Count)
  - Does not differentiate between organisms
  - Does not indicate source of contamination
  - Does not indicated effectiveness of processing
  - Typical range for raw milk: 1,000 to 300,000 CFU/mL

Microbial Enumeration of Milk and Dairy Products

- Thermoduric Bacterial Count
  - Most thermodurics can multiply in raw milk at ambient temperatures
  - High thermoduric counts indicate gross contamination from milking equipment

Microbial Enumeration of Milk and Dairy Products

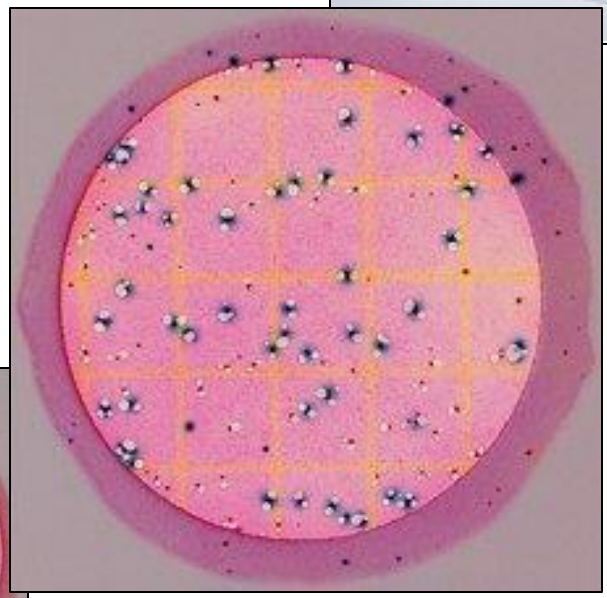
- Coliforms:
  - Gram-negative non-sporeforming rods
  - Facultative anaerobe (grows with or without oxygen)
  - Able to ferment lactose, with acid and gas formation, within 48 hours at 37°C.
- Coliforms include *Escherichia*, *Enterobacter, Citrobacter,* and *Klebsiella*.
- Coliforms are inhabitants of animal intestines and the environment.

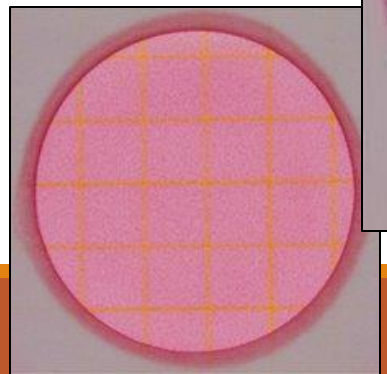
Microbial Enumeration of Milk and Dairy Products

- Fecal coliforms are coliforms derived from the intestine of warm-blooded animals, and can grow at 44.5°C.
- *Escherichia coli* is associated with the intestinal tract of humans. Some strains can be pathogenic (*E. coli* O157:H7).

Microbial Enumeration of Milk and Dairy Products

- Coliforms indicate potential under-processing or post-processing contamination.
- Enumeration of coliforms:
  - Violet Red Bile Agar (VRBA) and Brilliant Green Bile broth (BGB)
  - Petrifilm™





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Microbiological Quality Standards for Raw and Pasteurized Milk

Milk	Test	Maximum Limit
Raw (pre-pasteurized) milk for grade "A" use*	Total aerobic bacteria count	≤100,000 CFU/ml[1] individual[2]
		≤300,000 CFU/ml commingled[3]
	Somatic cell count	≤750,000 /ml[4]
	Coliform count	≤750 (California)
Raw milk for grade "B" (manufacturing) use**	Total aerobic bacteria count	≤500,000 CFU/ml individual
		≤1,000,000 CFU/ml commingled
	Somatic cell count	≤750,000 /ml
Pasteurized fluid milk	Total aerobic bacteria count	≤20,000 CFU/ml
	Coliform count	≤10 CFU/ml
Pasteurized cultured products	Total aerobic bacteria count	No standard
	Coliform count	≤10 CFU/ml

*Grade "A" milk is also known as "fluid grade" grade. It is milk that is intended for consumption in fluid form. Grade A milk is also typically used for cultured dairy products, such as cottage cheese and yogurt. More than 90% of milk produced in the US is grade A; much of the grade A supply is used in manufactured dairy products (as in Grade "B" below).

**Grade "B" or Manufacturing Grade milk can only be used for cheese, butter, and nonfat dry milk production. Standards are USDA "recommendations."

[1]CFU/m = colony forming units/milliliter. This unit estimates the number of viable microbial cells in a 1-milliliter sample that can multiply under the given growth conditions.

[2]Individual producer milk is milk that is still on the farm.

[3]Commingled milk has left the farm and has been mixed with other individual producer milk in a tank during shipment or at the processing plant. It includes the milk of more than one cow and/or more than one milking.

[4]Many states have set lower limits, generally in the range of 400,000 cells/ml.

Sourc: QualiTru, 2025

Control of Microorganisms in food

Three K's:

- Keep them out
- Keep them from growing
- Kill them