Food Microbiology 1

Indicators in Food Microbiology

Bacterial Groups Relevant to Food Microbiology

In Unit 1 we classified bacteria accordingly:

- **Good (Beneficial)**
- **Bad (Spoilage)**
- **Very Bad (Pathogenic)**

The primary interest in food microbiology is producing safe food with adequate shelf life

Indicators

- Looking for specific bacteria that cause spoilage or food-borne illness is like looking for a needle in a haystack

- An indicator (index) in food microbiology is needed to confirm that the food is safe and has adequate shelf life

Indicators

- Provide a gauge of product shelf life
- Highlight potential hazards
- An assessment of the previous history of food product
- Evaluation of the efficacy of control measures to prevent and/or inactivate microbial activity

Spoilage Indicators

Spoilage Indicators

Types of the bacterial counts will depend on the nature of the product

- Total Aerobic Count
- Psychrotrophic Count
- Lactic acid bacteria
- Yeast and molds

Total Aerobic Counts

- An assessment of the general levels of bacteria
- High numbers typically indicate significant bacterial activity
- Conditions plates are incubated under, reflect the food environment (for example, low incubation temperatures for samples derived from chilled foods)

Psychrotrophic Counts

- Grow at low temperatures
- Responsible for spoiling refrigerated foods
- Numbers provide an estimate of shelf life

Include:

- Yeast and molds
- Main concern is *Pseudomonas* spp

Pseudomonads

- Gram negative, rods
- Aerobes: *require* oxygen
- Non-fermentative

- Simple nutrition requirements and can metabolize a wide range of substrates (crude oil)

- A number form extracellular polysaccharide (biofilms) at low incubation temperatures and/or in high sucrose environments

Enzymes Produced by *Pseudomonas*

- **Proteinases**

Enzymes secreted into foods break down proteins leading to generation of ammonia, sulfur and/or organic acids (butyric, acetic)

- **Lipolytic**

Enzymes secreted by cells hydrolyze triglycerides and accelerate lipid oxidation leading to rancidity

- **Pectolytic**

Enzymes breakdown plant cell walls leading to loss of tugor pressure

Pigmentation

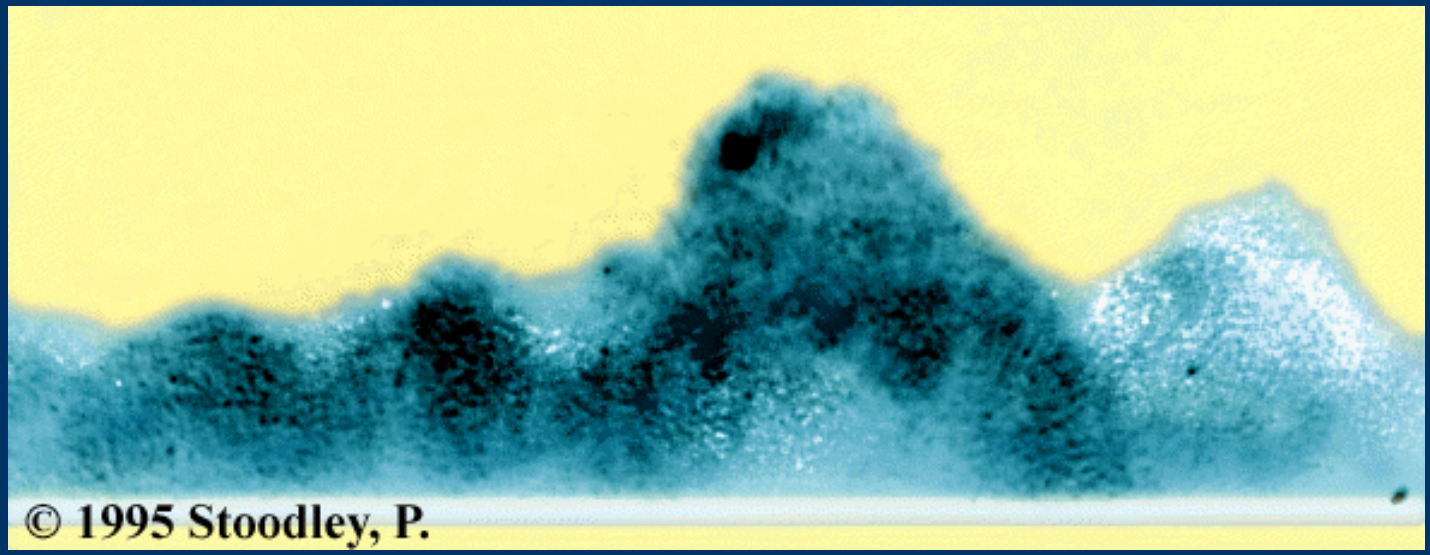
- Fluorescent Pseudomonas release siderophores to assimilate iron

- Siderophores are pigmented (fluorescent)

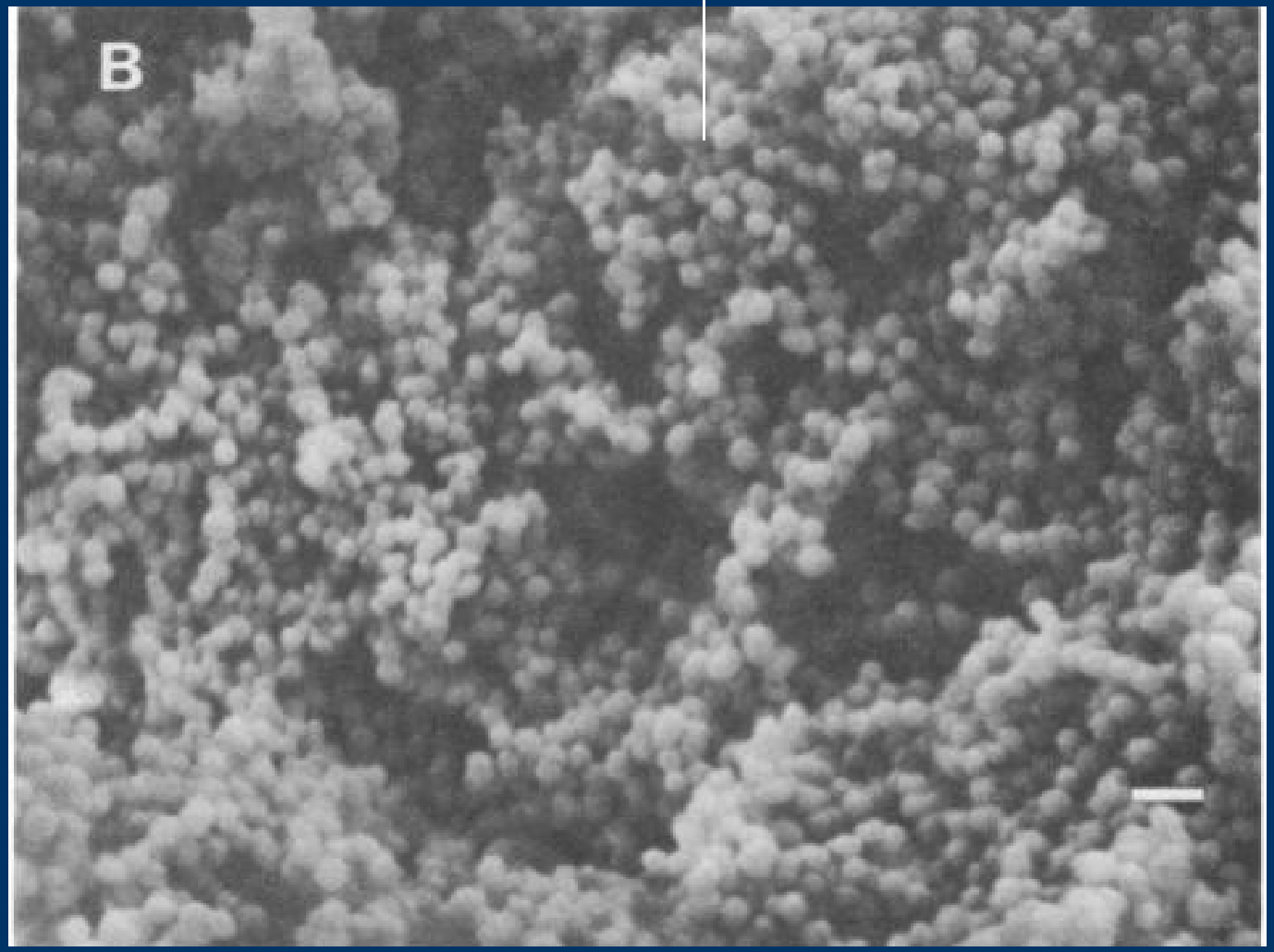
Biofilms

- Pseudomonas due to temperature or stress produce extracellular polysaccarides

- Method for the bacteria to utilize energy sources without growing



Bacteria with biofilm



Significance of Biofilms

- Cause slime layers
- Enable bacterial cells to resist drying and sanitizers
- Potentially can embed pathogenic bacteria that subsequently become protected from environmental stress and sanitizers

Representatives

- Opportunistic pathogens (uncommon pathogens)

E.g. *Pseuodomonas aueruginosa*

- Spoilage

E.g. *Pseuodomonas fluorescens*

Lactic Acid Bacteria

- Gram positive non-spore forming rods or cocci
- Facultative anaerobes: can use oxygen, but also can survive and grow without it

Genera of significance

Lactococcus

Lactobacillus

Leuconostoc

Pediococcus

Streptococcus

- Widespread in the environment (plants and GI tract of animals)
- Fastidious (complex nutritional demand)
- Tolerate low pH and high ethanol
- Can be Beneficial or Spoiling depending on fermentation products and food type

Lactic Acid Bacteria

1- **Homofermentative:** ferment carbohydrates to predominantly lactate

e.g. *Lactobacillus plantarium*

Lactobacillus delbruecki

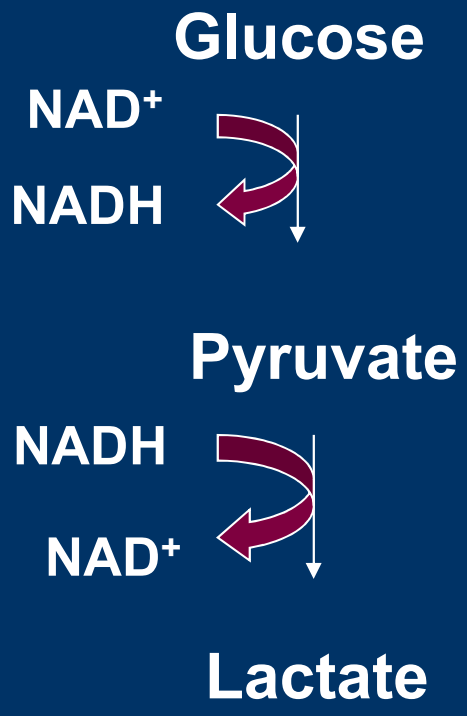
2- **Heterofermentative:** ferment carbohydrates to a mixture of products i.e. lactate, acetate, and ethanol

e.g. *Lactobacillus brevis*

3- Facultative homofermenters: prefer homolactic fermentation but can perform heterolactic

e.g. *Lactococcus lactis subsp. lactis*

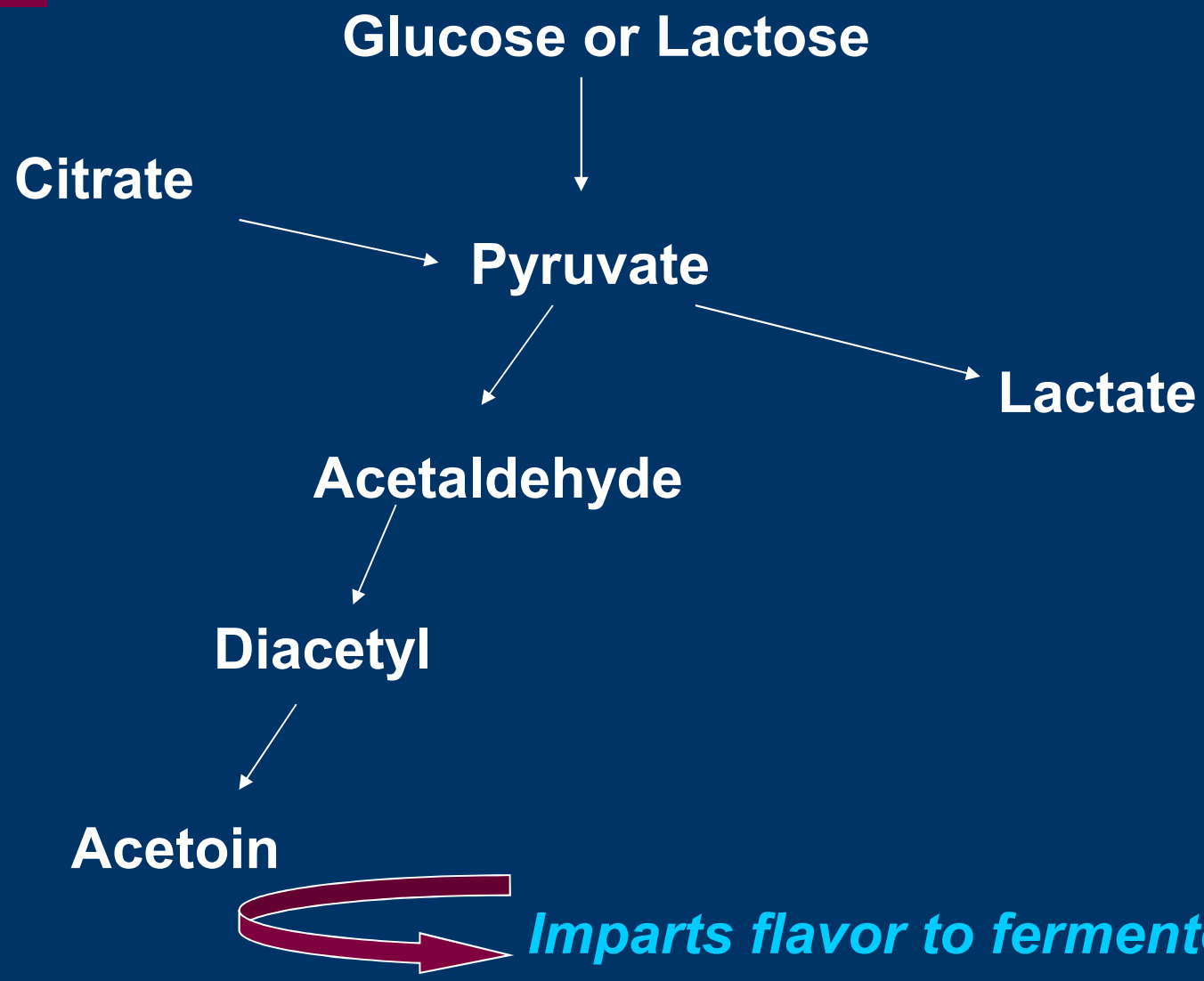
Homofermentative Metabolism



Embden-Meyerhof-Pamas Pathway

Food Fermentations

E. g. Yoghurt



Imparts flavor to fermented foods

Hetrolactic

Phosphoketolase Pathway

Glucose or Lactose

CO_2

Spoilage in vacuum packed foods

Xylulose 5-Phosphate

Pyruvate

Acetyl Phospate

Lactate

Ethanol

Acetate

Ch. 29: Food Microbiology

- The negative aspects of food microbiology
  - Economic loss
  - food safety
- Food preservation and microbial growth
- Foodborne diseases

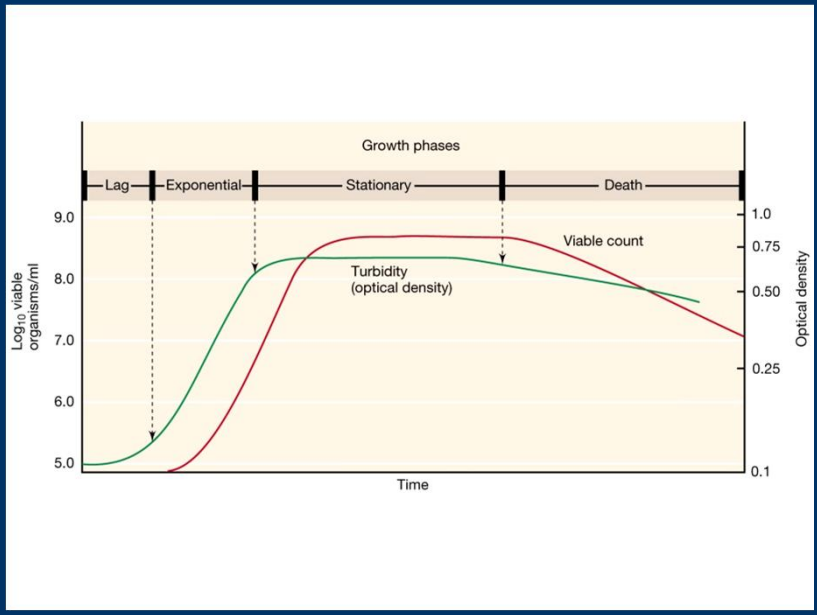
29.1 Food spoilage:

- Change in appearance, small, or taste that makes it unacceptable to consumers - not necessarily harmful to consumers
- Many food products are terrific microbial growth media
- We distinguish (Table 29.1):
  - Perishable food: meats, fish, poultry, eggs, milk, most fruit and vegetables
  - Semi-perishable food: potatoes, apples, nuts
  - Nonperishable food: sugar, flour, rice, dry beans

Related to water content (water activity) and implies storage conditions

- The microbial agent causing spoilage depends on the source of the food and its nutritional value:
  - Meats may be contaminated by intestinal pathogens released during slaughter
  - Dairy products - lactic acid bacteria
  - Fruit and vegetables - soil and water microbes
- Some microbes that cause spoilage may be human pathogens but the majority are not! (Table 29.2)

- Growth of microbes in food follows a typical microbial growth pattern
- Growth rate depends on the nutritional value and temperature of the food



- **Number of microbes depends on both inoculum size ar growth rate**
- **Food spoilage occurs at high populations density (at stationary phase) - retarding microbial growth dela spoilage**

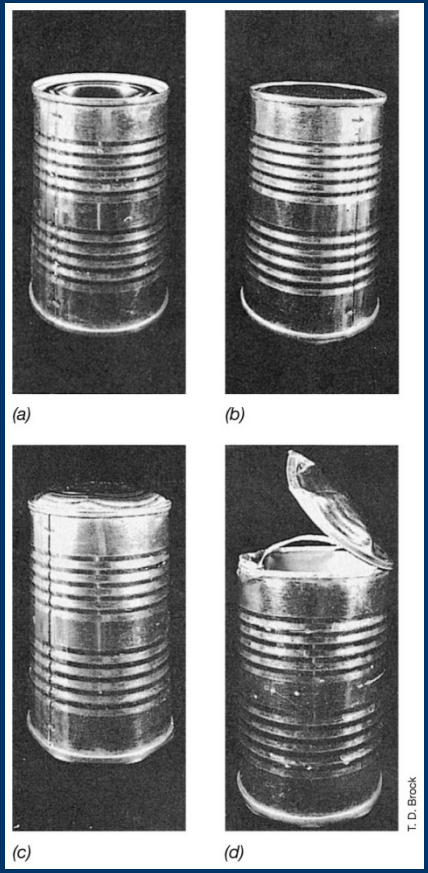
Food preservation:

- **Temperature**
  - Lower: decreased growth rate - but, psychrophilic microbes
  - Perishable food will only last for a few days at 4 ^{o}C
  - Freezing (- 20 ^{o}C) destroys the texture of many products and does not completely stop growth
  - Deep freezing (- 80 ^{o}C) is costly
- **Acidity**
  - Most foods at neutral or acidic pH
  - At pH < 5 microbial growth is inhibited
  - Pickling: Decreased food pH by the addition of vinegar (acetic acid bacteria); veggies, meats, fish
  - Fermented foods: acid is produced during food production (e.g., sauerkraut, yogurt, etc); lactic acid, acetic acid, and propionic acid bacteria; limited to pH > 4

Water activity (A_w):

- How available is water to microbes
- water is the most critical factor for life
- By reducing A_w we can slow down microbial growth; achieved by drying or by additions of solutes (sugars, spices, or salts)
- Freeze-drying (lyophilization) - the withdrawal of water from frozen food under vacuum
  - Milk, meat, fruit, vegetables, etc
- Increased solutes - Sugars for fruits; salts for meat and fish

Canning:



- Sealed food is heated to kill or inhibit microbial growth
- Acidic food easier to can; neutral food heated to > 100 ^{o}C; quality and nutritional value declines
- Spoilage of canned food by anaerobic organisms (*Clostridium* and toxin production); gas indicates problems

Chemical food preservation

- U.S. FDA “generally recognized as safe”
- Many are completely safe (sodium propionate); some may effect human health:
  - Nitrites (precursors of carcinogens)
  - Ethylene and propylene oxides (mutagens)
  - Antibiotics (spread of resistance)

Gamma radiation:

- Ionizing radiation - commonly used products need to be labeled as irradiated
- Meat - hamburgers (*E. coli* 0157:H7); chicken (*Campylobacter jejuni*)
- Varied but controlled dozes depending on purpose (e.g., NASA used 10 times more radiation to treat astronauts food than what we use to treat hamburgers - 44 kGy vs. 4.5 kGy)
- 12D factor

Foodborne diseases and microbial sampling:

- Food poisoning - Caused by preformed toxin in the food; organism may or may not be alive and growing; *Clostridium botulinum* and *Staphylococcus aureus*
- Food infection - Live cells delivered by contaminated food; organism multiply once food is ingested; *Salmonella*
- Sampling: Process food to release microbes; culturing and use of molecular probes (antibodies, gene probes, PCR) to detect specific microbes

Examples of foodborne diseases - most are infections and associated with animal products:

Organism	Number of cased per year (U.S.)	Foods to watch
Campylobacter jejuni	1,963,000	Poultry and diary products
Salmonella spp.	1,340,000	Poultry, meat, diary and eggs
Clostridium perfringens	248,000	Cooked and reheated meat products
Giardia lamblia	200,000	Contaminated meat
Norwalk-like viruses	9,200,000	Shellfish, other food

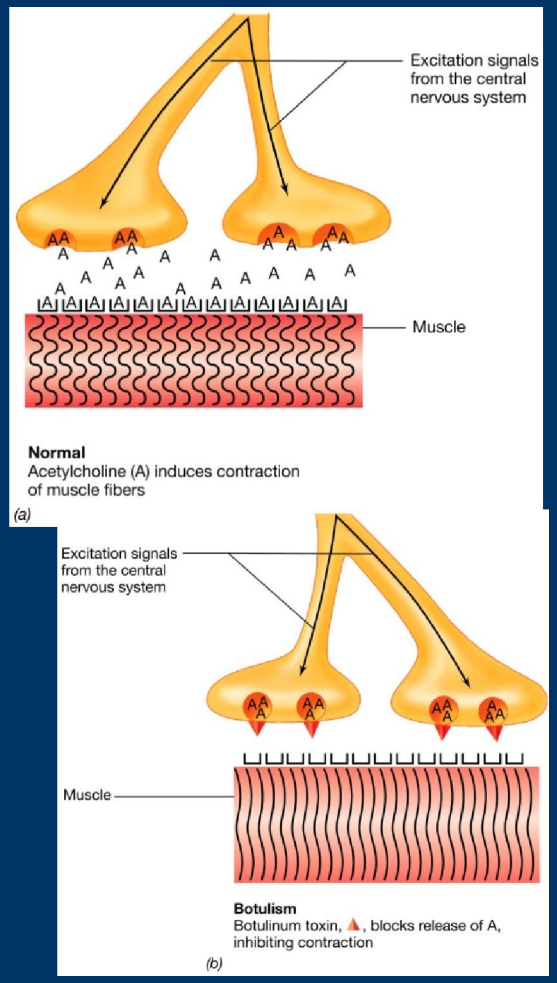
Staphylococcus aureus:

- Common skin, respiratory, and GI tract flora
- Grows readily in unrefrigirated meats and creamy foods; toxins are heat resistance
- Produces 7 entrotoxins; the most potent is A (*entA*); a superantigen (T cell stimulation ⮙ cytokines ⮙ intestinal inflammation ⮙ gastroenteritis)
- Severe but short response (1-6 hrs following ingestion; done by 48 hrs)
- Detection of toxins or the organism in food
- Antibiotics are useless

29.5 Clostridial diseases:

- Gram positive, spore-forming, anaerobes common in soil; *C. perfringens* and *C. botulinum*
- *C. perfringens* - food poisoning: ingestion of > 10^8 cells (inappropriate cooking followed by unrefrigirated storage in closed containers) ⮙ spore germination in the intestine leads to neurotoxin production
- Alteration of water permeability of intestinal lining ⮙ diarrhea and intestinal cramps (no vomiting or fever); onset within 7 - 16 hrs of ingestion but gone in 24 hrs
- Diagnosed by isolation of microbe or detection of toxin in feces

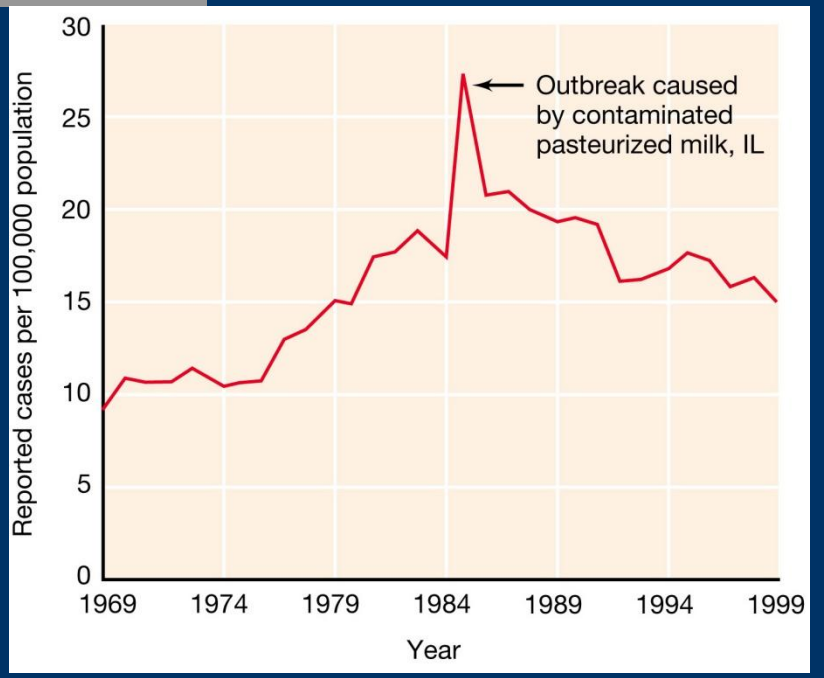
Botulism (*C. botulinum*):



- The most potent toxin known; few cases but high mortality (25%); destroyed by 10 min in 80 °C
- Flaccid paralysis of muscles
- Common in soil and water
- How? Improper canning ⮝ spore germination ⮝ toxin production ⮝ canned food used without cooking ⮝ disease
- Infant botulism: consumption of honey that is contaminated by spores (0 - 2 months)
- Treatment: antitoxin and ventilation

Salmonellosis:

- Gram negative enteric bacterium; all strains are pathogenic; transmission is from sources (eggs, meats) and by food handlers
- Colonization of of intestinal epithelium



- **Two diseases:**
  - **<u>Enterocolitis</u> (most commonly by *S. typhimurium*): 10^5 - 10^8 viable cells; disease onset within 8 - 48 hrs; headaches, chills, vomiting, diarrhea and fever (2-3 days); continuous shading of organism for months/years (Typhoid Mary); treatment - none**
  - **<u>Typhoid fever</u> (*S. typhi*): Septicemia leading to high fever that can last for several weeks; mortality is 15% if untreated; antibiotics**
- **Prevention: Cooked food (70 ^{o}C for 10 min); monitor for carrier state among food handlers**

Pathogenic *E. coli*:

- Some strains of *E. coli*; diarrhea and urinary tract infection; classification of pathogens is based on toxin and diseases
- Enterohemorrhagic (O157:H7) - colonization of the small intestine and verotxin production ⮙ diarrhea and kidney infection; uncooked and undercooked ground meat; occasional epidemics
- Enterotoxigenic (Travelers diarrhea) - heat labile toxin; water and produce in developing countries; immunity
- Enteropathogenic - diarrhea that afflicts young children
- Enteroinvasive - invasive colon infection; bloody diarrhea; survival in phagosomes; in developing countries
- Treatment and prevention: diseases are self-contained but antibiotics help; irradiation of ground beef!

Campylobacter:

- Gram negative microaerophile common in poultry and sometimes in beef
- *C. jejuni* and *C. coli* ➢ bacterial diarrhea; *C. fetus* ➢ spontaneous abortion in livestock
- Ingestion of 10^4 cells ➢ colonization of small intestine ➢ inflammation ➢ high fever (104 ^{o}C), headache, malaise, nausea, cramps, diarrhea ➢ subsides in 1 week; erythromycin to shorten infectious stage
- Prevention by proper cooking and hygiene (including utensils)

Listeriosis:

- *Listeria monocytogenes*: a gram (+) bacillus; Cold and salt tolerant; wide distribution; found in soil water and raw milk; contaminates all food products either at source or during processing; mostly in processed food
- Pathology (2500 per year):
  - Uptake by phagocytes ⮝ growth ⮝ lysis of phagocyte ⮝ infection of nearby cells
  - Immunity due to cell-mediated T_H1 cells ⮝ macrophage activation
  - In normal individuals - gastrointestinal food infection; in immuno-compromised individuals - acute bacterimia and meningitis (20% death rate)
- Prevention: cleanliness during food processing; avoiding outdated foods
- Diagnosis by culturing from blood and spinal fluid; treated with trimethoprim drugs

Other foodborne infectious diseases

- **Bacterial diseases**
  - *Yersinia enterocolitica* - enteric fever
  - *Bacillus cereus* - food poisoning by heat stable toxin
  - *Shigella* spp. - shigolosis (100,000 per year)
  - *Vibrio* spp. - contaminated seafood
- **Viral diseases** - the most common cause of gastrointestinal diseases; “24-hour flu” - fast and self-containing; fecal contamination
  - Norwalk viruses, rotaviruses, astroviruses, hepatitis A

- **Protists**
  - *Giardia lamblia, Cryptosporidium parvum, Cyclospora caytanensis*
  - Spread by use of water contaminated with fecal metter; mostly by consumption of fresh produce
  - Cyclosporiasis - an emerging disease (acute gastroenteritis)
  - *Toxoplasma gondii* (toxoplasmosis)- transmitted by cats; of concern with immunocompromised individuals
- **Prions**
  - Slow-progressing degenerative diseases - "mad cow disease"
  - The infectious agent is a protein; causes a conformational change in homologes ⮙ cell death⮙ "holes" in brain tissue
  - Transmission from animals by consumption of meat
  - Variant Creutzfeldt-Jacob Disease, Bovine Spongiform Encephalitis
  - Control by destruction of infected animals