Food Microbiology

The good, the bad and the ugly

- Good-bacteria are important in food production
- Bad-some bacteria cause food poisoning
- Ugly-some bacteria cause food spoilage

Some factors that influence growth in foods…**temperature**

- Remember that some microbes grow well at cooler temperature, others more slowly

Some of the factors that influence growth in foods… **Water Availability (a_w)**

Food	**(a_w)**	**Microbe**	**Minumum (a_w)**
Fresh meat	0.99	Spoilage microbes	0.91
Hot dog	0.92	*Pseudomonas*	0.97
Ham	0.91	*Staphylococcus aureus*	0.86
Dried fruit	0.72-0.8	Yeasts	0.81
		Molds	0.80

Some factors that influence growth in foods….**pH**

Foods	**pH of food**	**Microbe**	**Minimum pH of microbe**
beef	5.5	Most spoilage microbes	4.0
milk	6.3	molds	1.5
spinach	5.5	yeast	2.5
apples	3.0	*E. coli*	4.0

Some factors that influence growth in foods…. **Atmosphere**

- Presence or absence of oxygen
  - *Pseudomonas* are obligate aerobes
  - *Clostridium* are obligate anaerobes

Some factors that influence growth in foods…. **Nutrients**

- If a food lacks a nutrient then the organism must be able to make it on their own to grow

Some factors that influence growth in foods…. **Biological barriers**

- Foods that have skins, rinds, shells protect from spoilage….prevent bacterial growth

Some factors that influence growth in foods…..**Antimicrobial chemicals**

- Some food have naturally occurring enzymes, etc

Microbes in food production

- Lactic Acid bacteria
- Yeasts…*Saccharomyces cerevisiae*
- Molds

Lactic Acid Bacteria

TABLE 31.1 Foods Produced Using Lactic Acid Bacteria

Food	Characteristic
Milk Products	
Cheese (unripened)	Uses a starter culture usually containing *Lactococcus cremoris* and *L. lactis*
Cheese (ripened)	Uses rennin and a starter culture containing *Lactococcus cremoris* and *L. lactis;* ripened for weeks to years; other bacteria and/or fungi may be added to enhance flavor development
Yogurt	Uses a starter culture containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subspecies *bulgaricus*
Sweet acidophilus milk	*Lactobacillus acidophilus* added for possible health benefits
Vegetables	
Sauerkraut	Cabbage; succession of naturally occurring bacteria including *Leuconostoc mesenteroides, Lactobacillus brevis,* and *Lactobacillus plantarum*
Pickles	Cucumbers; naturally occurring bacteria
Poi	Taro root; naturally occurring bacteria; Hawaii
Olives	Green olives
Kimchee	Cabbage and other vegetables; Korea
Meats	
Dry and semidry sausages	Uses a starter culture containing species of *Lactobacillus* and *Pediococcus*; meat is stuffed into casings, incubated, heated, and then dried

Yeasts

TABLE 31.2 Foods and Beverages Produced Using Alcoholic Fermentation by Yeast

Product	Characteristic
Alcoholic Beverages	
Wine	Sugars in grape juice are fermented by *Saccharomyces cerevisiae.*
Sake	Amylase from mold (*Aspergillus oryzae*) converts the starch in rice to sugar, which is then fermented by *S. cerevisiae.*
Beer	Enzymes in germinated barley convert starches of barley and other grains to sugar, which is then fermented by *S. cerevisiae.*
Distilled spirits	Sugars, or starches that are converted to sugars, are fermented by *S. cerevisiae*; distillation purifies the alcohol.
Vinegar	Alcohol produced by fermentation is oxidized to acetic acid by species of *Gluconobacter* or *Acetobacter.*
Breads	*S. cerevisiae* ferments sugar; expansion of CO_2 causes the bread to rise; alcohol evaporates during baking.

Molds

TABLE 31.3 Foods Produced Using Molds

Food	Characteristic
Soy sauce	Koji is produced by inoculating soybeans and cracked wheat with a starter culture of *Aspergillus oryzae* or *A. sojae;* the mixture is then added to a brine and incubated for many months.
Tempeh	Soybeans are fermented by lactic acid bacteria and then inoculated with a species of the mold *Rhizopus;* Indonesia.
Miso	Rice, soybeans, or barley are inoculated with *Aspergillus oryzae;* Asia.
Cheeses	
Roquefort, Gorgonzola, and Stilton	Curd is inoculated with *Penicillium roquefortii.*
Brie and Camembert	Wheels of cheese are inoculated with selected species of *Penicillium*.

Food Spoilage

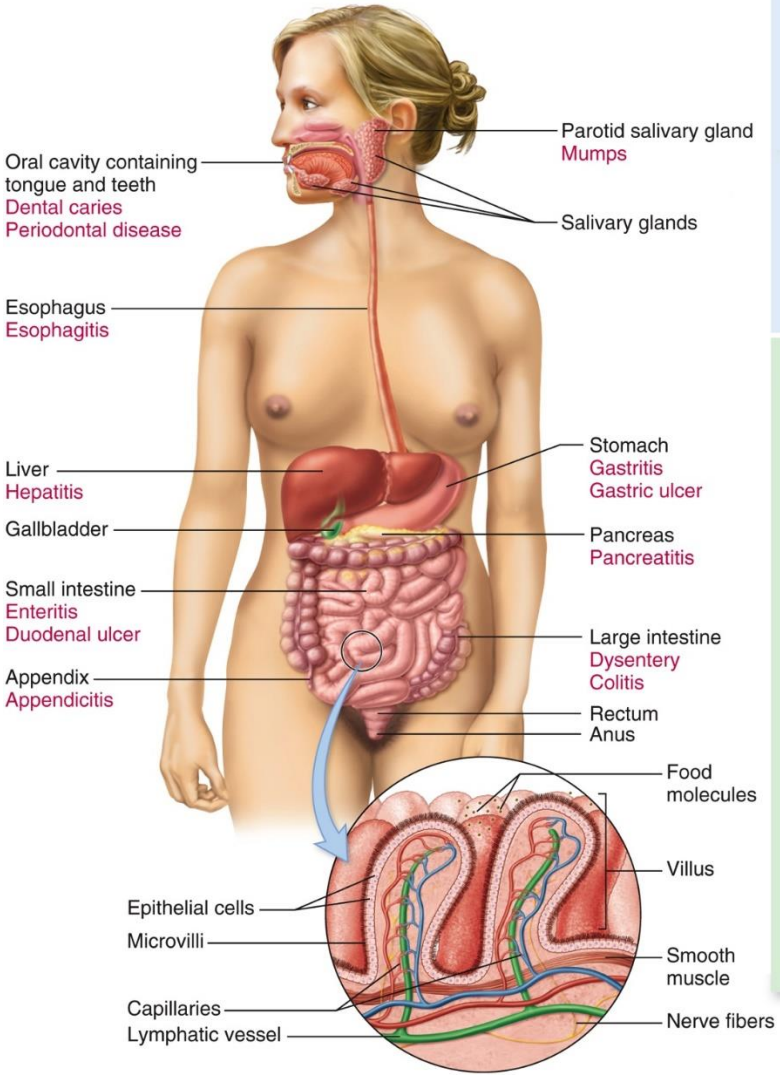
- Spoilage is due to the bacteria that contaminate foods

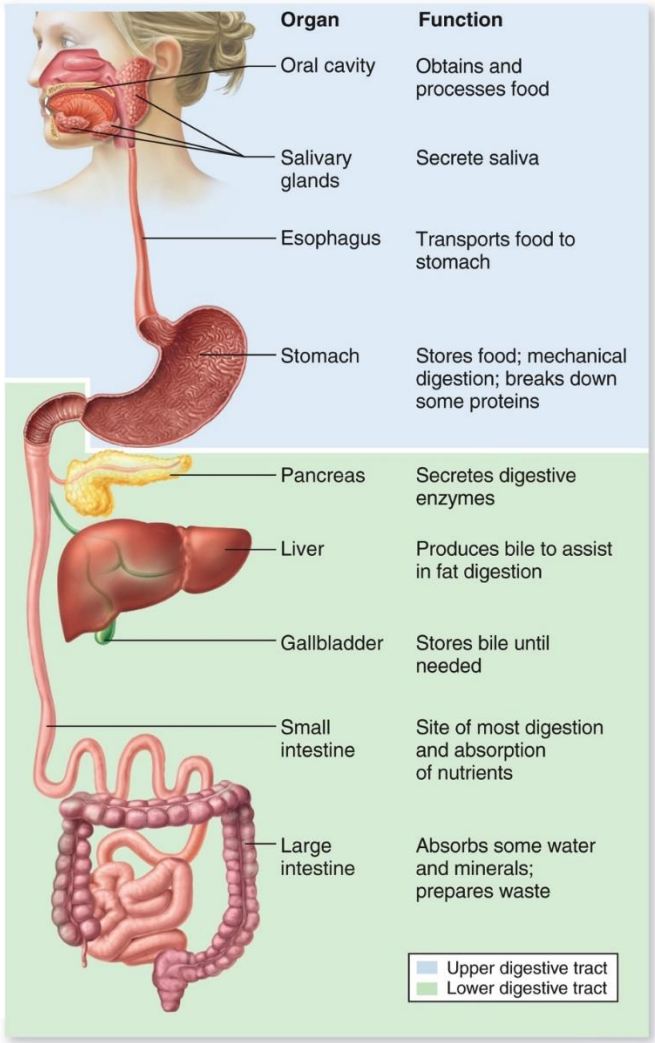
  -meats

  -fish

  -grains

Overview of Digestion





How do organisms cause food poisoning?

- **Food borne intoxication**: bacteria grow within the food and produce **toxins**, the toxins are what lead to food poisoning symptoms
- Examples: *Clostridium botulinum*

  Staphylococcus aureus

Clostridium botulinum : botulism

- General characteristics: gram positive rod, anaerobe, spore former
- Produces a toxin (neurotoxin)
  - Heat sensitive
  - One gram can kill 1 million
- Toxin inhibits the release of acetylcholine from neurons…..what happens next?

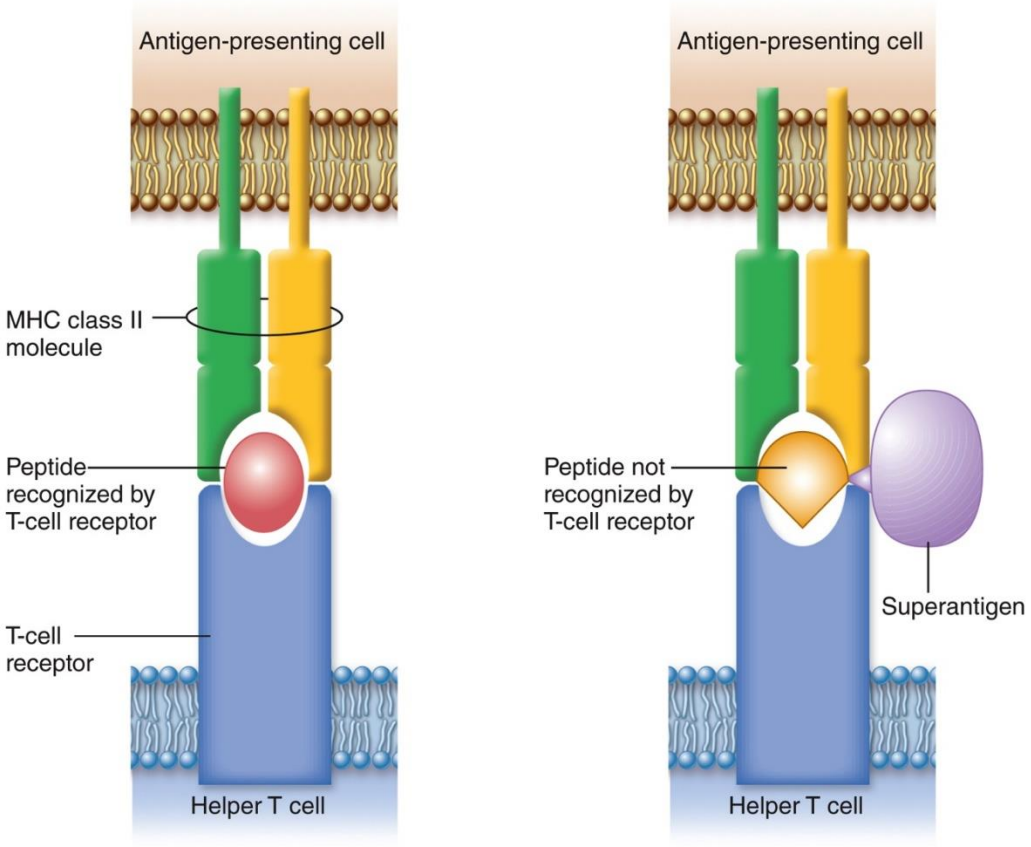
Botulism

- **Foods associated:** home canned "low acid" vegetables, honey
- **Symptoms**:12-36 hours after ingestion vomiting, diarrhea, blurred vision, double vision, trouble swallowing, and descending muscle weakness
- **Treatment**: antitoxin not antibiotics..why?

Staphylococcus aureus: 24 hour Flu?

- **General characteristics**: gram positive coccus in clusters, facultative anaerobe, part of normal skin flora
- Strain that cause food poisoning
  - Produce an enterotoxin (targets the GI tract)
  - Enterotoxin acts as a **superantigen**

Superantigens activate T cells



a Helper T cell that recognizes peptide is activated; it proliferates and releases cytokines.

b Helper T cell that does not recognize peptide is activated because of superantigen; it proliferates and releases cytokines.

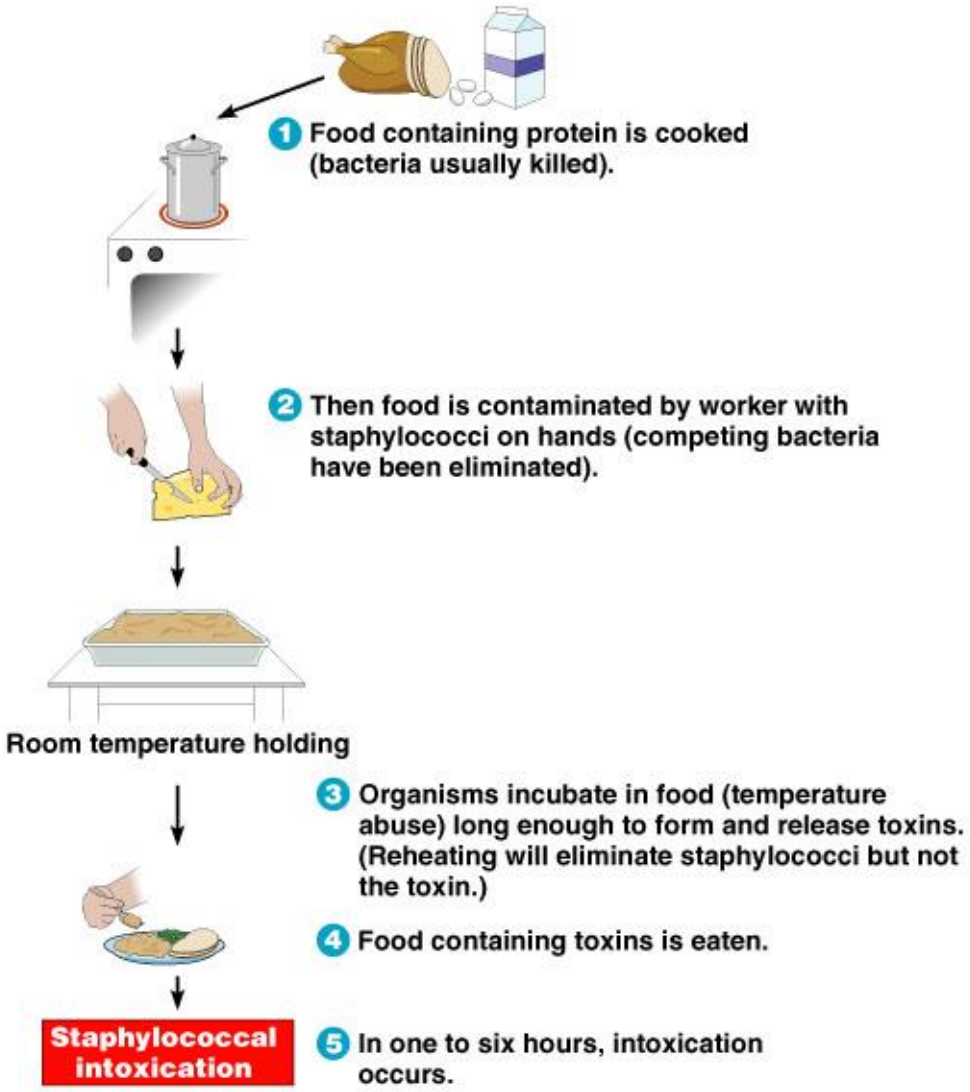
Staphylococcus aureus

- **Foods associated**: cream based desserts, custards, potato and egg salads
  - Key is to have a food handler with the organism as part of the normal skin flora
  - Remember a_w coefficient for this organism is low
  - Food left at 28^0C for 2-4 hours with *S. aureus* will have enough cells grown to cause food poisoning

Staphylococcus aureus

- **Symptoms**: appear 1-6 hours after ingestion and include vomiting, diarrhea, and intense abdominal pain/cramping, usually no fever

  -last approximately 24 hours
- **Treatment**: none, supportive care



Some organisms cause food poisoning after ingestion

- **Food borne infection**: bacteria enter food, **infected food is ingested**, bacteria grow within the intestines and produce toxins
- Examples: *Campylobacter jejuni*, *Salmonella*, *Salmonella typhii*, *Shigella*, *Vibrio cholerae, Vibrio parahemolyticus, Vibrio vulnificus, E. coli, Listeria monocytogenes*

Mechanisms of pathogenesis

- **Attachment**: pili or adhesins
- **Toxin** production: two kinds of toxins

  1)increase secretion of water and electrolytes

  2)cause cell death
- **Alterations** in host cells
- **Cell invasion**

Campylobacter jejuni

- **General characteristics**: gram negative curved rod, microaerophile, one or two polar flagella, no capsule
- Part of the normal flora of poultry and dairy cattle

  -unpasteurized milk, undercooked poultry

  -may be found at a concentration of 10^9cells/gram of chicken

Campylobacter jejuni

- **Incubation period**: 2-5 days
- **Symptoms**: Diarrhea (which may be bloody), abdominal pain, fever (104^0F), vomiting not common
  - Last 2-10 days
  - Some cases lead to Guillain-Barre (0.1%) syndrome and Rheumatoid Arthritis weeks after the illness

Campylobacter jejuni

- **Treatment**: none, mostly supportive care

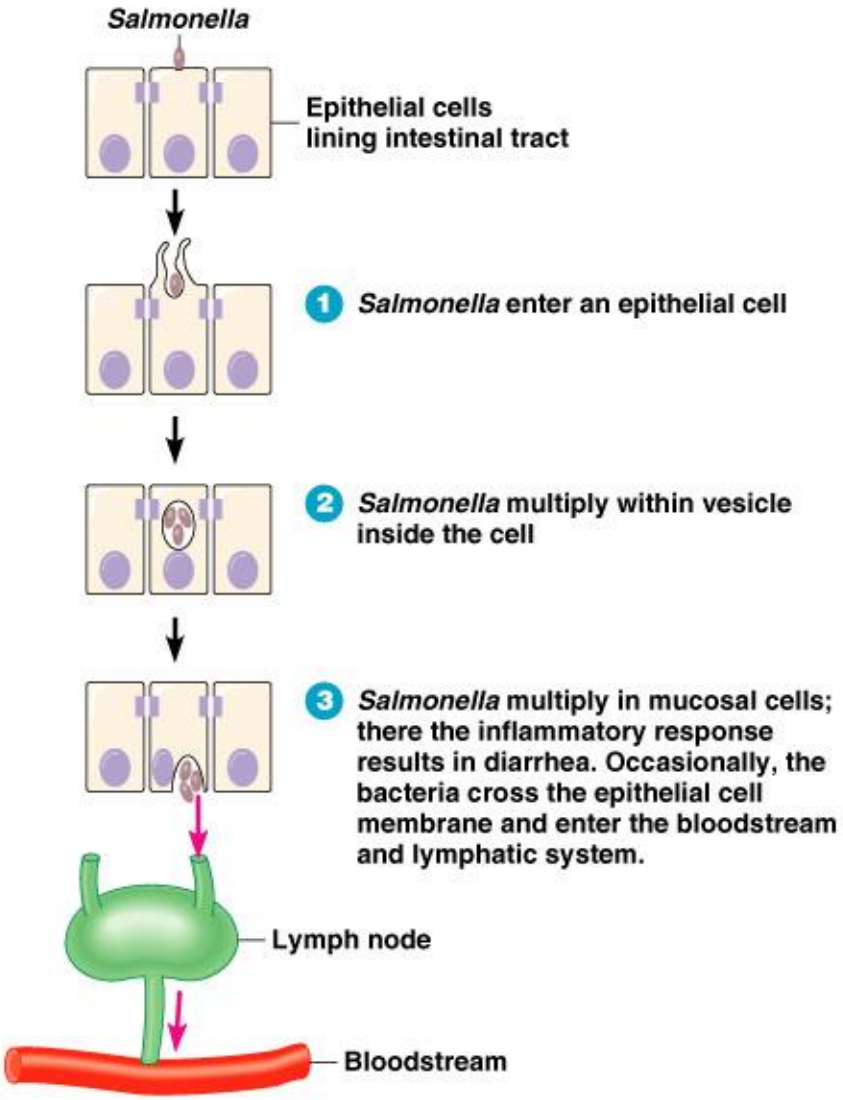
  -some cases require antibiotics (erythromycin, quinolones)
- Avoid undercooked poultry and watch the use cutting boards in food prep

Salmonella enterica

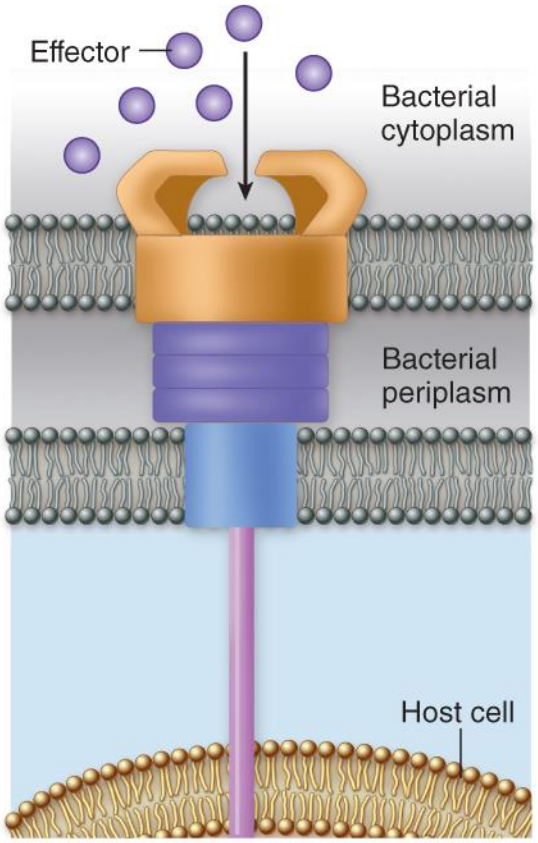
- **General characteristics**: gram negative rod, facultative anaerobe, peritrichous flagella
- Over 2000 closely related serovars
- Part of the normal flora of poultry, reptiles

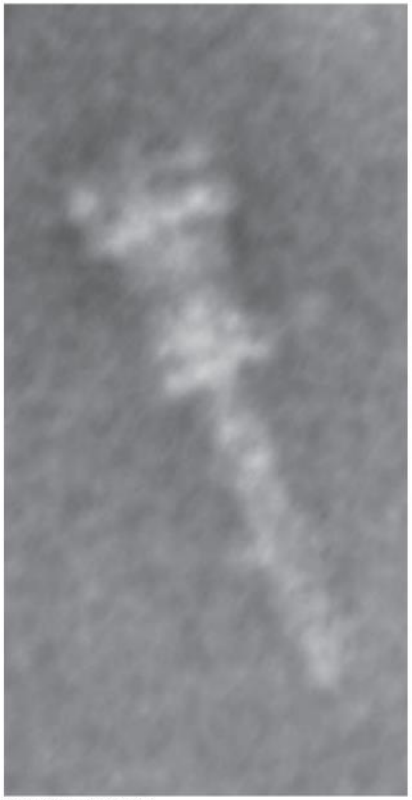
Salmonella

- Incubation time: 6-72 hours
- Symptoms: include diarrhea, abdominal pain, and a moderate fever
- Full recovery in a few days but may shed the organism for 6 months
- Approx. 2-4 million cases/year, only 40-50,000 are actually reported



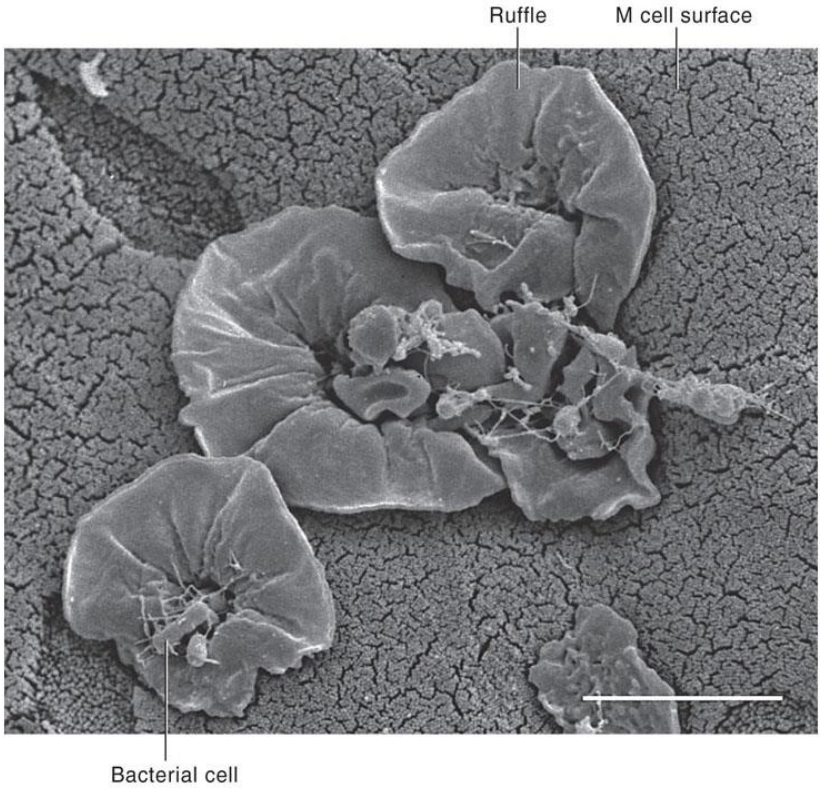
Type III Secretion System





Courtesy of ChihiroSasakawa, University of Tokyo

Membrane ruffling



Courtesy of Mark A. Jepson, from Trends in Microbiology (98)v6:359-365, "Studying M cells and their role in infection"; M.A. Jepson and M.A. Clark, Elsevier Press

Salmonella serotype Typhi and *Salmonella* serotype Paratyphi

- *Salmonella* serotype Typhi causes typhoid fever
- *Salmonella* serotype Paratyphi causes paratyphoid fever
- General characteristics: Both are gram negative rods, only found in humans (fecal-oral transmission)

Salmonella serotype Typhi and *Salmonella* serotype Paratyphi

- **Incubation time**: 1-3 weeks
- **Symptoms:** high fever (104^0F), headache, constipation, abdominal pain, internal bleeding, shock, death
- Some individuals become carriers (1-3%), only 350-500 cases annually in US
- **Treatment**: antibiotics

Shigellosis caused by *Shigella*

- **General characteristics**: gram negative rod, facultative anaerobe, non-motile
- Only found in the feces of other humans
- Organisms transmitted by the five F's

  -food, fingers, feces, flies, and fomites

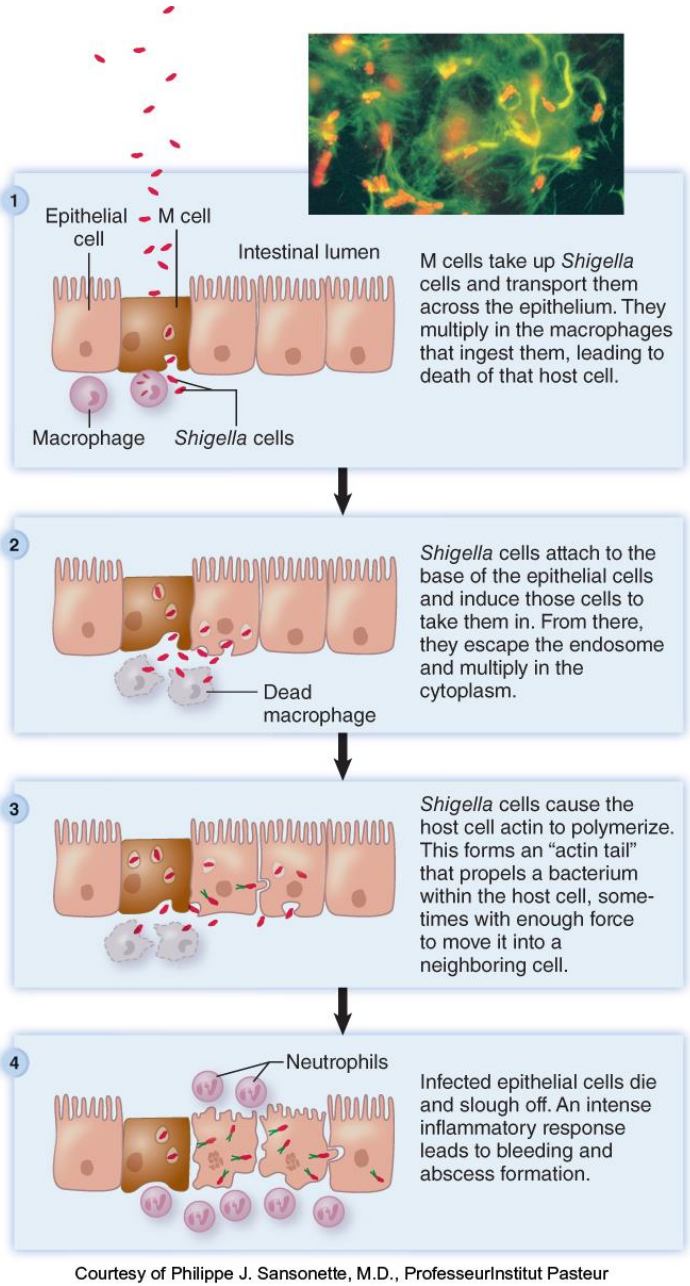
Different species of *Shigella*

- *Shigella sonnei*-most common species found in the US, least virulent of all *Shigella* responsible for bacillary dysentery
- *Shigella dysenteriae*-most common in developing countries, causes more serious infection due to the production of a Shiga toxin

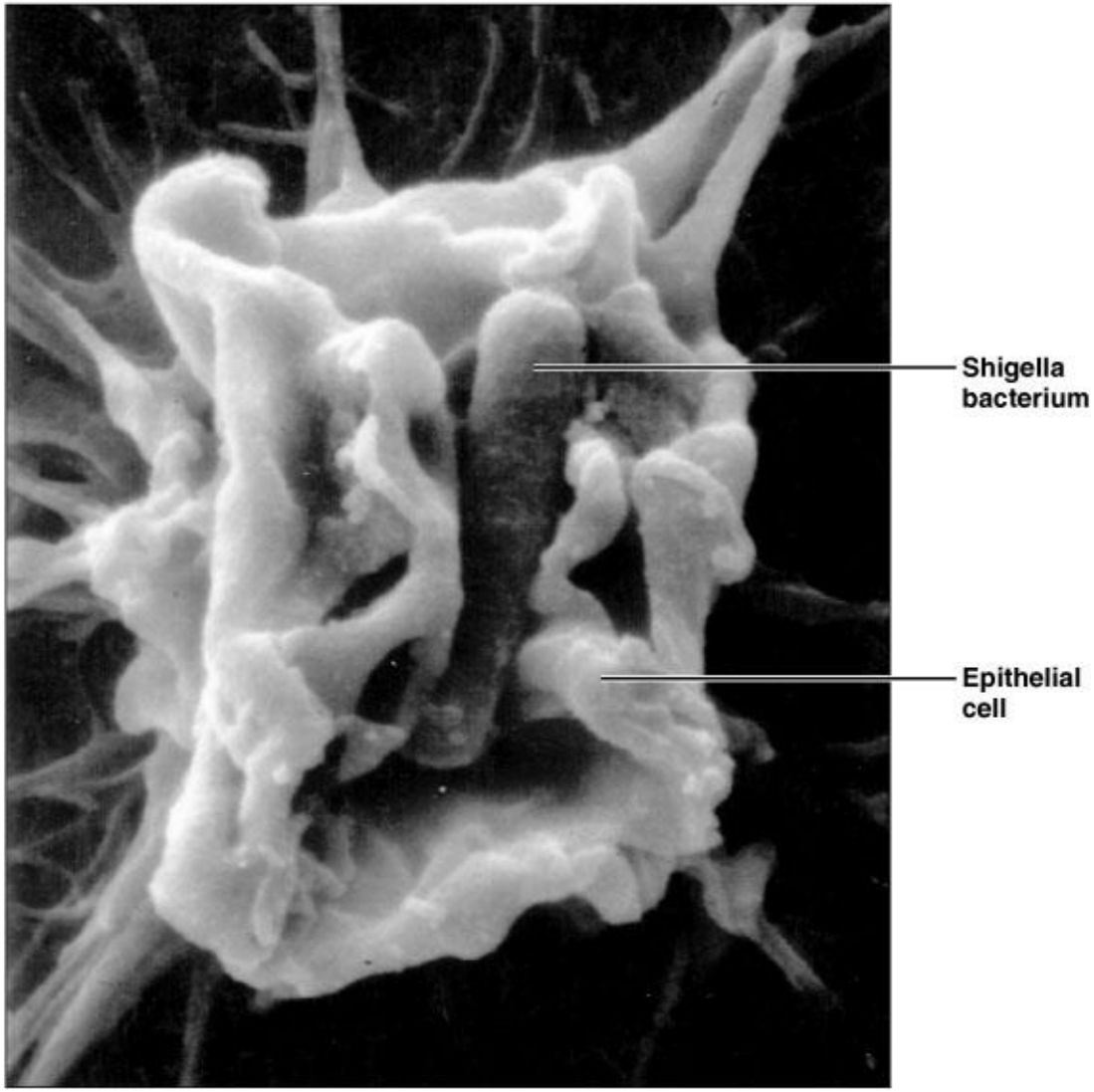
Shigella

- **Incubation time**: 3-4 days
  - Only need 10 cells to cause infection
- **Symptoms**: fever, abdominal pain, diarrhea (may contain blood and mucus which is why it is also called dysentery)
- See passage of small volume bloody stools (20/day)

Shigella enters via M cells



Courtesy of Philippe J. Sansonette, M.D., ProfesseurInstitut Pasteur

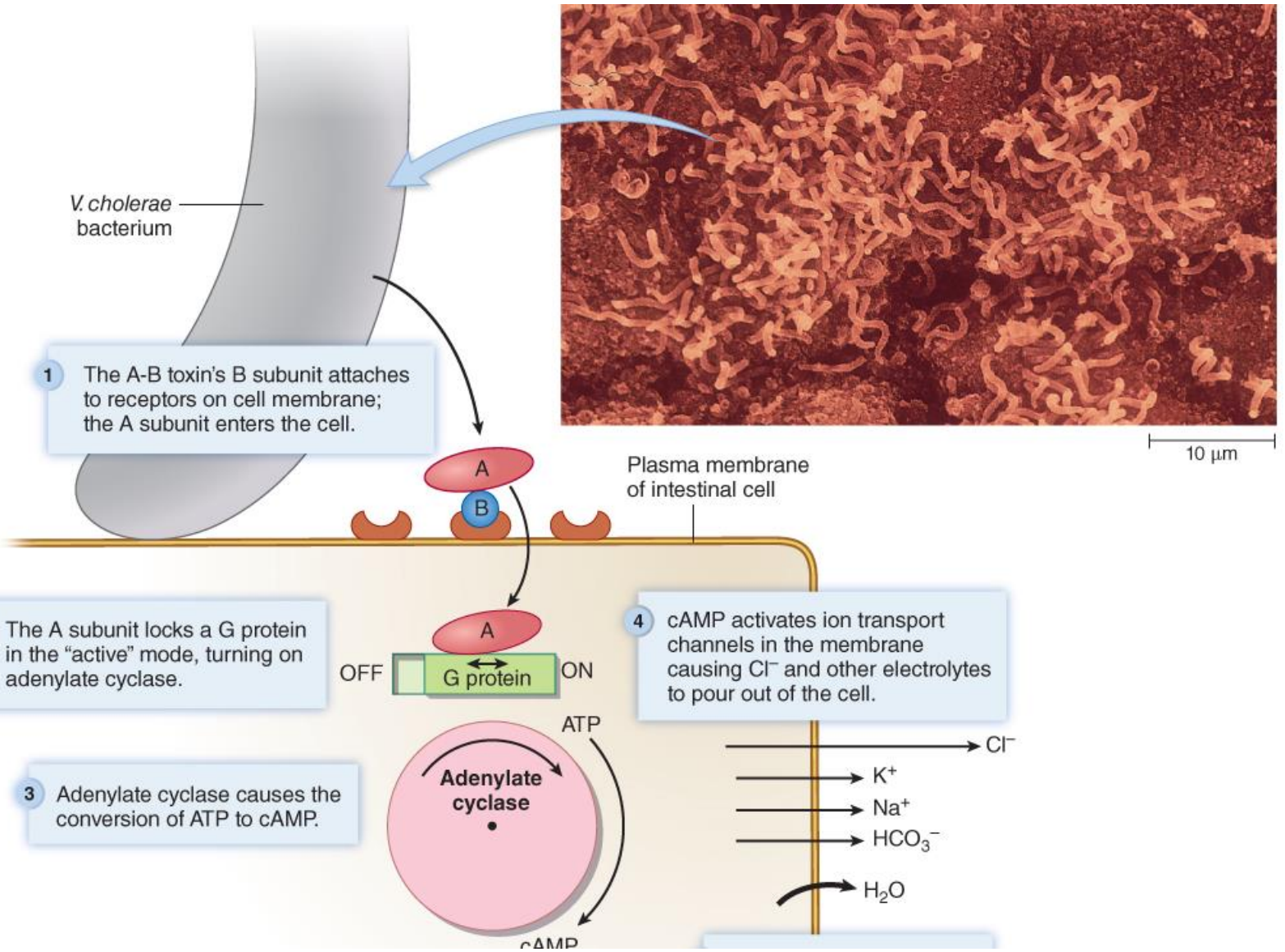


Vibrio cholerae

- Causative agent of cholera
- **General Characteristics**: Curved gram negative rod, facultative anaerobe, single polar flagella, pili
- Can exist in saltwater for extended periods of time, halotolerant
- Different serotypes based on O antigen, O1 is current serotype circulating

Vibrio cholerae

- Incubation time: 12-48 hours
- Symptoms: rice watery stools, sudden onset of explosive watery diarrhea with vomiting and pain
- Cholera toxin is the key pathogenic feature


V. cholerae bacterium
10 µm
1 The A-B toxin's B subunit attaches to receptors on cell membrane; the A subunit enters the cell.
A
B
Plasma membrane of intestinal cell
2 The A subunit locks a G protein in the "active" mode, turning on adenylate cyclase.
A
OFF
G protein
ON
4 cAMP activates ion transport channels in the membrane causing Cl⁻ and other electrolytes to pour out of the cell.
ATP
Adenylate cyclase
3 Adenylate cyclase causes the conversion of ATP to cAMP.
cAMP
Cl⁻
K⁺
Na⁺
HCO3⁻
H2O

Non-cholera Vibrios

- *V. parahemolyticus*
  - Cause vomiting, diarrhea, abdominal pain
  - Incubation time 4-96 hours
  - Duration of illness 2.5 days
- *V. vulnificus*
  - Cause vomiting, diarrhea, abdominal pain
  - Immunosuppressed individuals leads to septic shock

Diarrhea causing *E. coli*

- Classified according to virulence
  - Entertoxigenic *E. coli* (ETEC*)*
  - Enterpathogenic *E. coli* (EPEC*)*
  - Shiga toxin-producing *E. coli* (STEC*)*
  - Enteroinvasive *E. coli* (EIEC*)*
  - Enteroaggregative *E. coli* (EAEC)
  - Diffusely adhering *E. coli* (DAEC)

Entertoxigenic *E. coli* (ETEC)

- Also known as traveler's diarrhea
- Enterotoxin promotes the pumping of Cl^- and inhibition of NaCl which results in diarrhea, similar to cholera toxin
- No invasion
- Pili used for attachment
- Can develop immunity

Enterpathogenic *E. coli* (EPEC)

- Attacks the small intestine
- Inject effector proteins that cause A/E lesions
- In developing countries accounts for 20% of diarrhea in bottle-fed infants

Shiga-toxin *E. coli* (STEC)

- Obtain from the consumption of animal products
- Attacks the colon, produce A/E lesions
- Produces Shiga toxins
- O157:H7 causes bloody diarrhea which may lead to hemolytic uremic syndrome

Enteroinvasive *E. coli* (EIEC)

- Invade the intestinal lining
- Disease is similar to Shigella

Enteroaggregative *E. coli*

- Use pili to attach to intestine
- Grow as aggregates in a biofilm
- Produce cytotoxins and enterotoxins which damage host epithelium

Diffusely adhering E. coli

- Similar to EAEC but grow in a diffuse layer

Listeria monocytogenes

- Motile, non-spore forming facultative anaerobe, gram positive rod
- Grows at 4^{o}C
- Causes Listerosis

Listeria monocytogenes

- Symptoms
  - Fever, muscle aches, sometimes nausea and diarrhea
- Incubation
  - Few days to months

Listeria monocytogenes

- Watch foods such as soft cheeses, non-pasteurized cheeses, coleslaw, hot dogs
- Prevention: high risk groups should avoid the food listed above and reheat and refrigerated leftovers

Clostridium perfringens

- Problem with poorly prepared meats and poultry
- Incubation time 8-16 hours, lasts 24 hours
- Symptoms: intense abdominal cramps, diarrhea
- Enterotoxin mediates symptoms