Pathogen Genomics—What makes an Organism a Pathogen

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FOOD SAFETY SUMMIT CONFERENCE & EXPO
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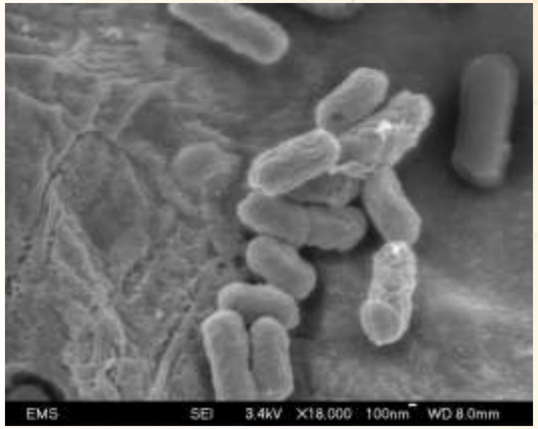
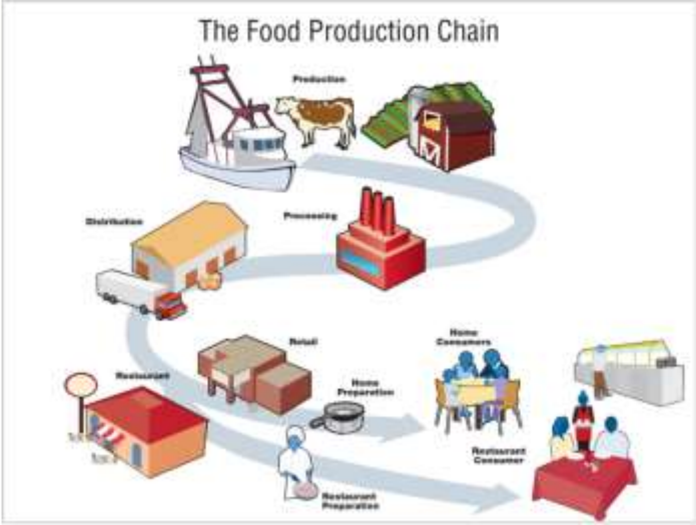
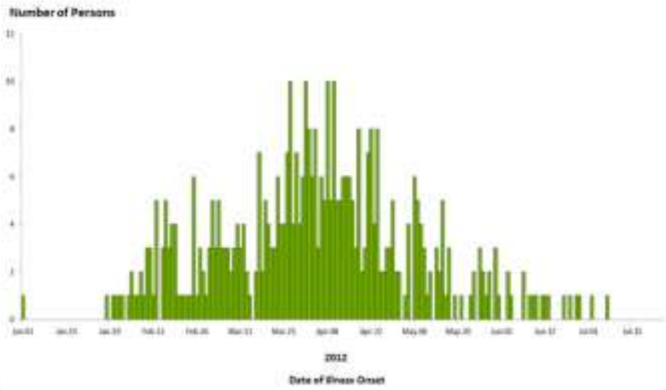
What is a Pathogen?

Overview



- Microbial relationships and some definitions
- How foodborne illness occurs
- Pathogenesis
- Stress and Injury Adaptation
- Genetic Responses





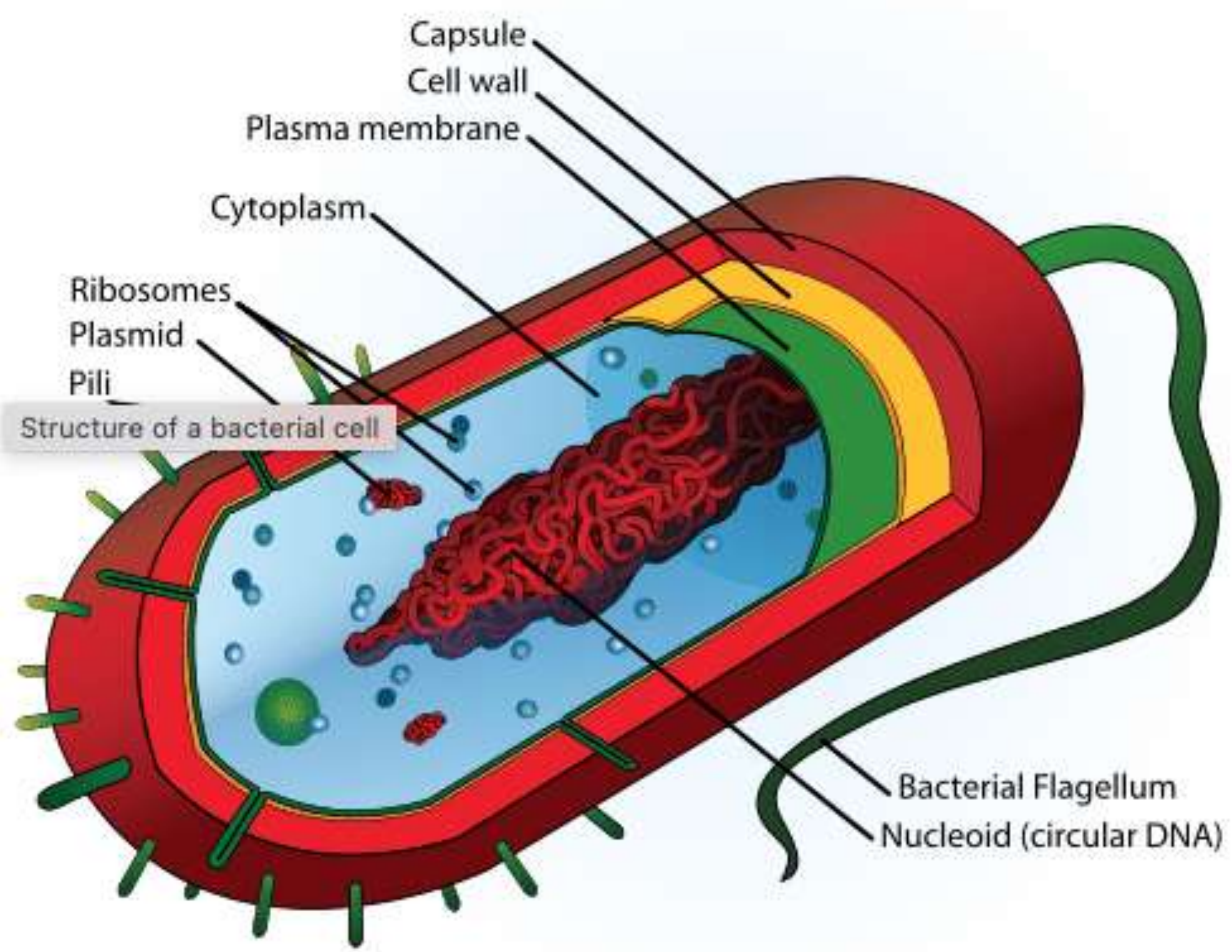
What is Foodborne Illness

Diseases, usually either infectious or toxic in nature, caused by agents that enter the body through the ingestion of food.

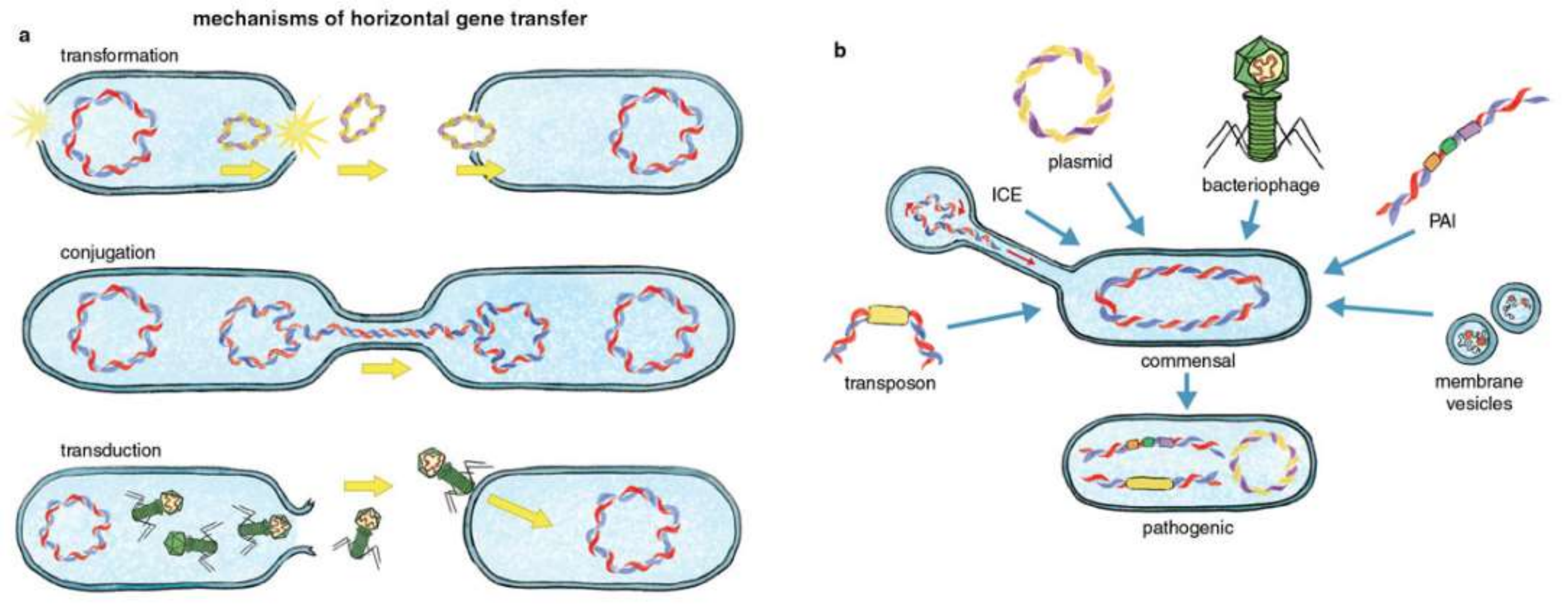
- World Health Organization

A long time ago,
in a galaxy far, far away

The Bacteria



How It All Begin … There’s This One Time …



Scientific American

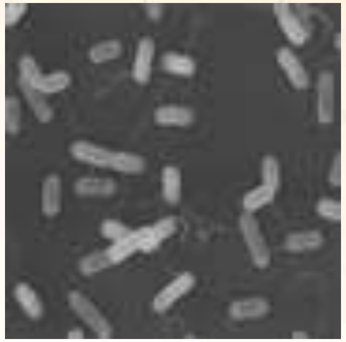
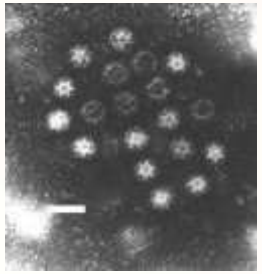
What makes a Pathogen?

- *Yersinia pestis* (Bubonic plague) (1347-1351)
- Wiped out 1/3 of Europe
- *Yersinia pseudotuberculosis* (ancestor)
- 5,000 years ago in Eurasian continent
- Acquired gene cluster to cause the respiratory from of disease
- Acquired *ymt* gene to transmission via fleas

American Scientist, *Vibrio cholerae*

Foodborne Pathogens

- > 250 different types described
- Infections: viable pathogens
  - Bacteria
  - Viruses
  - Parasites
- Intoxications: pre-formed toxins
  - Chemicals
  - Biological toxins
    - Microbial
    - Plant
    - Animal
- Allergenic reactions

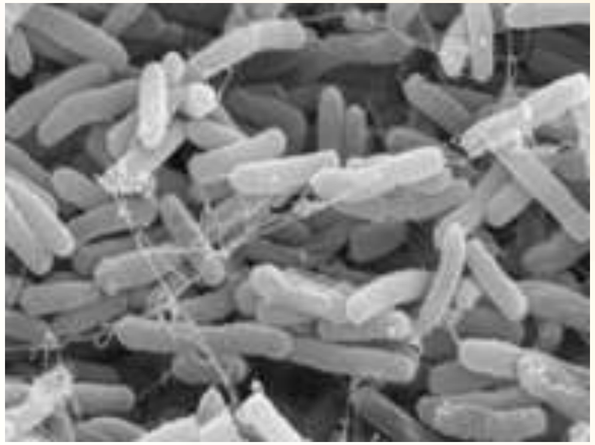


Definitions

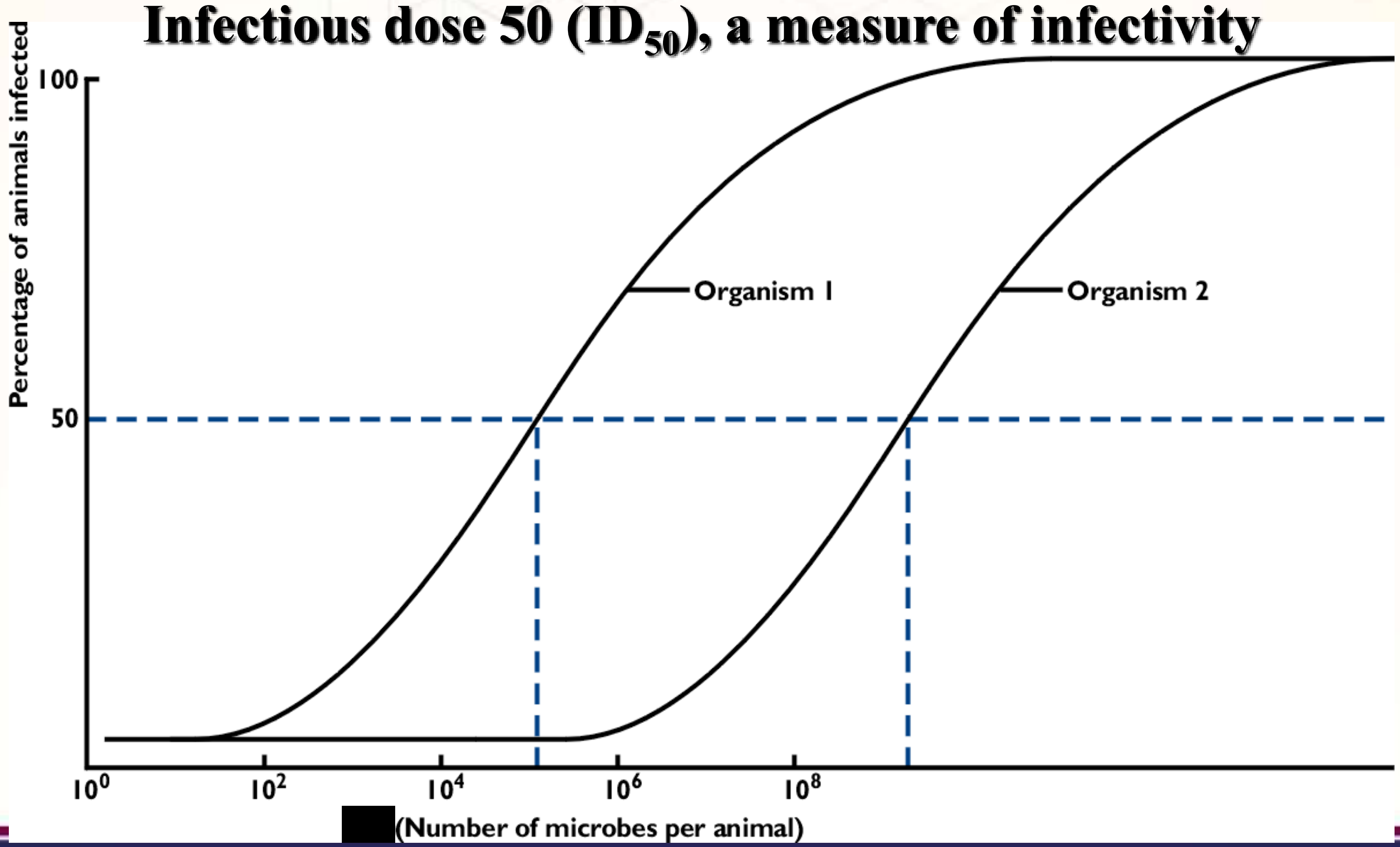
- **Infectious dose**: number of pathogen cells needed to be ingested to cause illness
- **Incubation period**: time between eating and appearance of symptoms

Nature of the Pathogen

- Virulence or Pathogenicity = capacity of a pathogen to cause disease
- Characterized by:
- Ability to infect a person
- Severity of disease
- No complications
- Complications with need for hospitalization
- Ability to cause death



Nature of the Pathogen: Ability to Infect



Ways of Exposure: Pathogens in *raw or undercooked* foods

Pre-harvest contamination

- Fields & manure
- Contaminated water
- Vectors
  - Wild animals: birds, deer, rodents, insects …
  - Domesticated animals: cows, pigs, chickens …

Ways of Exposure: Pathogens in *processed* foods

Post-harvest contamination

- Cross-contamination during processing
  - contaminated facility environment
- Process failure
  - inadequate cooking, preservatives, packaging
- Pathogen growth in food
  - improper holding temperatures – “temperature abuse”

Ways of Exposure: *humans* as agents of infection
Transfer by *fecal-oral route*

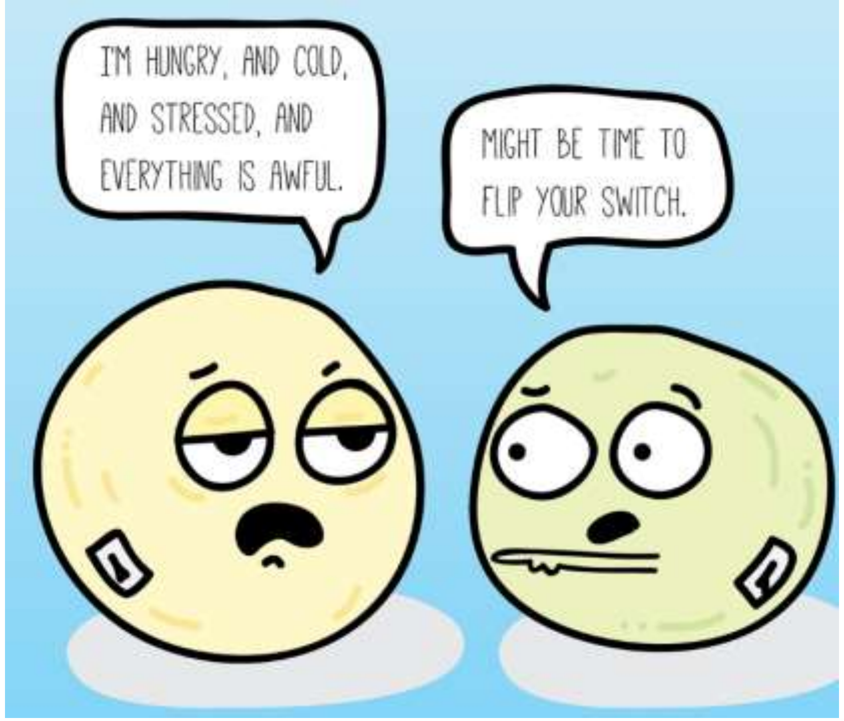
Feces contaminate hands → **Hands contaminate food** → **Person eats contaminated food** → **Feces contaminate hands**

Wash hands before preparing and eating food

Proper Handling and Preparation

Microbial Goal?

Adapt & Survive



Stress Adaptation

"A situation whereby a brief exposure to a suboptimal physical or chemical environment that enables the cells to resist subsequent exposure to the same or other types of harsher treatment to which the species is normally susceptible."

Ray et al

Stress Adaptation

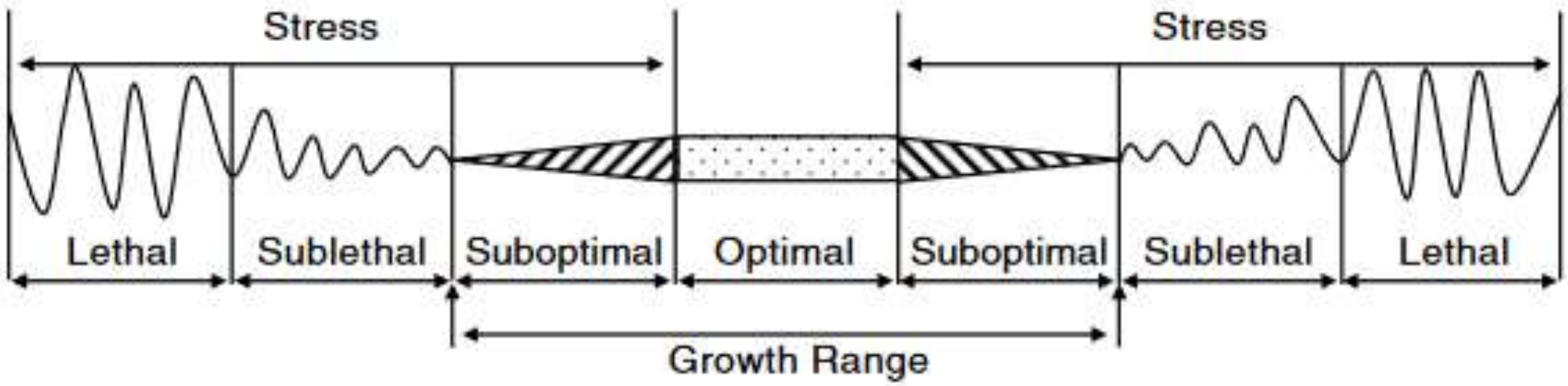


Figure 9.1 Different levels or degrees of environmental stresses to which bacterial cells can be exposed during processing and preservation of food. Bacterial cells exposed to a suboptimal growth condition show stress adaptation. Beyond the growth range, the cells are usually either sublethally or lethally stressed. See text for further explanations.

Ray et al

Sublethal Injury

F-T

Nonselective

selective

Importance of Injured Microorganisms in Food

Injured microorganisms likely to occur during processing, storage, and preservation

Hazards

-Potential capability of multiplying
-Undetectable in selective media

Preventive Controls

- Short repair phase prior to detection
- Storage in preservative conditions

Types of Stress

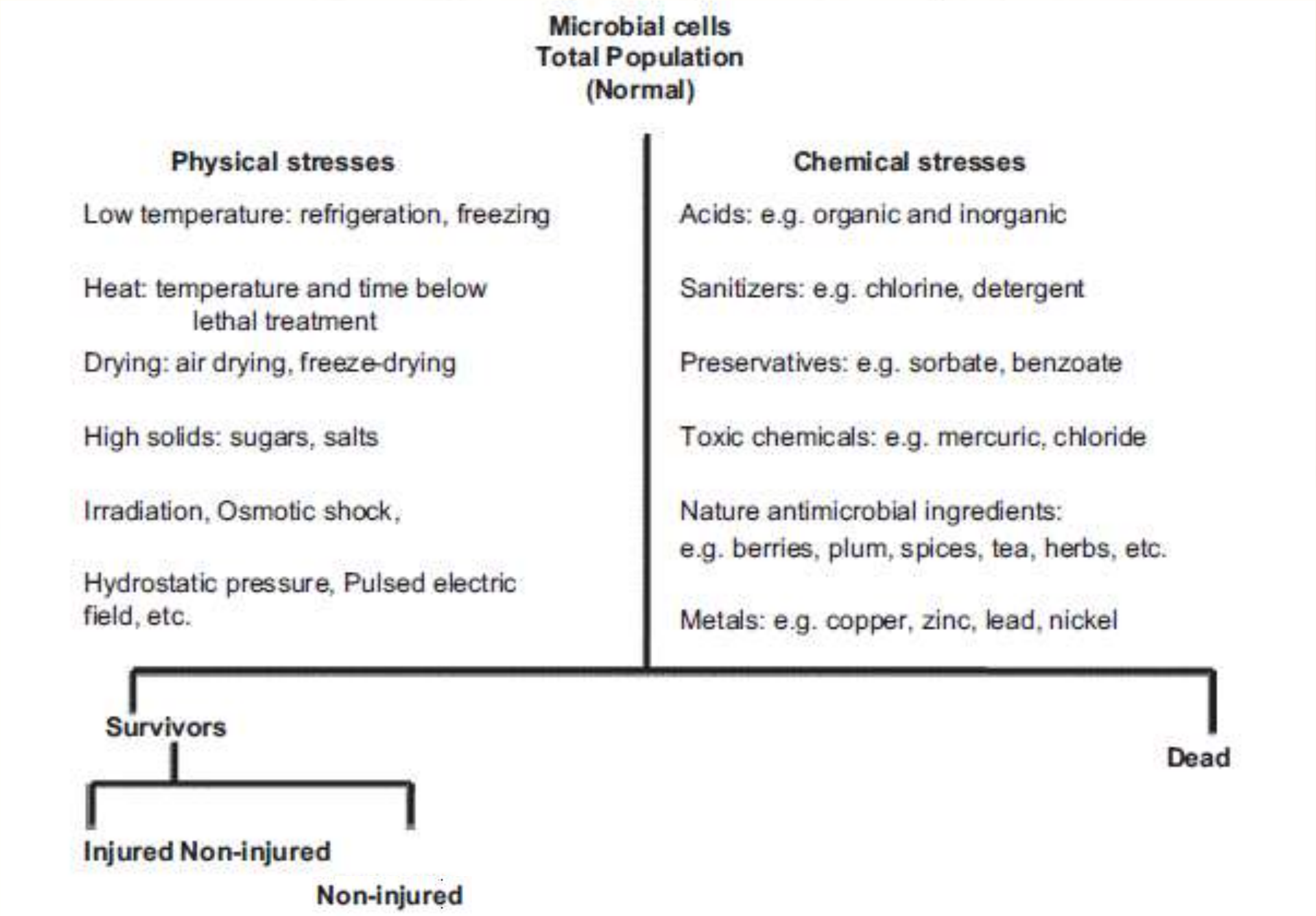
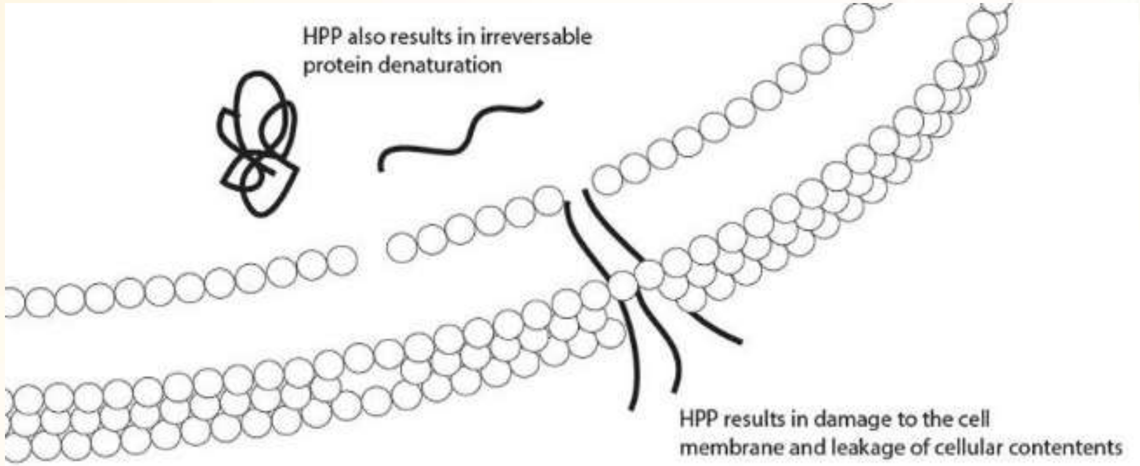


Fig. 1. Effects of sublethal treatments on microbial cells (Ray, 1979, 1989; Russell, 1984; McFeters, 1989; Bozoglu et al., 2004).

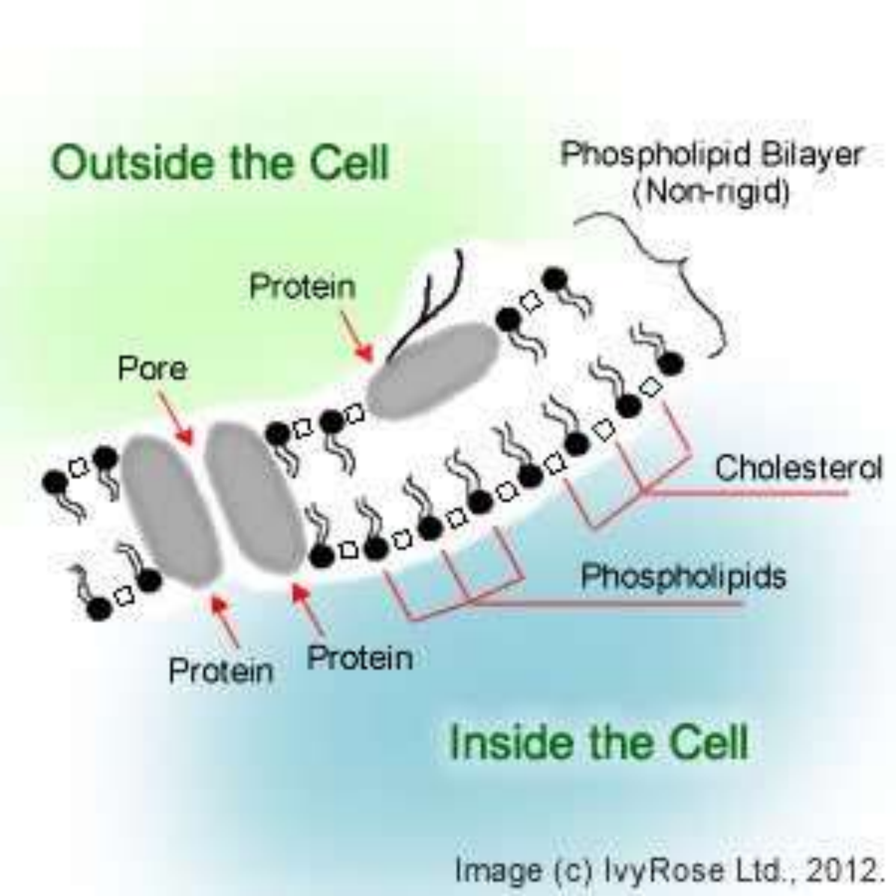
Cellular Changes

- Gram (+) and Gram (-) → cytoplasmic membrane remains intact, loses permeability



- Degradation of Genetic Materials
  - rRNA digested by RNase
  - Breakage of DNA
  - Autolytic enzymes activated, cell lysis

J. Ronholm et al. Front. Microbiol. 2016;7:350



Bacterial cells alter lipid composition (membrane)

→ Maintained fluid state

Mechanisms of Stress Adaptation

Current Concept:

Gene Response
→ Shock Proteins
→ Stress Proteins

- **Specific or nonspecific**
- **Inducible or constitutive**

❖ **Aid for adaptation to other stressors**

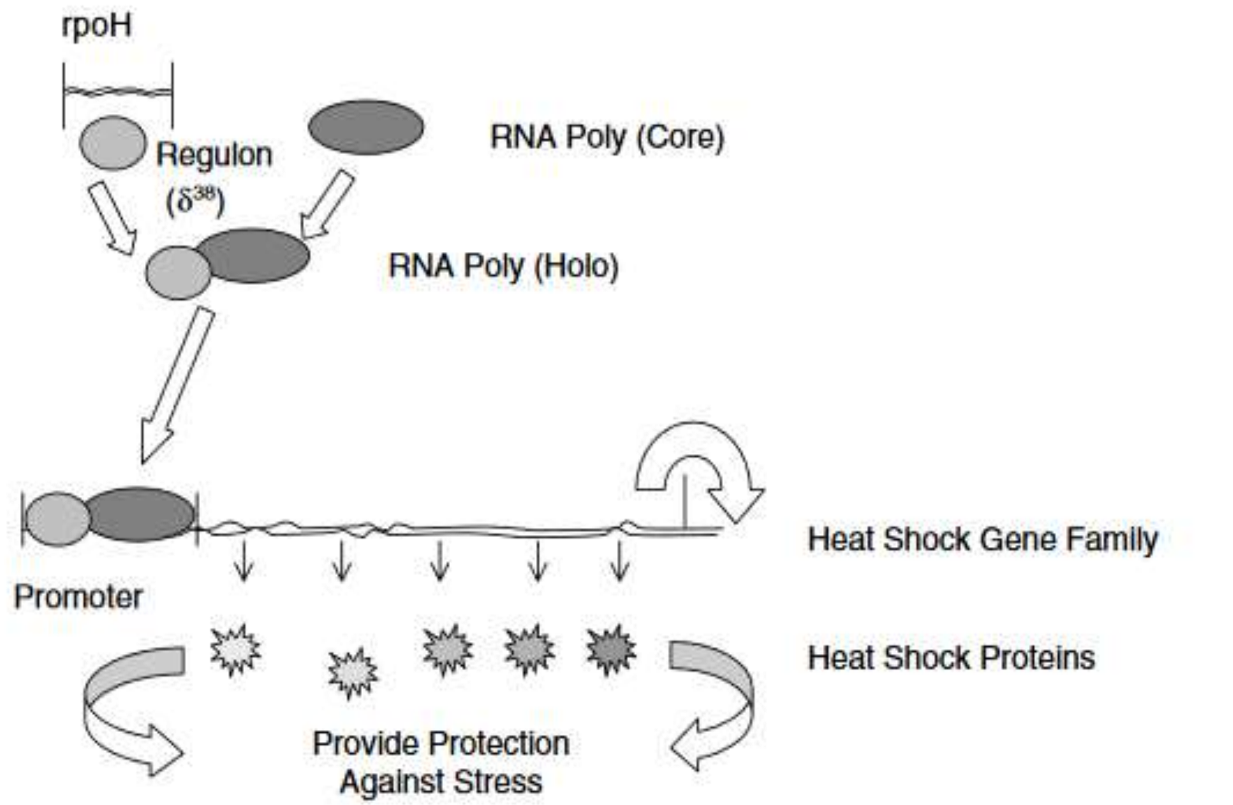


Figure 9.2 Genetic basis of coping stress with sigma factors by bacterial cells. See text for explanations.

Ray et al

Stress-Related Genes

- Bacteria contain a primary σ factor that is responsible for transcription of housekeeping genes necessary for growth and survival

- Many bacteria encode multiple alternative σ factors
  - The level and activity of the alternative σ factors are highly regulated and can vary depending on environmental or developmental signals

- Example: *rpo* systems
  - Usually regulated by a regulatory gene
  - Gram (-) – *rpoH* and *rpoE* for heat response and *rpoS* for general stress, cell density and starvation

Sigma Factors

Organism	σ	Gene	Function
E. coli	σ^{70} (σ^{D})	*rpoD*	Housekeeping genes
	σ^{H} (σ^{32})	*rpoH*	Heat shock
	σ^{E} (σ^{24})	*rpoE*	Extreme heat shock, periplasmic stress (ECF)
	σ^{F} (σ^{28})	*fliA*	Flagellar-based motility
	σ^{S} (σ^{38})	*rpoS*	Stationary phase adaptations
	σ^{N} (σ^{54})	*rpoN, glnF*	Nitrogen-regulated genes
	σ^{fecI}	*fecI*	Ferric citrate uptake (ECF)

J. Helmann. Sigma factors in gene expression. 2005; 10.1038/npg.els.0003829

- ***E. coli*** **can choose between 7 σ factors to fine tune its transcriptional output**

Applied and Environmental Microbiology, May 1996, p. 1822–1824 Vol. 62, No. 5
0099-2240/96/$04.00+0

rpoS Regulation of Acid, Heat, and Salt Tolerance in *Escherichia coli* O157:H7

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Received 11 December 1995/Accepted 9 March 1996

An *rpoS* mutant (*rpoS*::pRR10) of *Escherichia coli* O157:H7 ATCC 43895 was generated. Stationary-phase acid, heat, and salt tolerance was significantly reduced, and starvation-induced acid tolerance did not develop in the mutant. RpoS was also important for survival of *E. coli* O157:H7 in dry, fermented sausage.

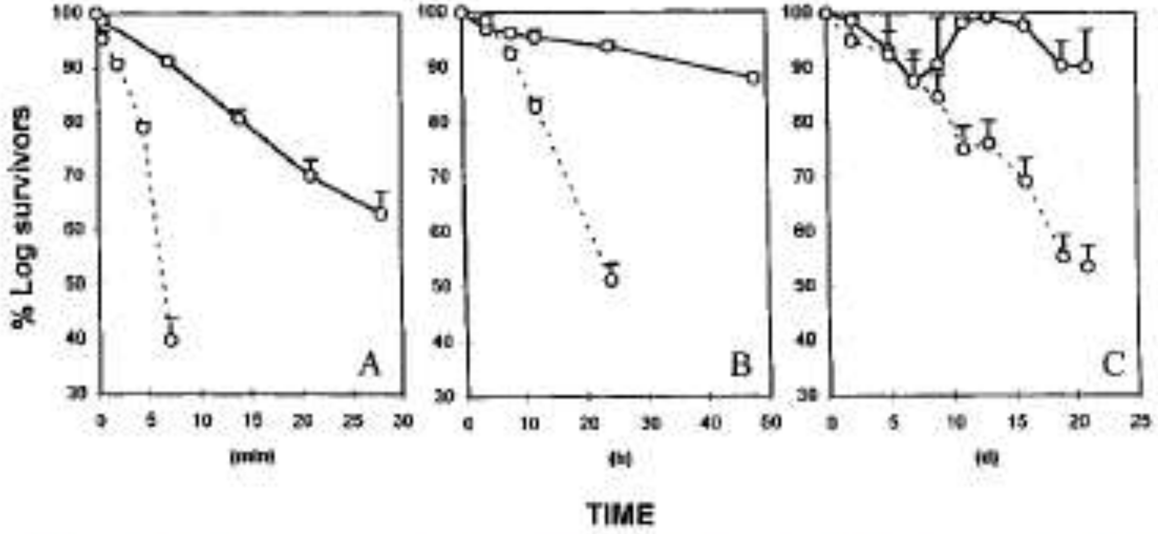


FIG. 1. Heat (A) and salt (B) tolerance and survival in dry, fermented sausage (C) of stationary-phase *E. coli* O157:H7 ATCC 43895 (○—○) and *rpoS* mutant FRIK 816-3 (○----○). Error bars represent standard deviations of the means.

Acid Stress

Classic example by Foster *et al*. in 1990s on *Salmonella* ATR

JOURNAL OF BACTERIOLOGY, Feb. 1990, p. 771–778 Vol. 172, No. 2
0021-9193/90/020771-08$02.00/0

Adaptive Acidification Tolerance Response of *Salmonella typhimurium*

JOHN W. FOSTER* AND HOLLY K. HALL

Department of Microbiology and Immunology, College of Medicine, University of South Alabama, Mobile, Alabama 36688

Received 14 August 1989/Accepted 1 November 1989

pH 7.6 → pH 5.8 → pH 3.3

pH 7.6 → pH 3.3

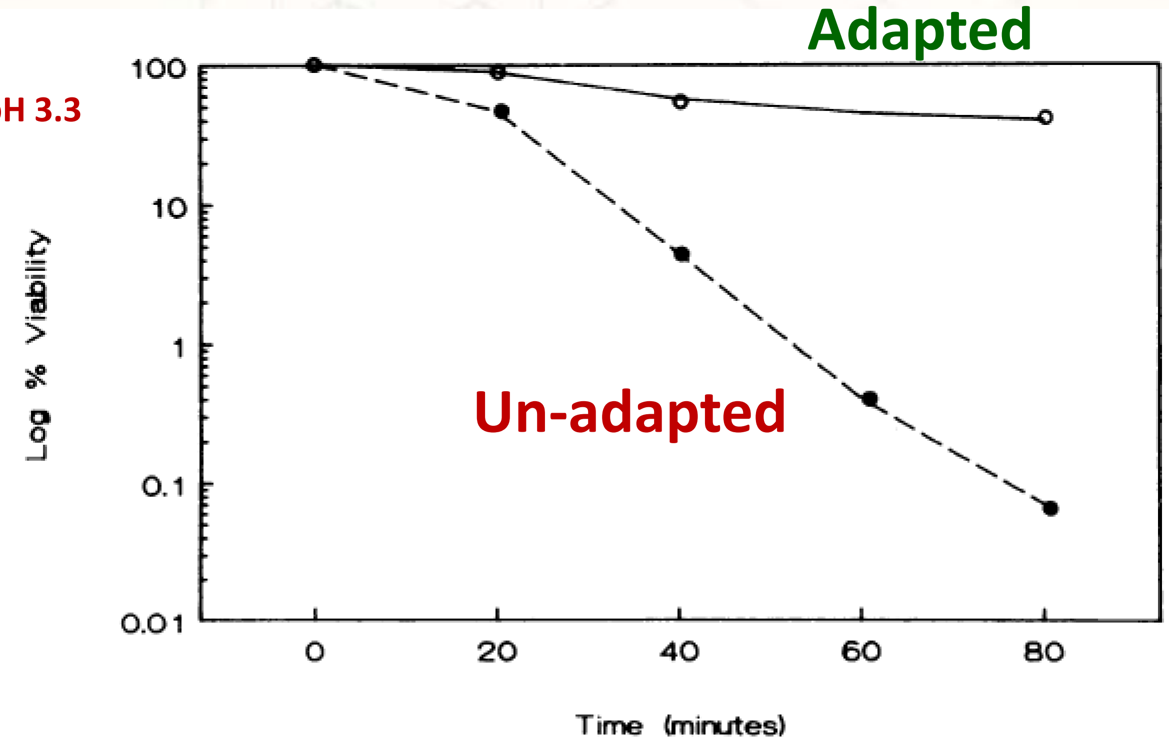


FIG. 1. ATR. Cells grown to 10^8 cells per ml in pH 7.6 minimal glucose medium were adapted by adjusting the medium pH to 5.8 (○). After one doubling, the cells were challenged by readjusting the pH to 3.3 (t_0). Unadapted cultures (●) remained at pH 7.6 until achieving a cell density of 2×10^8 cells per ml and then were directly challenged at pH 3.3 (t_0). Viable counts were determined at timed intervals. The results are expressed in terms of log percent survival.

Heat Resistance after Exposed to Acid Stress

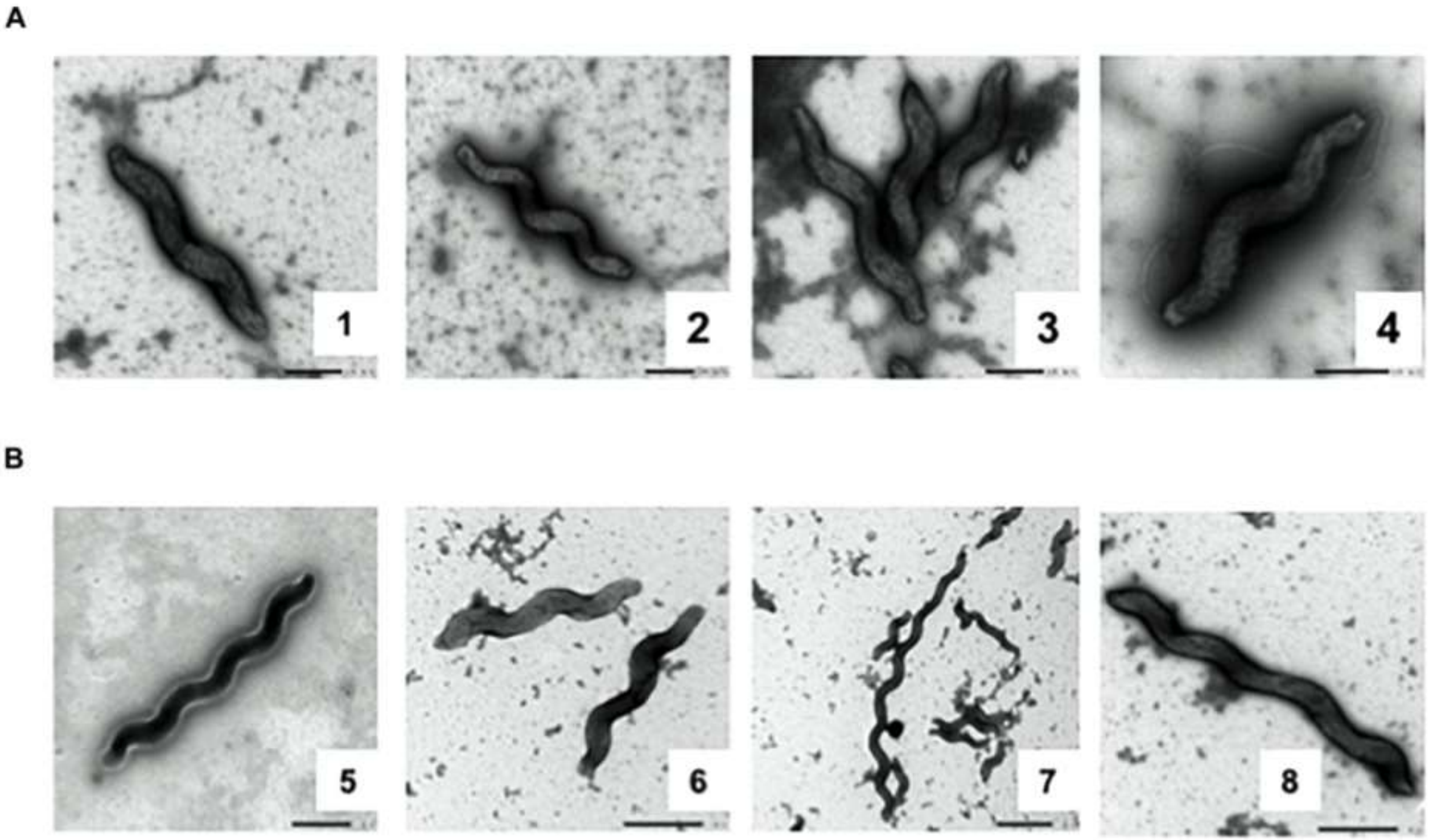
	D-Value (min)*		
	In Orange juice	In Apple Juice	In Grape juice
L. m. (un-treated)	2.1	1.6	2.3
L. m. exposed to pH 5.0	3.8	5.0	4.6

- **D-Value: the time needed at a given temperature for destroying 90% of microbial population**

Stress adaptation can occur in a food system!

Table 9.1 Cells and Spores of Some Microorganisms Important in Food in Which Sublethal Injury is Detected

Gram-positive pathogenic bacteria: *Sta. aureus*, *Clo. botulinum*, *Lis. monocytogenes*, *Clo. perfringens*, *Bac. cereus*
Gram-negative pathogenic bacteria: *Salmonella*, *Shigella* spp., enteropathogenic *Esc. coli*, *Esc. coli* O157:H7, *Vib. parahaemolyticus*, *Cam. jejuni*, *Yer. enterocolitica*, *Aer. hydrophila*
Gram-positive spoilage bacteria: *Clo. sporogenes*, *Clo. bifermentum*, *Bac. subtilis*, *Bac. stearothermophilus*, *Bac. megaterium*, *Bac. coagulans*
Gram-negative spoilage bacteria: *Pseudomonas* spp., *Serratia* spp.
Gram-positive bacteria used in food bioprocessing: *Lac. lactis* spp., *Lab. delbrueckii* subsp. *bulgaricus*, *Lab. acidophilus*
Gram-positive indicator bacteria: *Ent. faecalis*
Gram-negative indicator bacteria: *Esc. coli*, *Ent. aerogenes*, *Klebsiella* spp.
Bacterial spores: *Clo. botulinum*, *Clo. perfringens*, *Clo. bifermentum*, *Clo. sporogenes*, *Bac. cereus*, *Bac. subtilis*, *Bac. stearothermophilus*, *Des. nigrificans*
Yeasts and molds: *Sac. cerevisiae*, *Candida* spp., *Asp. flavus*



frontiers in Microbiology

ORIGINAL RESEARCH
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Experimental Evolution of *Campylobacter jejuni* Leads to Loss of Motility, *rpo*N (σ54) Deletion and Genome Reduction

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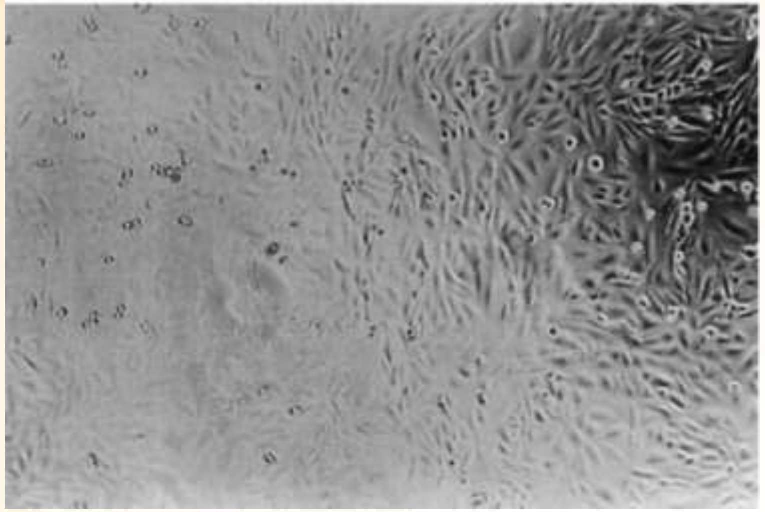

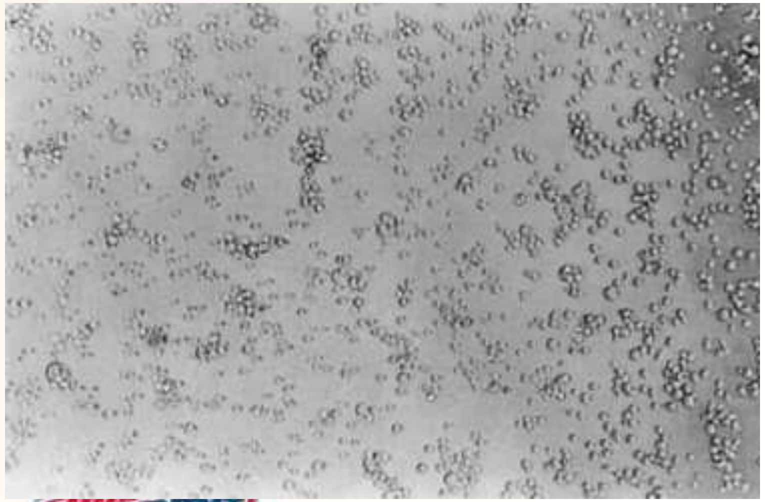

Journal of Applied Microbiology 2000, **89**, 719–725

Detection of a novel campylobacter cytotoxin

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297/03/2000: received 29 March 2000, revised 7 June 2000 and accepted 19 June 2000

Table 1 Screening of campylobacter strains for the presence of cytotoxin in crude fractions using a variety of tissue culture cell lines. Cytotoxicity on the various tissue culture cell lines was determined at 72 h

Campylobacter species	Strain	Origin of isolate	Morphological changes on cell lines used				
CHO	HeLa	Vero	J774	Int407			
C. jejuni	108	Chicken	R(++)	R(++)	—	R(++)	R(++)
C. jejuni	331	Chicken	—	—	—	—	—
C. jejuni	H413	Chicken	—	—	—	—	—
C. jejuni	S007	Chicken	—	—	—	—	—
C. jejuni	351	Human	R(+++)	R(+++)	—	R(+++)	R(+++)
C. jejuni	957	Human	R(+++)	R(+++)	R(+)	R(++)	R(+++)
C. jejuni	81116	Human	R(+++)	R(+++)	R(++)	R(+++)	R(+++)
C. jejuni	FF3	Human	R(+++)	R(+++)	R(++)	R(+++)	R(+++)
C. coli	H424	Chicken	R(++)	R(++)	—	R(++)	R(++)
C. coli	832	Human	R(+)	R(+)	—	R(+)	R(+)
Uninoculated Brucella broth	—	—	—	—	—	—	—

R, Tissue culture cell rounding and the degree of rounding after 72 h incubation; +, 30–50% rounding; ++, 50–80% rounding; +++, > 80% rounding of tissue-cultured cells; —, no visible morphological changes; CHO. Chinese hamster ovary.

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Review

A review of microbial injury and recovery methods in food

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ABSTRACT

The existence of injured microorganisms in food and their recovery during culturing procedures is critical. Microbial injury is characterized by the capability of a microorganism to return to normalcy during a resuscitation process in which the damaged essential components are repaired. Injury of microorganisms can be induced by sublethal heat, freezing, freeze-drying, drying, irradiation, high

- **Resuscitation → The restoration of lost capabilities in injured cells (revived from apparent death)**

Enhancing Viability of Starter Culture

- Commercial starter cultures
  - Frozen or freeze-dried culture
  - ✓ GOAL → High number of survivors…However, viability tends to be low (especially when freeze-dried)

↓

Starter cultures exposed to mild stress conditions

↓

Stress proteins expressed → **Increased survival**

Note: Genetic engineering can be utilized to develop strains capable of producing cryoproteins and other stress proteins

Questions?

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Gram positive bacteria

Listeria monocytogenes

- Gram positive, 13 serotypes. Most foodborne illness associated with 1/2a, 1/2b, and 4b
- Hardy, salt-tolerant, cold tolerant. Grows at refrigeration temperatures, ubiquitous in the environment, readily forms/participates in durable biofilms.
- Many foods associated, including raw milk, inadequately pasteurized milk, chocolate milk, cheeses (particularly soft cheeses), ice cream, raw vegetables, raw poultry and meats (all types), fermented raw-meat sausages, hot dogs and deli meats, and raw and smoked fish and other seafood.

Listeria monocytogenes

- Potential routes of contamination: workers, incoming air, raw materials, food contact surfaces, food-processing environments.
- Post-processing contamination is serious risk
- Persistence as biofilms
- Responsible for ~255 deaths annually (US)
- Notable outbreaks
  - Cantaloupe, 2011. 147 illnesses, 33 deaths
  - Salads, 2021. 16 illnesses, 2 deaths
  - Enoki mushrooms, 2020. 36 illnesses, 4 deaths
  - Caramel apples, 2014-15. 35 illnesses, 7 deaths

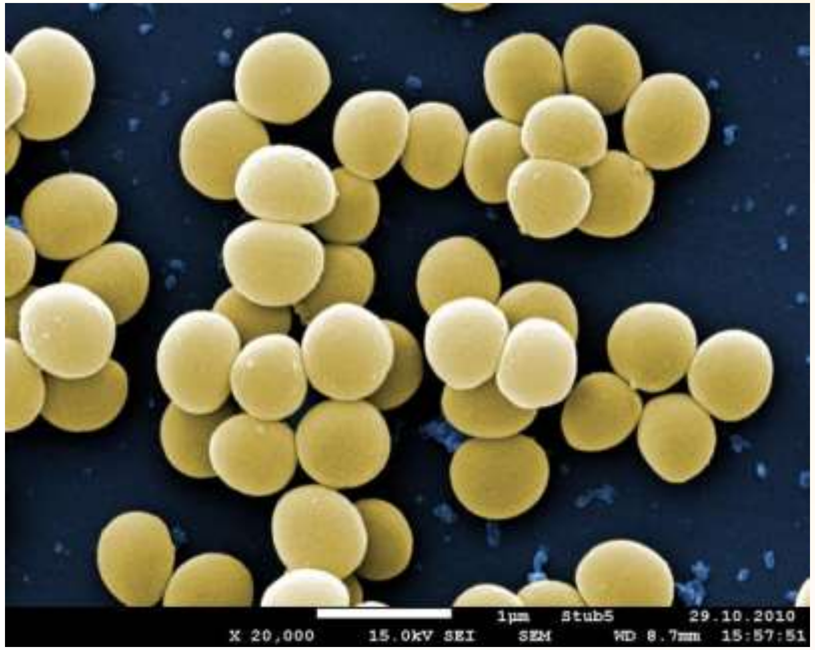
https://www.fda.gov/food/foodborne-pathogens/listeria-listeriosis

Staphylococcus aureus

- Gram positive, produces fast-acting enterotoxins which are heat-stable, i.e. not degraded by cooking temperatures
- Survives dry conditions, salt-tolerant, can grow in low moisture foods, as low as 0.83 a_w
- Prepared foods that require much handling are greater risk potential.
- Common food vectors: meat, poultry, and egg products; egg, tuna, chicken, potato, and macaroni salads; pastries, pies, éclairs; sandwich fillings; and milk and dairy products.

Staphylococcus aureus

- Potential routes of contamination: improper handling of food, broken cold chain, raw materials, food-processing environments.
- Post-processing contamination is serious risk, especially when combined with mild temperature abuse
- Annually: 241,000 illnesses, ~1000 hospitalization, 6 deaths (US)
- Methicillin-resistant *Staphylococcus aureus* (MRSA) is a serious public health concern



https://www.eurekalert.org/multimedia/605406

Viruses

CELEBRATING 25 YEARS

Produced by FoodSafety magazine

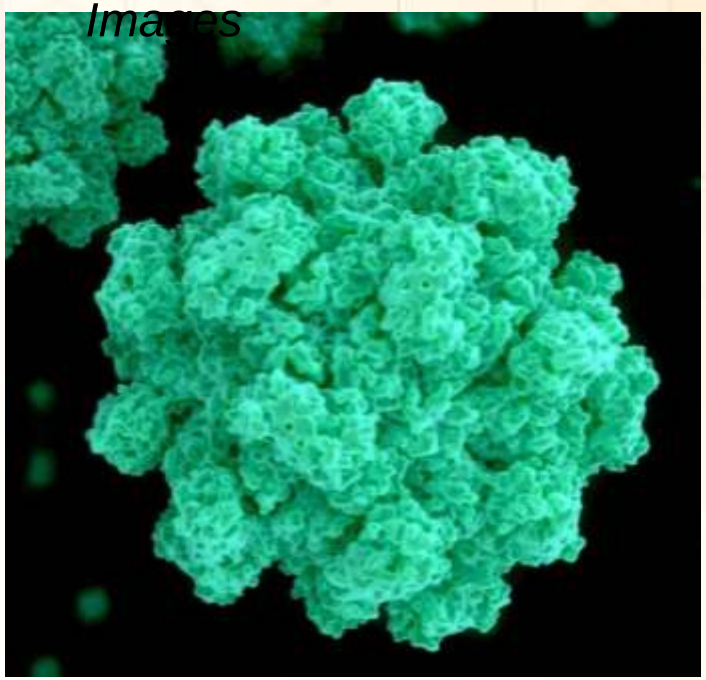
Human norovirus

- Calicivirus family, multiple genogroups
- Environmentally hardy, tolerates dry and cold conditions. Easily spreads from person to person
- Spreads via water, air, food contact surfaces, environmental surfaces. Very low infective dose.
- Variety of food vectors – meats, fruits and vegetables, salads, etc. Prepared by an infected person and spreads via contact.
- Contaminated water – commercial ice, floodwater, surface water

Human norovirus

- Improper sanitation, worker hygiene often key factors
- Prepared RTE foods a key risk factor, due to processing and handling
- Disinfectants and alcohol-based hand sanitizers work on bacteria, but are not effective against these viruses
- Annually: ~5,500,000 illnesses, ~15,000 hospitalization, ~150 deaths (US)

Selvanegra | Getty Images



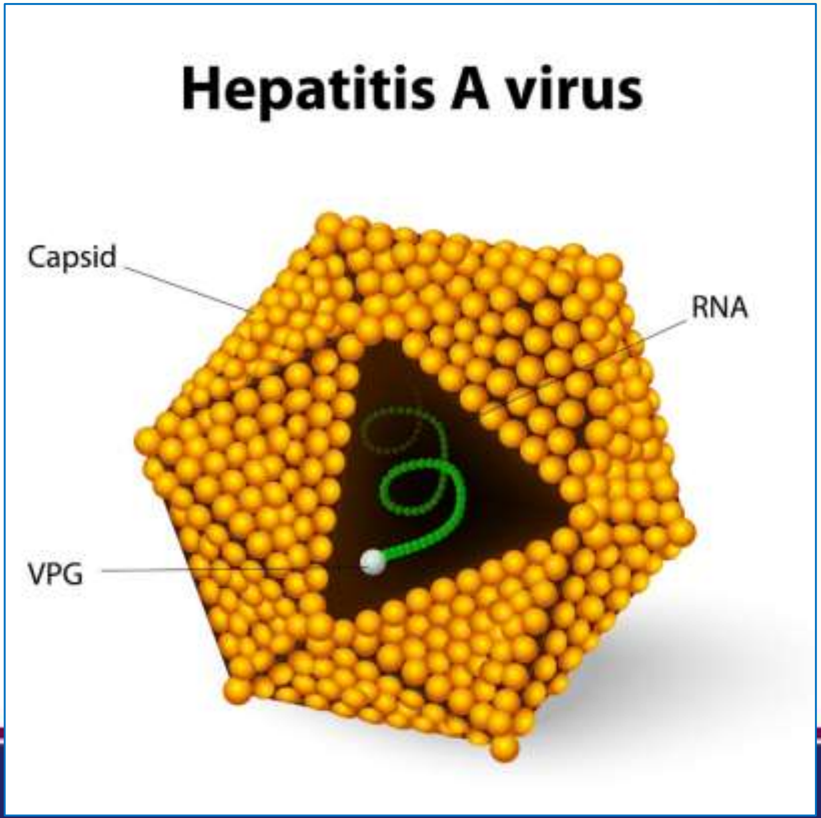
City of Minneapolis

Hepatitis A

- Enterovirus (Picornaviridae family). Six genotypes, infecting humans and other primates
- Environmentally hardy. Tolerates freezing, heat, desiccation, chemical sanitizers. Most commonly spreads from person to person
- Spreads via water, air, food contact surfaces, environmental surfaces. Very low infective dose.
- Variety of food vectors – shellfish, fruits and vegetables, frozen fruits, salads, etc. Prepared by an infected person and spreads via contact.
- Contaminated water – commercial ice, floodwater, surface water

Hepatitis A

- Low infectious dose, typically oral exposure
- Most Hep A infections are not food related: ~1570 of ~25,000 annually
- Notable food outbreaks and recalls in US:
  - 2003 - Tennessee, North Carolina, Georgia, Pennsylvania – green onions
  - 2005 - Tennessee, Alabama – oysters
  - 2020 – northeast states – frozen raspberries
  - 2022 – California, Minnesota, Canada – fresh strawberries
  - 2023 – Washinton - frozen strawberries



www.hepmag.com/basics/hepatitis-a-basics/

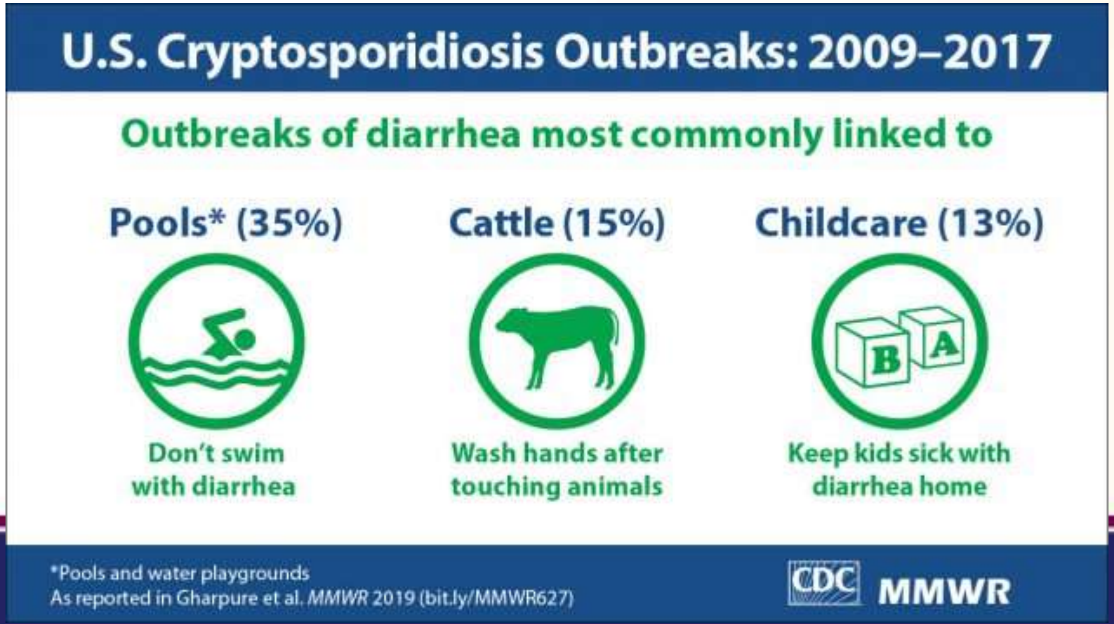
Parasites

Cryptosporidium parvum

- Protozoan parasite, obligate life cycle. Several species, but only C. parvum commonly causes disease.
- Highly resistant to chlorine, but susceptible to desiccation and UV
- Fecal-oral is common route of contamination.
- Foodborne – washing or irrigating with contaminated water is highest risk factor. Almost any food can be a vector.
- Water-borne – irrigation, surface water, recreational water (pools, water parks)

Cryptosporidium parvum

- Mechanism of disease remains unclear, as are underlying genetics/epigenetics. Toxin? Surface antigens for intestinal wall?
- Improper sanitation, worker hygiene, poor water quality often key factors
- Prepared RTE foods a key risk factor, due to processing and handling
- Annually: ~20,000 illnesses (est.), ~7,500 hospitalizations



Cyclospora cayetanensis

- Protozoan parasite, obligate life cycle. Several species, but only C. cayetanensis causes disease in humans.
- Difficult to study properly. Susceptibility to chlorine is presumed high, but not certain. Survival under desiccation, freezing, UV, etc. is unknown.
- Fecal-oral is a likely route of contamination.
- Common food vectors include fresh produce, lettuce, berries, other fruits

Cyclospora cayetanensis

- Outbreaks are uncommon and sporadic. Difficult to construct proper epidemiology, due to lack of research material
- Extremely difficult to isolate and detect in foods

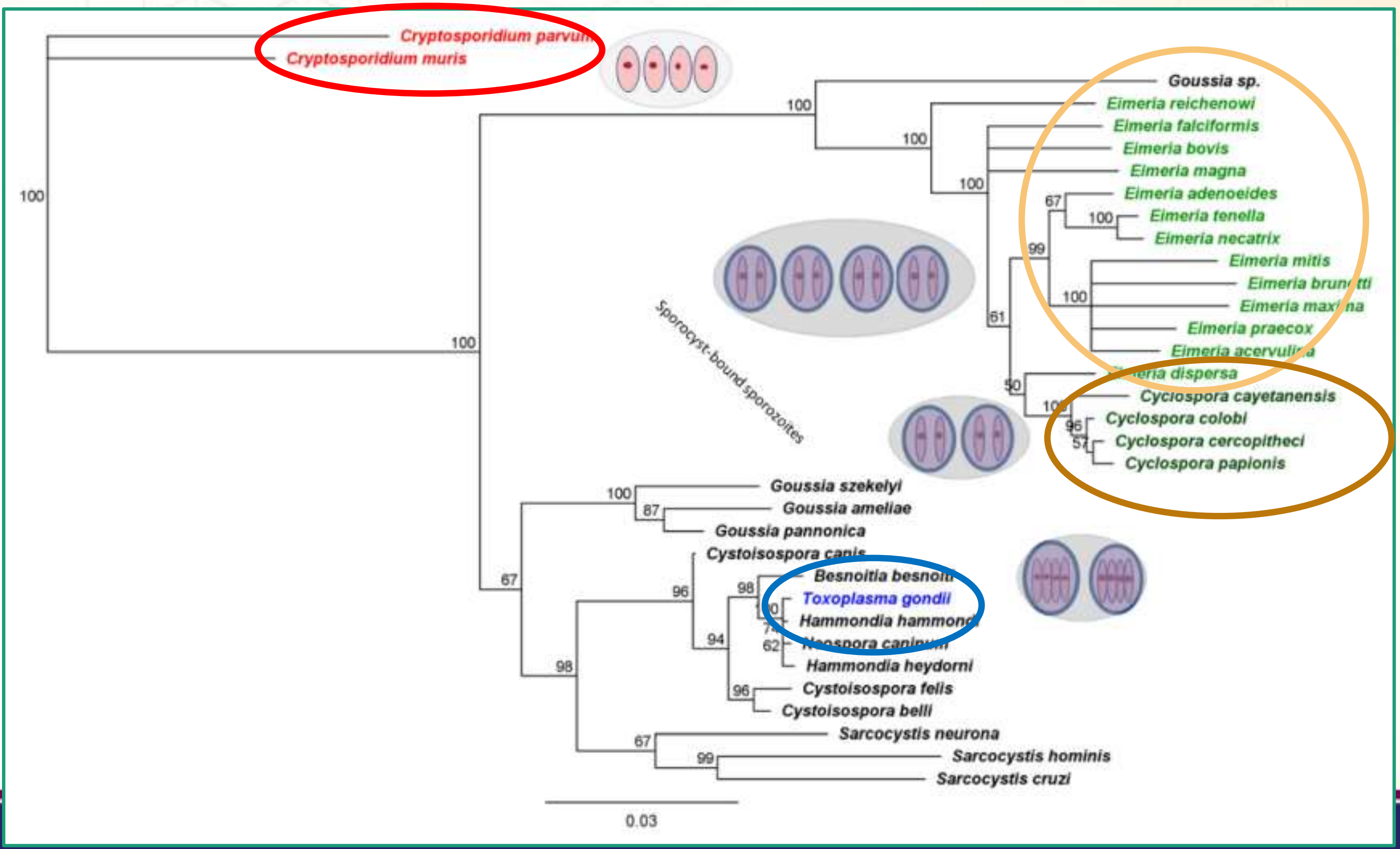
www.cdc.gov/parasites/cyclosporiasis/index.html

Eimeria – a surrogate for parasite research

- *Cyclospora cayetanensis* is difficult to grow. Hard to do research on effective controls, as oocysts are limited and expensive
- *Eimeria acervulina* is a pathogen of poultry, harmless to people. Easy to grow, collect abundant research material. Very similar genetics.
- Using *Eimeria* oocycts would greatly speed research
- **Question:** does *Eimeria* respond to sanitation treatments the way Cyclospora would? Is it a good surrogate?
- Conditions of growth, response to treatments, genetics, epigenetics

Eimeria – a surrogate for parasite research

- *Eimeria* is closely related to *Cyclospora*, less closely to *Toxoplasma*, even less to *Cryptosporidium*
- Genetic differences
- Behavioral, epigenetic differences



Tucker et al., 2022. *Microorganisms*, *10*(10), 1977

Biofilms

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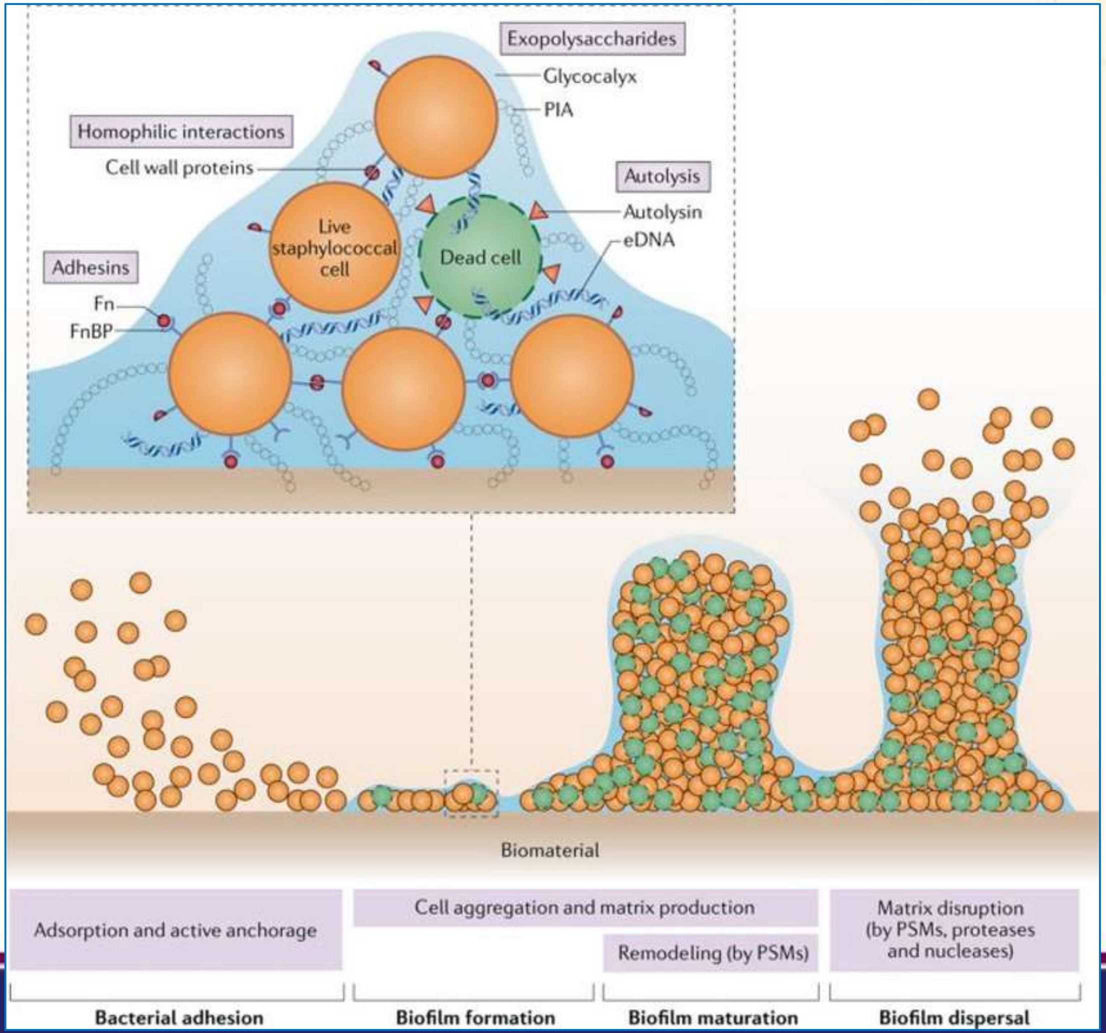
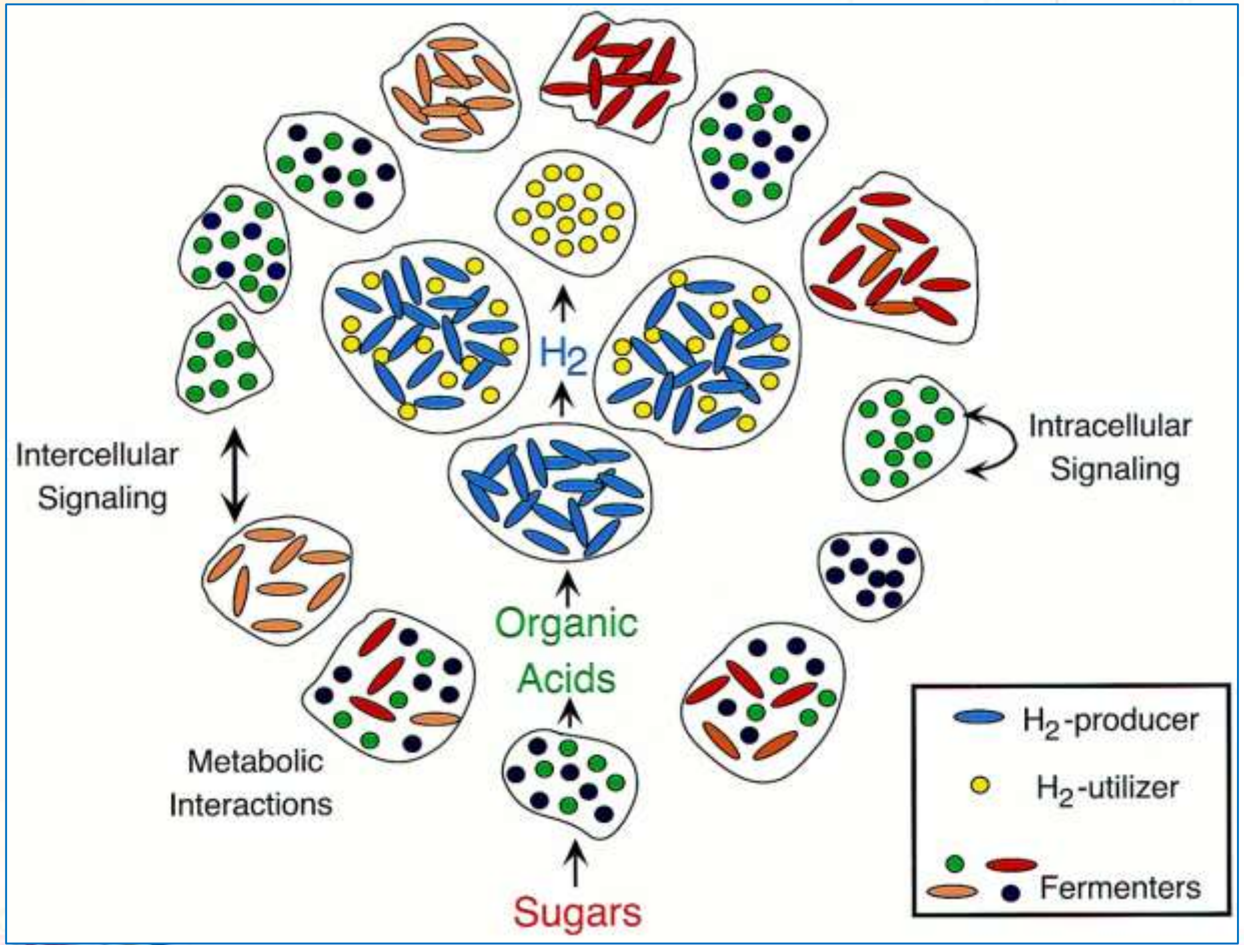
Biofilms – a complex challenge for genomics

- Bacteria live in communities
    - Multiple species
    - Multiple strains
- Adherent extracellular matrix of proteins, carbohydrates, biopolymers
- Exchange of metabolites, DNA, RNA
- Protection from sanitizers, desiccation, freezing, heat



Ahmed et al. 2019. Materials Today Bio. 2. 100017.

Biofilms – a complex challenge for genomics



- Pathogens can participate in mostly non-pathogenic biofilms
- Toxigenic strain may be weak, sensitive, poor biofilm former. Not much risk?
- In partnership with a non-toxigenic, non-pathogen which forms a strong, durable biofilm, risk is increased.
- Does the pathogen actively participate in biofilm formation? Or is it just along for the ride?
- Are genes traded? Are new genes expressed in a biofilm which are NOT expressed by free-living bacteria?

Davey and O'Toole, 2000. Microb Mol Biol Rev. 64(4)

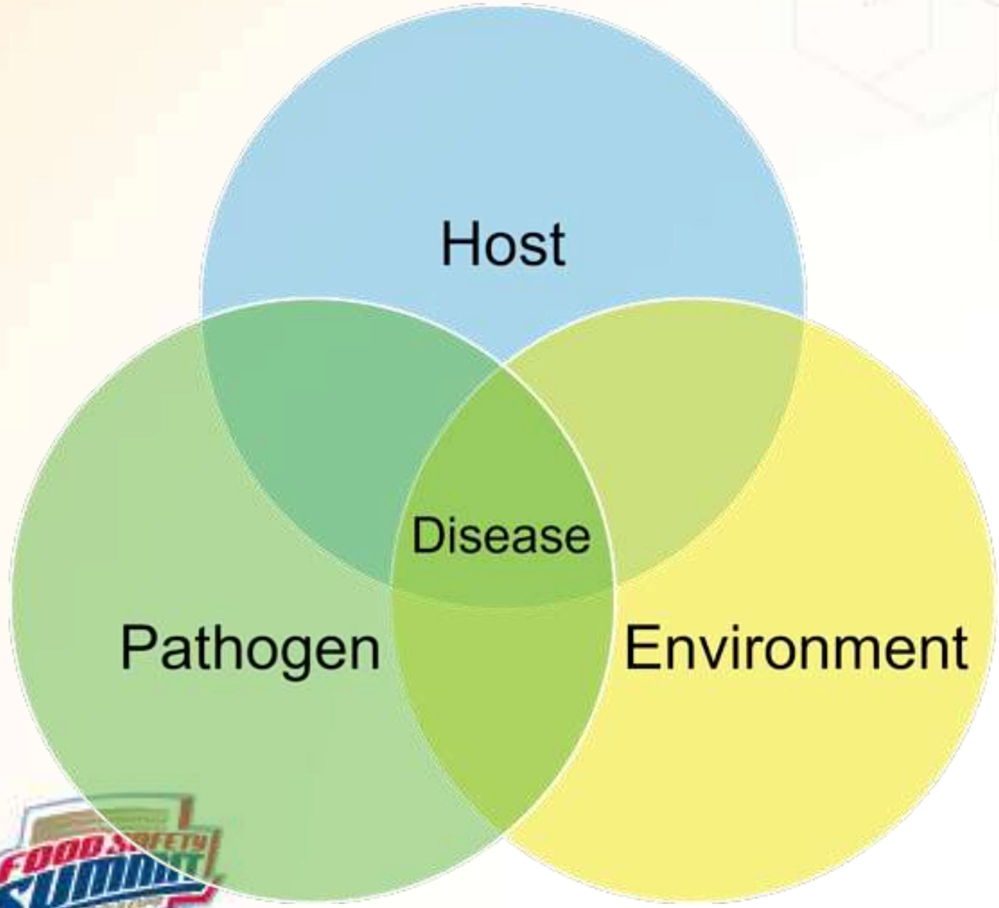
Biofilms – quorum sensing

- Bacterial populations grow – accumulation, reproduction
- Intercellular communication, signaling. Sensing of environment and each other.
- Which critical thresholds are reached, new genes activate
- Changes to the behavior of individual participants and the biofilm as a whole
- Inhibition and/or deregulation of QS is a strategy for advanced antimicrobial interventions
- Challenge: need to know what happening before you can effectively interfere with it

Biofilms – quorum sensing, microbial ecology

- What makes an organism a pathogen? It might be who it's hanging out with
- Biofilm microbial ecology remains only partially understood
- Multispecies interactions, dynamically changing environment
- Persistent, resistant
- Sanitation programs use combination of conventional tools and novel interventions

Conclusions



- Organisms cause disease when present in the right environment, in sufficient numbers
- What makes an organism a pathogen:
  - Genes for harmful toxins
  - Circumstances that promote expression of those genes
  - Exposure/transfer to food and people
- Controls for a pathogen
  - General, broadly effective
  - Specific, targeted

Resources and further reading

- FDA: Foodborne Pathogenic Microorganisms and Natural Toxins Handbook, 2nd edition – **"The Bad Bug Book"**
  - Sorted by type of organism. Relevant information on food vectors, common contamination pathways, illness symptoms and treatments
  - Downloadable PDF
  - www.fda.gov/food/foodborne-pathogens/bad-bug-book-second-edition
- CDC – Current Outbreaks: www.cdc.gov/outbreaks/index.html
- CDC's "Solve the Outbreak" online game
  - "Become a disease detective" – learn about dozens of infectious organisms and diseases while investigating outbreaks, foodborne and otherwise
  - www.cdc.gov/mobile/applications/sto/web-app.html

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