The Genomic and Environmental Stressors that Impact Microbial Pathogenesis

Alvin Lee

Food Safety Summit 2024

Food Environment

- **Physical/chemical environments**
  - Production
  - Processing
  - Preservation
  - Storage
  - Transportation
  - Consumption

- **Microbiological quality evaluations**
  - Standardized procedures

Classic acid stress example by Foster & Hall in 90s on *Salmonella* ATR

JOURNAL OF BACTERIOLOGY, Feb. 1990, p. 771–778
0021-9193/90/020771-08$02.00/0

Vol. 172, No. 2

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

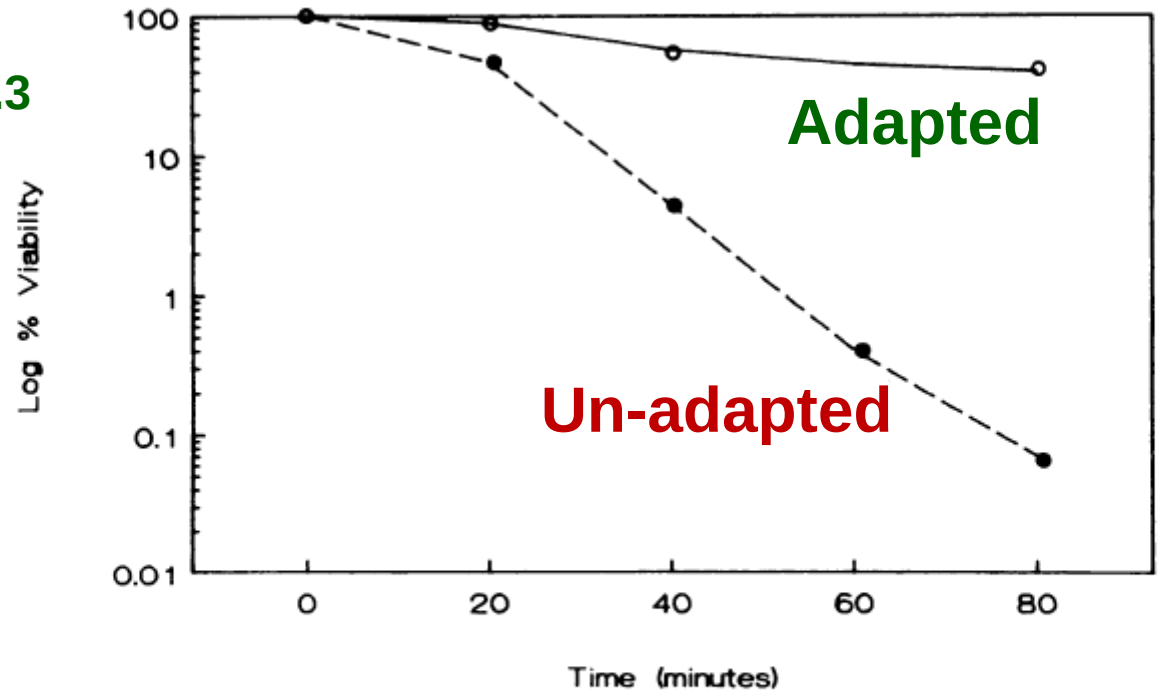


FIG. 1. ATR. Cells grown to 10^8 cells per ml in pH 7.6 minimal glucose medium were adapted by adjusting the medium pH to 5.8 (○). After one doubling, the cells were challenged by readjusting the pH to 3.3 (t_0). Unadapted cultures (●) remained at pH 7.6 until achieving a cell density of 2×10^8 cells 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.

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

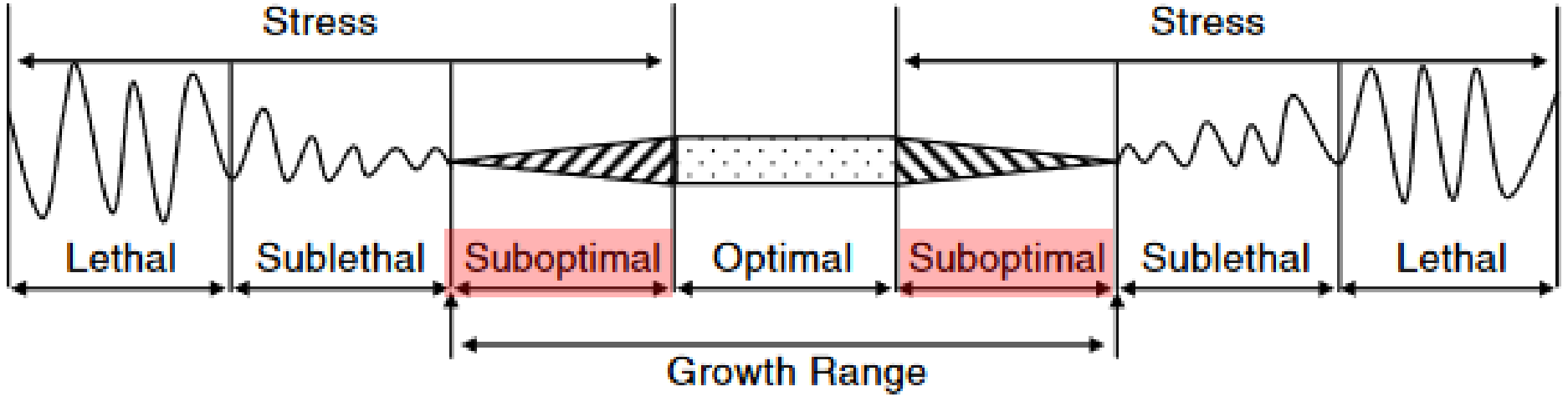


Figure 9.1 Different levels or degrees of environmental stresses to which bacterial cells can be exposed during processing and preservation of food. Bacterial cells exposed to a suboptimal growth condition show stress adaptation. Beyond the growth range, the cells are usually either sublethally or lethally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

Ray et al

Types of Stress

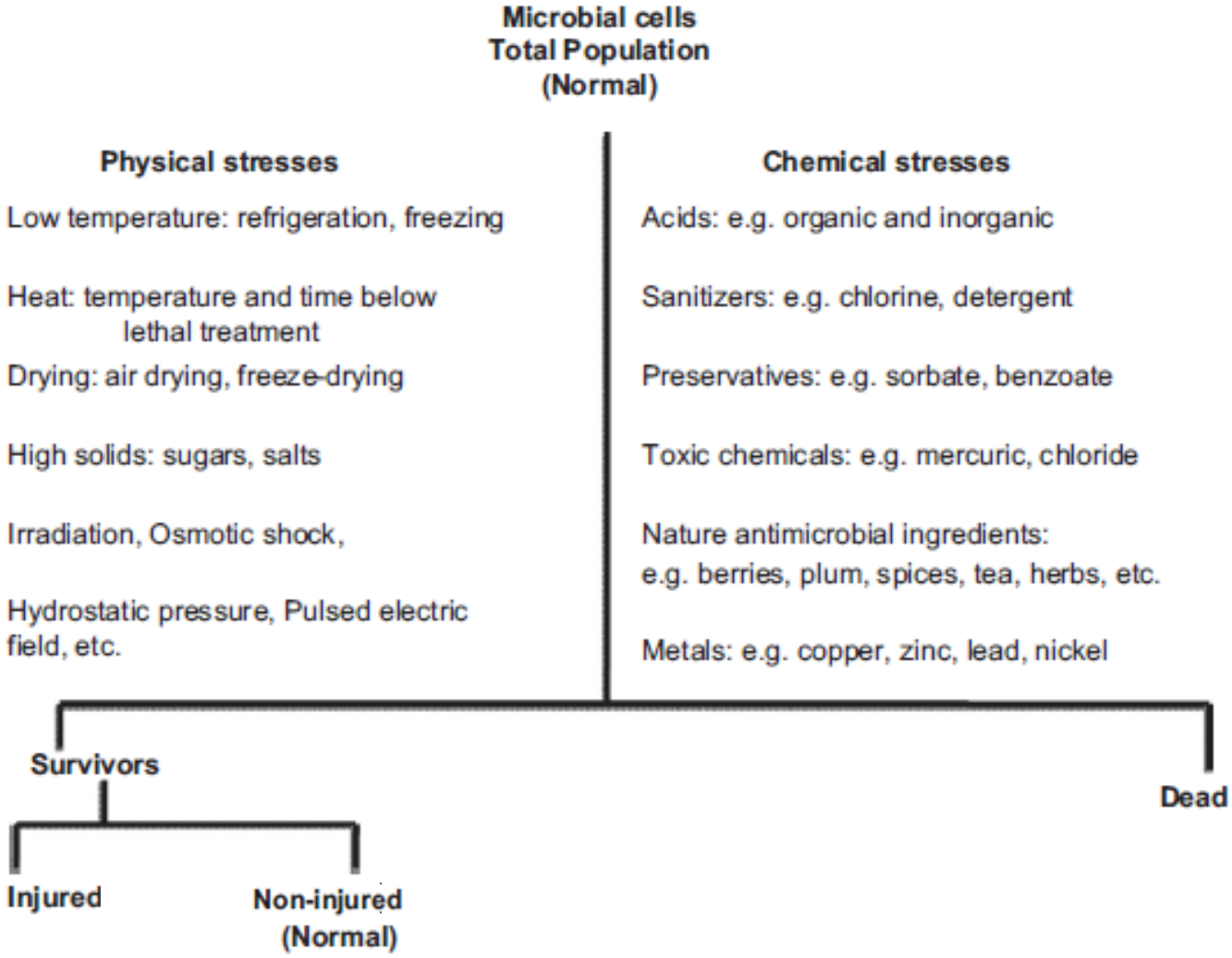
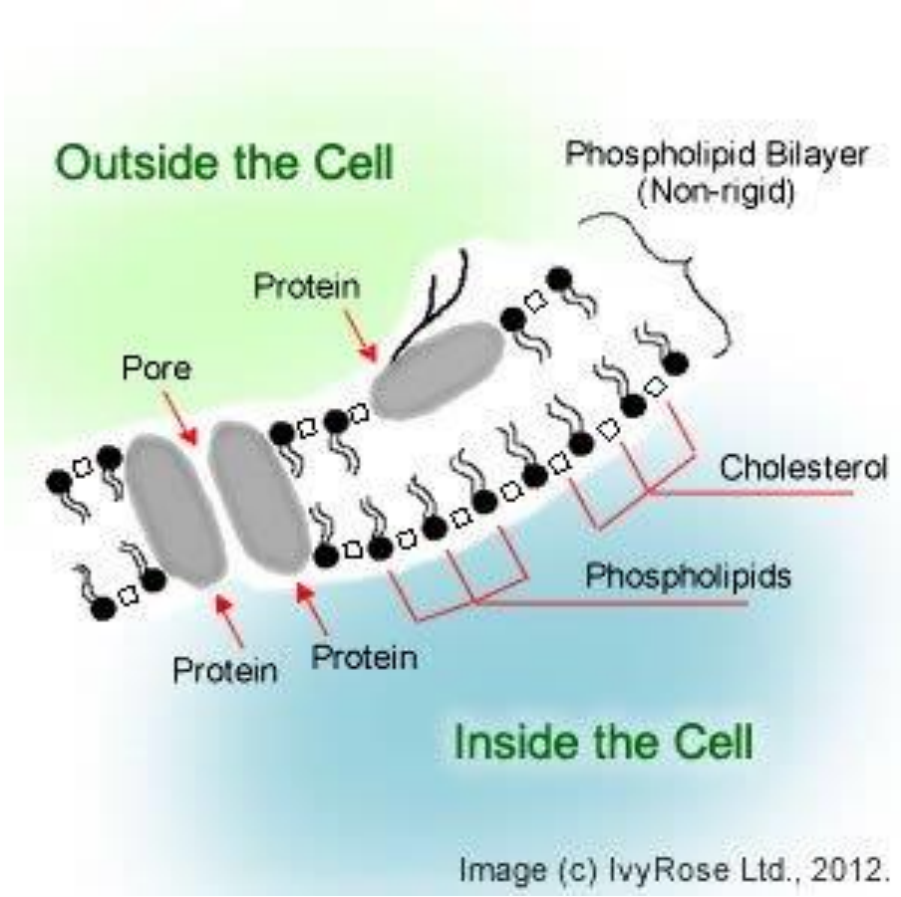


Fig. 1. Effects of sublethal treatments on microbial cells (Ray, 1979, 1989; Russell, 1984; McFeters, 1989; Bozoglu et al., 2004).

Mechanisms of Stress Adaptation



- Bacterial cells alter lipid composition (membrane)
  → Maintained fluid state
- Proportion of cyclopropane fatty acids in the cell membrane regulated by cyclopropane fatty acid synthase
  - Leads to Gram negative pressure, acid and oxidative resistance

Mechanisms of Stress Adaptation

Current Concept:

Gene Response
→ Shock Proteins
→ Stress Proteins

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

❖ **Aid for adaptation to other stressors**

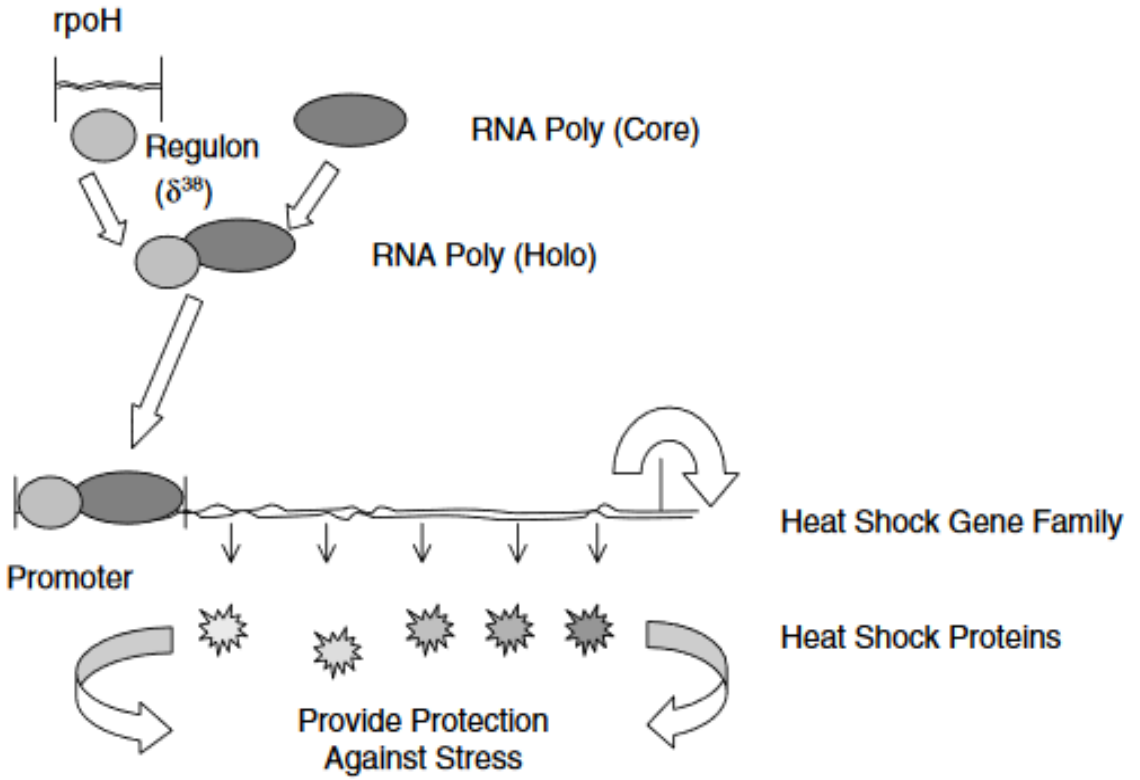


Figure 9.2 Genetic basis of coping stress with sigma factors by bacterial cells. See text for explanations.

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

A. M. CHEVILLE, K. W. ARNOLD, C. BUCHRIESER, C.-M. CHENG, AND C. W. KASPAR*

Food Research Institute, Department of Food Microbiology and Toxicology, University of Wisconsin, Madison, Wisconsin 53706-1187

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.

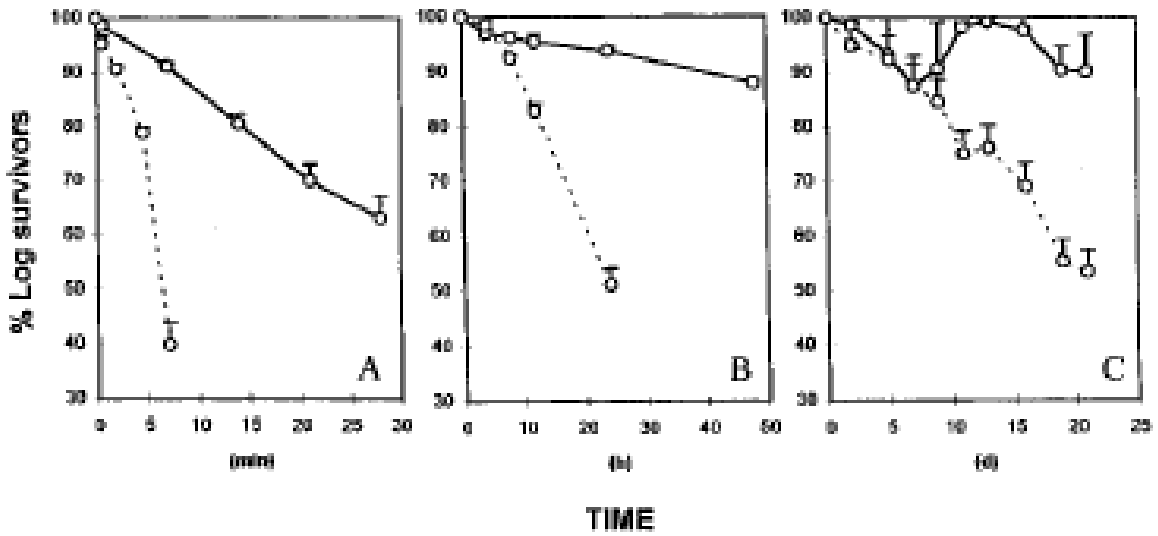


FIG. 1. Heat (A) and salt (B) tolerance and survival in dry, fermented sausage (C) of stationary-phase *E. coli* O157:H7 ATCC 43895 (○——○) and *rpoS* mutant FRIK 816-3 (○----○). Error bars represent standard deviations of the means.

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Sept. 2010, p. 5911–5917 Vol. 76, No. 17
0099-2240/10/$12.00 doi:10.1128/AEM.01532-10

Survival of *Campylobacter jejuni* under Conditions of Atmospheric Oxygen Tension with the Support of *Pseudomonas* spp.[▽]

Friederike Hilbert,[1]* Manuela Scherwitzel,[1] Peter Paulsen,[1] and Michael P. Szostak[2]

Institute of Meat Hygiene, Meat Technology and Food Science, Department for Farm Animals and Veterinary Public Health,[1] and Institute of Bacteriology, Mycology and Hygiene, Department for Pathobiology,[2] University of Veterinary Medicine Vienna, Vienna, Austria

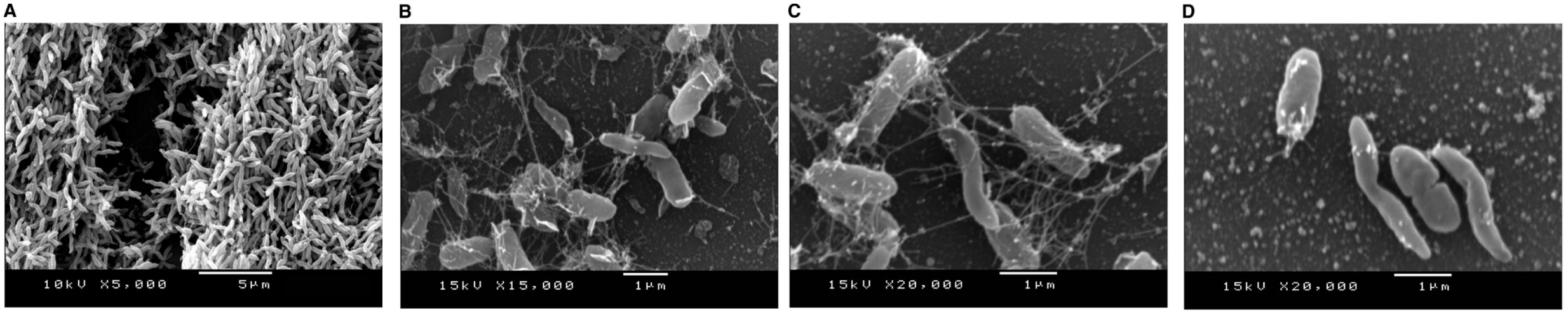
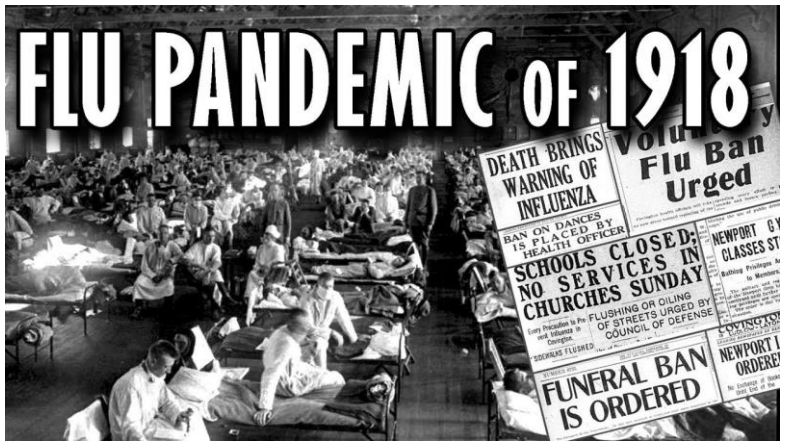


FIG. 3. Representative scanning electron microscopy. (A) SEM of *C. jejuni* DSM 4688 cultured under microaerobic conditions. (B and C) SEM of *P. putida* DSM 50198 and *C. jejuni* DSM 4688 cocultured under aerobic conditions for 32 h. *C. jejuni* cells are spiral shaped when cocultured with *P. putida*. (B to D) Cells of *Pseudomonas* and *Campylobacter* can be distinguished by the shape and thickness of the cells; fiberlike structures form cobwebs around the bacteria. (D) Both *P. putida* and *C. jejuni* seem to interact by close contact.

Emerging or Re-Emerging

- Novel etiological agents that have been recently introduced in a population
- Often zoonotic in origin
- Microorganisms include Gram +, Gram – bacteria, parasites and viruses
- Acquisition of new virulence factors
- Acquisition of antibiotic resistance
- Genetic assortment
- Factors – human host, environment, processing, travel, novel companion animals etc…



Spanish flu – 1918

1957 - Asian flu
1968 – Hong Kong flu
1977 – Russian flu
2009 –swine flu

Several other flu outbreaks

Veterinary Microbiology 140 (2010) 56–62

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Detection and analysis of bovine rotavirus strains circulating in Australian calves during 2004 and 2005

D.L. Swiatek [a,b], E.A. Palombo [c], A. Lee [d,1], M.J. Coventry [d], M.L. Britz [a], C.D. Kirkwood [a,b,*]

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[c] Environment and Biotechnology Centre, Faculty of Life and Social Sciences, Swinburne University of Technology, Hawthorn, Victoria, Australia
[d] Food Science Australia, Werribee, Victoria, Australia

Circulation of bovine G6 and G10 strains in calves and humans

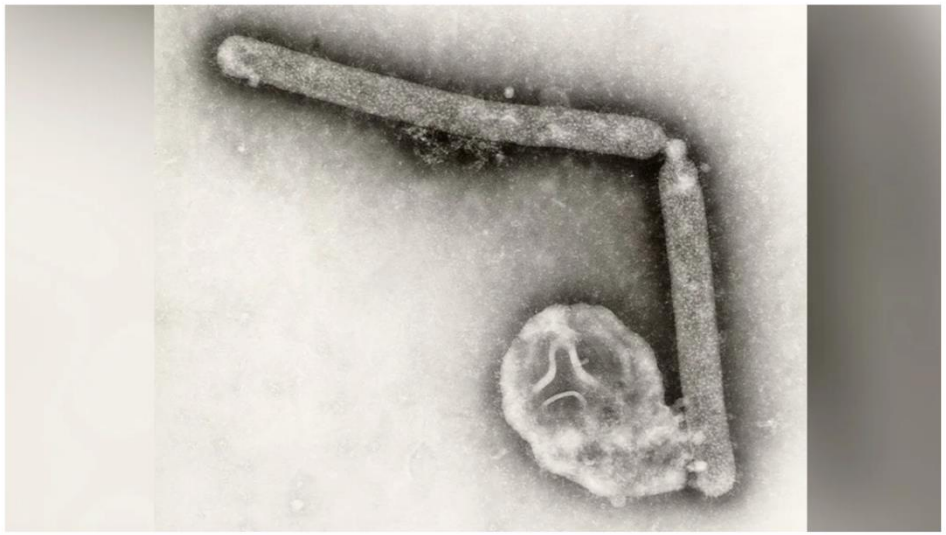
Updates on Highly Pathogenic Avian Influenza (HPAI)

CNN Health | Life, But Better | Fitness | Food | Sleep | Mindfulness | Relationships | Watch | Listen | Live TV | Sign in

What a US farmworker's case of bird flu tells us about tracking the infection

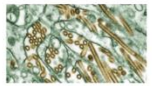
By Brenda Goodman, CNN

4 minute read · Published 10:31 AM EDT, Fri May 3, 2024



A dairy worker's infection is important because it confirms that humans can be infected with H5N1 after

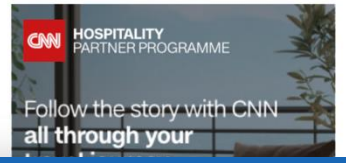
MORE FROM CNN



H5N1 bird flu was circulating in dairy cows for four months before it ...

Continued FDA testing finds no active bird flu virus in variety of dairy ...

USDA says it is testing beef for H5N1 bird flu virus



Fragments of bird flu virus genome found

BIZ & IT | TECH | SCIENCE | POL

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FDA says

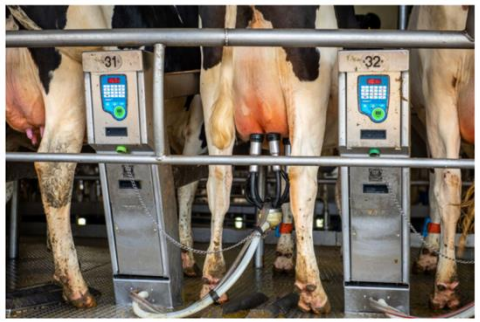
The test cannot tell if the virus is live. The FDA still assess milk supply as safe.

BETH MOLE - 4/23/2024, 8:20 PM

USDA Animal and Plant Health Inspection Service
U.S. DEPARTMENT OF AGRICULTURE

Detection of Highly Pathogenic Avian Influenza (H5N1) in Dairy Herds: Frequently Asked Questions

Updated April 16, 2024



ILLINOIS TECH

IFSH INSTITUTE FOR FOOD SAFETY AND HEALTH

Adaptation to Stress – A Good Idea?

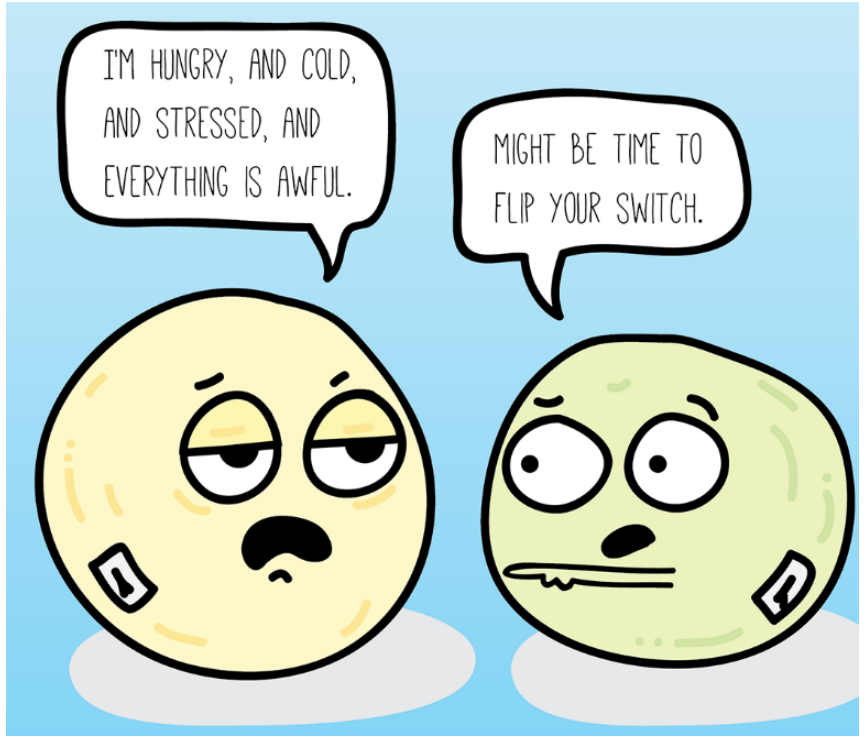
- **Beneficial bacteria**
  - Fermentation bacteria – new and novel foods
- **Pathogens and spoilage bacteria**
  - Antibiotic resistance
  - More virulent strains
- **Impact on characteristics of food**
  - New flavors – starter cultures
  - Food as medicine??

Control Measures

- Clean and disinfect environmental surfaces
- Hand washing and personal hygiene (asymptomatic transmission control)
- Interventions for reduction of pathogen load
- Improve diagnostics for detection and characterization
- Understand and evaluate food safety systems

Microbial Goal?

Adapt & Survive OR Perish



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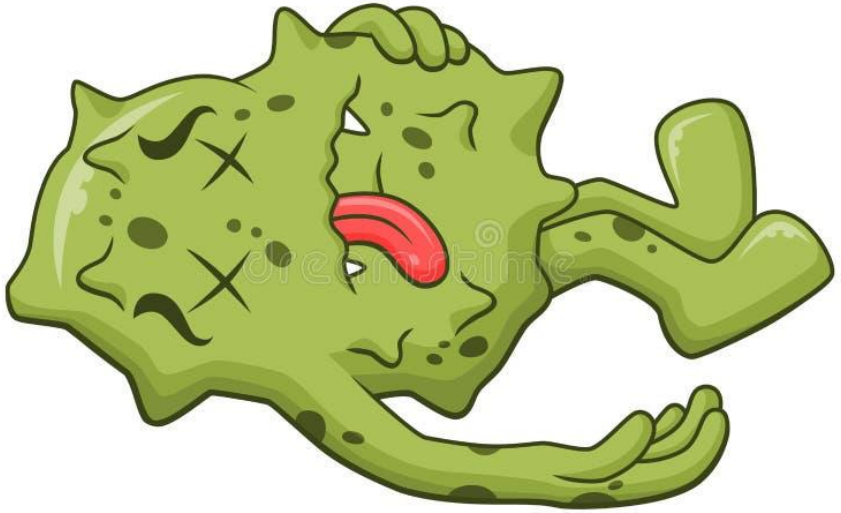

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Evolution and Pathogenesis: What time has done to microorganisms - what we know and don't know

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Agenda

- Introduction: Emerging, Reemerging Pathogens- What is in the name?
- Mechanism of Bacterial Pathogenicity
- Evolution of microbial Pathogens
- What we know and don’t know
- Summary

Emerging, Reemerging Pathogens- What is in the name?

The Challenge of Emerging Pathogens

Infectious Diseases: Considerations for the 21st Century

Anthony S. Fauci
National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland

Clin Infect Dis. 2001;32:675–85.

The challenge of emerging and re-emerging infectious diseases

David M. Morens, Gregory K. Folkers & Anthony S. Fauci

National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services, Bethesda, Maryland 20892-2520, USA (e-mail: afauci@niaid.nih.gov)

Infectious diseases have for centuries ranked with wars and famine as major challenges to human progress and survival. They remain among the leading causes of death and disability worldwide. Against a constant background of established infections, epidemics of new and old infectious diseases periodically emerge, greatly magnifying the global burden of infections. Studies of these emerging infections reveal the evolutionary properties of pathogenic microorganisms and the dynamic relationships between microorganisms, their hosts and the environment.

***Nature,* volume 430**, pages 242–249 (2004)

Emerging, Reemerging and Opportunistic Pathogens

- Emerging pathogens are New, reemerging, or drug-resistant infections whose incidence in humans have increased within the past two decades or threatened to increase in the near future

- Reemerging Pathogens- involved in the reappearance of a known disease following a decline in incidence including newly recognized pathogens, new diseases caused by known organisms, and the extension of the geographic or host range of a pathogen

- Opportunistic Pathogens- are microbes that usually do not cause disease in healthy people, but may become virulent with immunocompromised and unhealthy individuals

Emerging, Reemerging and Opportunistic Pathogens

Early 1900

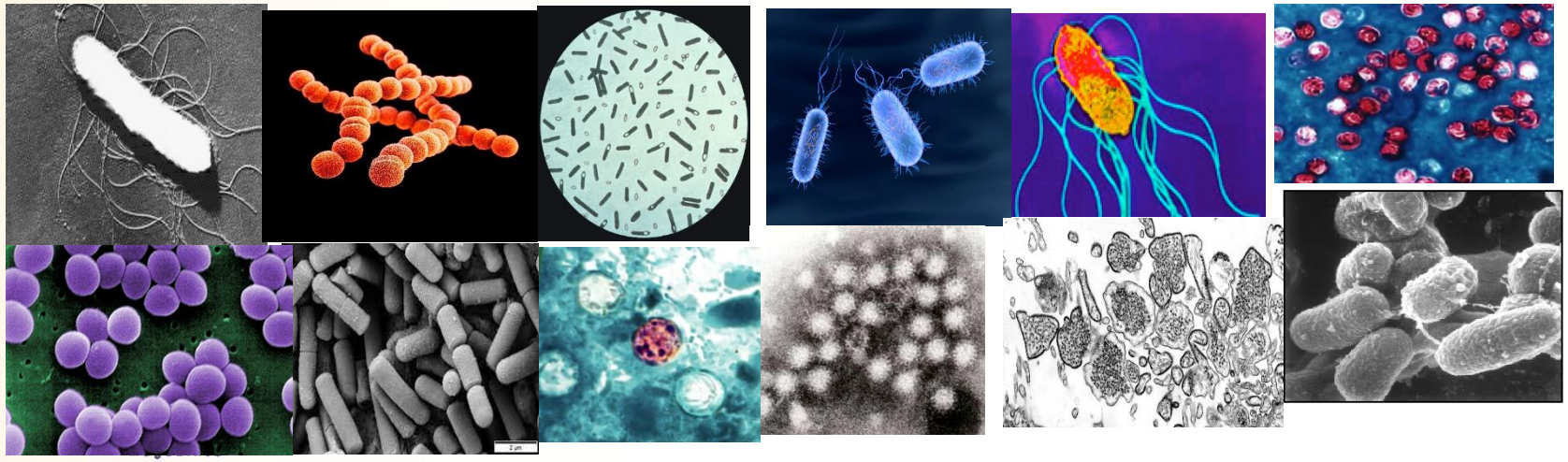
- Typhoid fever
- Tuberculosis
- Septic sore throat
- Diphtheria
- Brucellosis

1940s-1960s

- Clostridium botulinum
- Salmonella Spp.
- Staphylococcus aureus
- Streptococci

1960s - 1990s

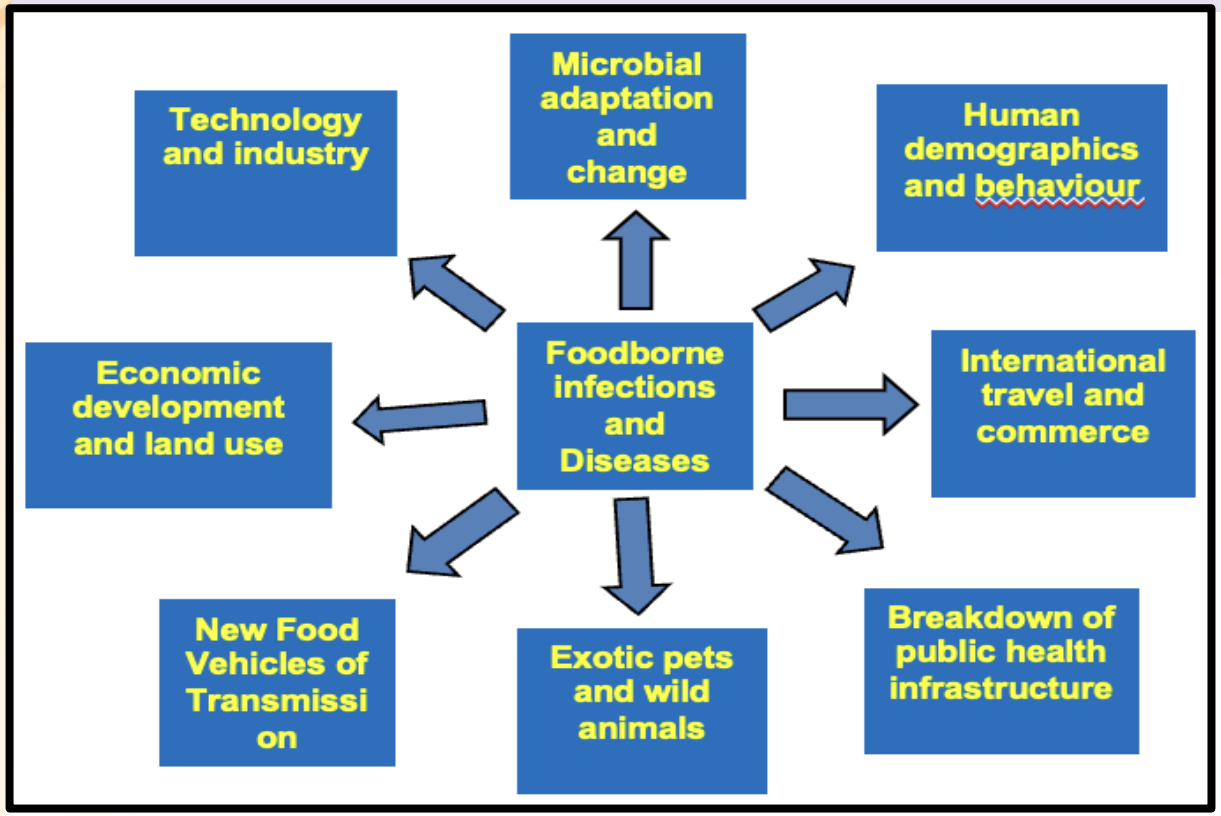
- Salmonella
- Clostridium perfringens
- Vibrio parahaemolyticus
- Bacillus cereus
- Pathogenic E. coli
- Campylobacter jejuni



2000 - s

- Salmonella Spp.
- E. coli 057:H7
- Non 0157 EHEC/STECs
- Listeria monocytogenes*
- Yersinia enterocolitica*
- Bacillus cereus*
- Staphylococcus aureus
- Cronobacter sakazakii
- Vibrio parahemolyticus
- V. vulnificus
- Cryptosporidium, Cyclospora, Toxoplasma gondii
- Norovirus, Hep A. Nipah Virus

Factors Contributing to the Emergence of Foodborne Pathogens and Diseases



- Microbial adaptation and change
- Human susceptibility to infection
- Climate and weather
- Changing ecosystems
- Human demographics and behavior
- Economic development and land use
- International travel and commerce
- Technology and industry
- Breakdown of public health measures
- Other social, political and economic factors e.g. War and famine, Lack of political will, and Intent to harm

Mechanisms of Bacterial Pathogenicity

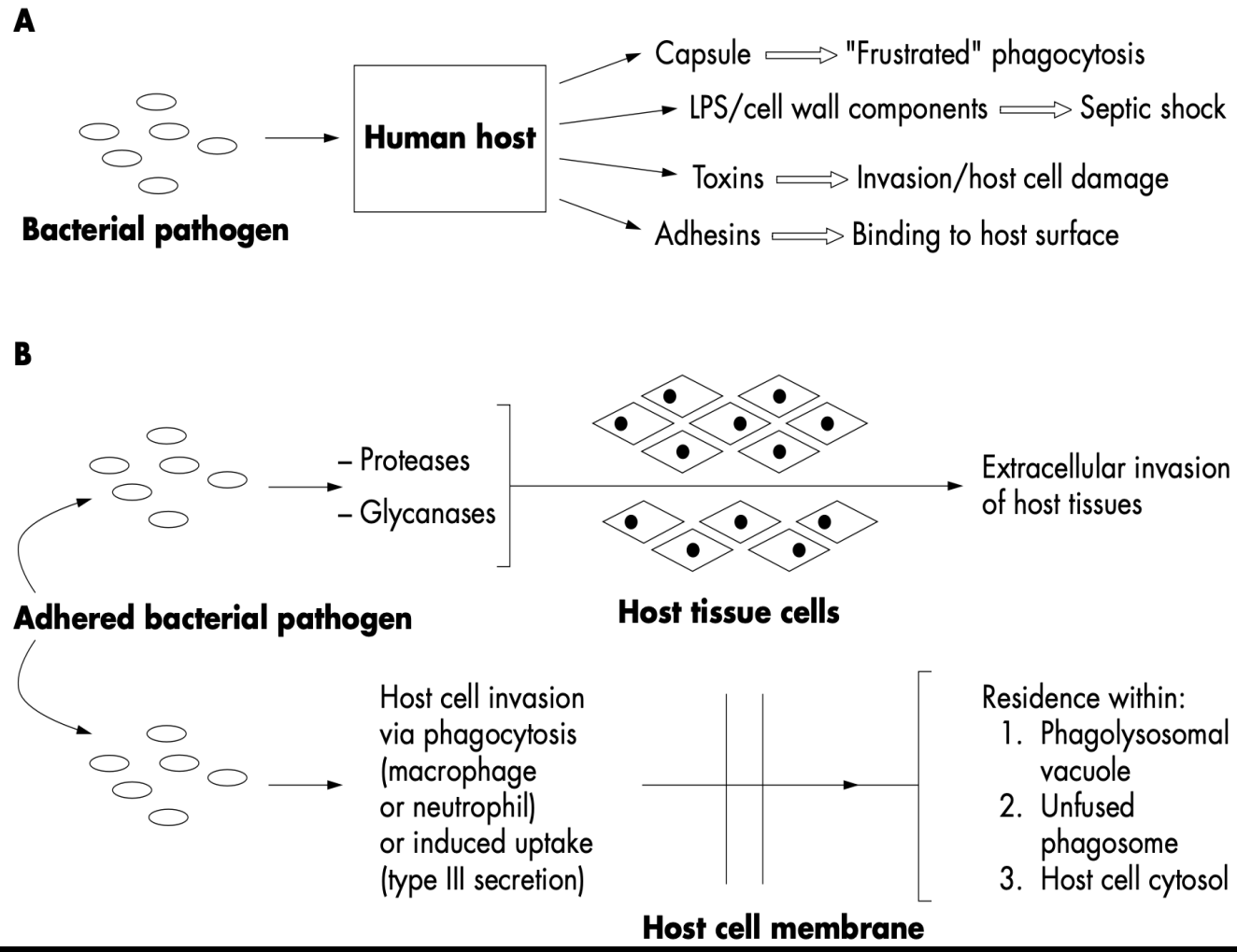
Pathogenesis and Pathogenicity

- Pathogenesis refers to the development of a disease
- Pathogenicity refers to the ability of an organism to cause disease.
- Commensals and opportunistic pathogens lack this inherent ability to cause disease
- Virulence refers to the degree of pathology caused by the organism.
- Virulence genes are involved in horizontal (lateral) transfer
- The extent of the virulence is usually correlated with the ability of the pathogen to multiply within the host and may be affected by other factors.
- Pathogenicity is used as a qualitative term, virulence is used more as a quantitative term.

Mechanisms of Bacterial Pathogenicity

- 4 Stages of Pathogenesis
- Exposure (contact)
- Adhesion (colonization)
- Invasion
- Infection

- Survival and replication after invasion
- Adaptation to the new environment
- Antibiotic resistance
- Biofilm



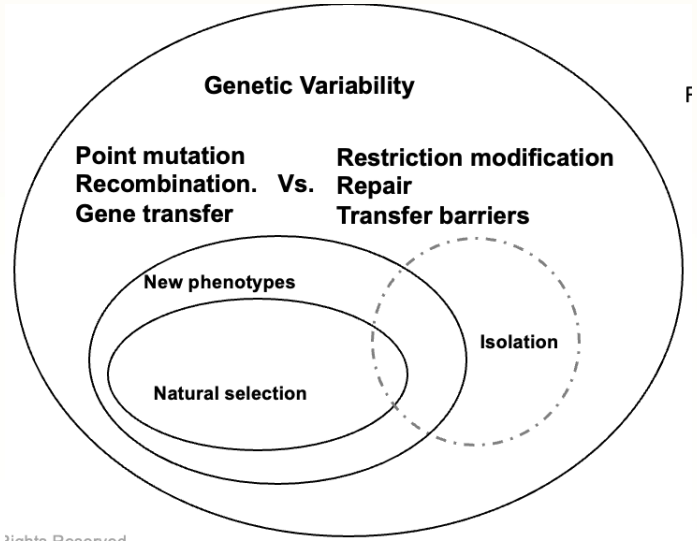
Wilson, Schurr, LeBlanc, et al.2002 Postgrad Med J: **78**:216–224

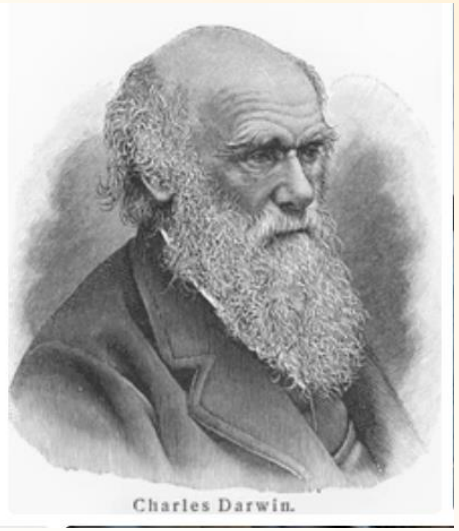
Evolution of microbial Pathogens

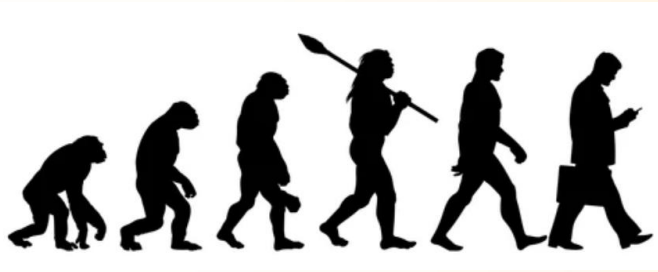
Evolution

Darwinian Principles of Evolution

- Genetic variability
- Phenotype formation
- Selection, and
- Isolation







Pathogens evolve over time following natural selection.

Morschhìuser, Kohler, Ziebuhr et al. 2000. Phil.Trans. R. Soc. Lond. B : 355, 695-704

Evolution of Microbial Pathogens

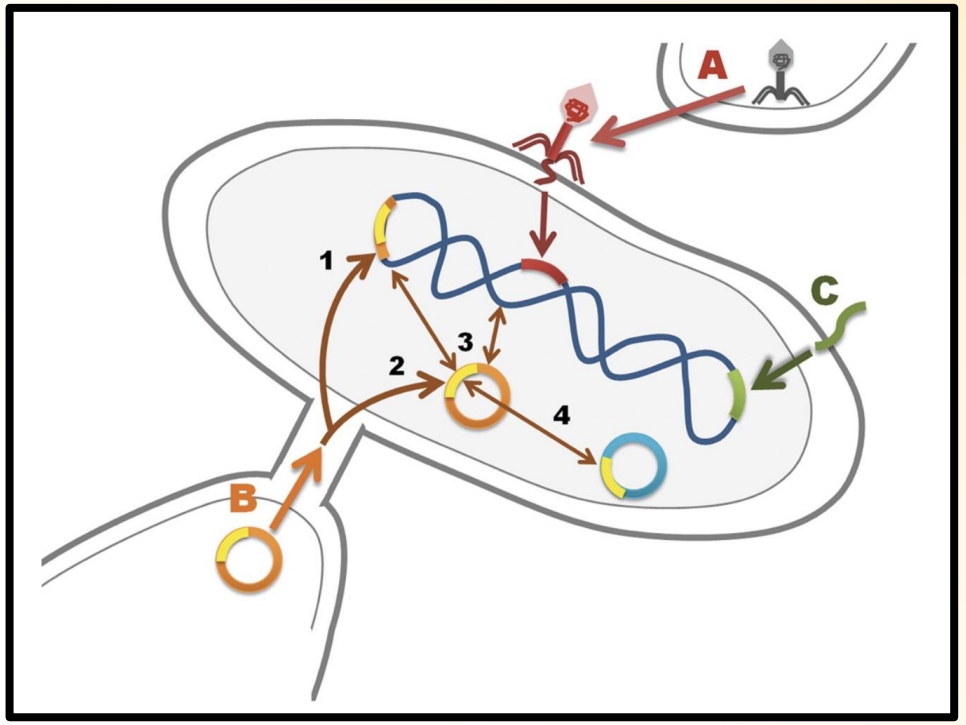
- Bacteria can evolve from non-pathogens to become pathogens through acquisition of new genetic material that enables them to colonize a host species and have detrimental effects on the host
- Bacterial evaluation occurs through mechanisms including natural selection and genetic drift and can result in adaptations to environmental change or host immunity
- The genetic makeup of bacterial genomes is subject to rapid and dramatic change through a variety of processes collectively referred to as “horizontal gene transfer” (HGT), which plays a principal part in the molecular evolution of novel bacterial pathogens
- Pathogenic bacteria can undergo further genetic modification that leads to altered virulence and changes in their genome

Genetic Mechanisms in Bacterial Evolution

- Macro evaluation- Long-term processes leading to the development of new species or subspecies
- Microevolution- Short-term developments, which occur during days or weeks
- Both processes, macro- and microevolution need horizontal gene transfer, which is particularly important for the development of pathogenic microorganisms.
- Horizontal or Lateral gene transfer (HGT) and Mobile Genetic Elements (MGE) are important in evolution of pathogen from non-pathogenic ancestor

Genetic Mechanisms in Bacterial Evolution

- Transfer of foreign DNA –
  - Horizontal or Lateral gene transfer (HGT)
    - Transformation
    - Transduction,
    - Conjugation
  - Mobile genetic elements (MGE)



Genetic Mechanisms in Bacterial Evolution

- Horizontal Gene Transfer (HGT)
- Mobile Genetic Elements (MGE) - the DNA mobilized into the host bacterium
  - Plasmids, bacteriophages, Integrative and conjugative element, prophages, and Pathogenicity islands (PAIs) play a crucial role in the evolution of pathogens.
  - Virulence genes transferred via HGT include genes for bacterial adherence to host cells, type 3 secretion systems, toxins, iron acquisition, and antimicrobial resistance

Mobile Genetic Elements in Selected Bacterial Pathogens

Organism	Mobile Element	Virulence mechanism
EHEC, EPEC, ETEC	Plasmids, Phage, Pathogenic Island	Adherence, type III secretions, stx
Salmonella enterica	Plasmid, Pathogenic Island	Invasion of nonphagocytic cells, intracellular survival and replication
Clostridium perfringens	Plasmid, Pathogenic Island	Toxins
Listeria monocytogenes	Plasmid, Pathogenic Islands	Adherence, Invasion, enzymes
Enterococcus spp.	Plasmid, Pathogenic Islands	Biofilm, toxins, pili
Staphylococcus aureus	Pathogenic Islands, Phages	Superantigen, leukocidin

Gyles and Boerlin.2014. Veterinary Pathology. Vol. 51(2) 328-340

Genomic Islands and Pathogenicity Islands

- Genomic islands— blocks of DNA containing mobile genetic elements transferred from the donor organisms to recipient.
- Widely distributed in GM + and GM – bacteria and known to encode a variety of functions
- Depending on the functions which are encoded by genomic islands, they may also be called symbiosis islands, metabolic or resistance islands and pathogenic islands

Genomic Islands and Pathogenicity Islands

- Occur as distinct units on the core chromosome with a general genetic structures characterized by a set of unifying features.
  - Present in the genomes of many bacteria but absent from the genomes of closely related strains
  - Often large (10–200 kb); however, smaller inserts (1–10 kb) can occur
  - They differ in GC content and in their codon usage from the rest of the chromosome
  - They are flanked by specific sequences (direct repeats)
  - They are usually associated with tRNA loci.

Pathogenicity Islands

- Best known Genomic islands
- Represent compact, distinct genetic units, often flanked by direct repeats
- Encode clusters of genes whose products contribute to virulence
- Different G+C content in comparison to DNA of host bacteria
- Occupy large chromosomal regions (often > 30 kb).
- Found in GM – and GM + bacteria and known to encode a variety of functions
- They are present in the genome of pathogenic strains of a given species but absent or only rarely present in those of non-pathogenic variants of the same or related species.

Functions Coded by Pathogenic Islands

Function	Organism	Increased pathogenicity
iron uptake	Salmonella enterica Klebsiella spp. Yersinia spp., Bacillus cereus	+
toxin production	Vibrio cholerae	+
Adhesins	urinary E. coli	
Methicillin resistance	Staphylococcus aureus	
type III-system	Salmonella enterica Shigella flexneri Yersinia spp.	+
type IV-system	Helicobacter pylori	+

Hacker and Carmiel (2001

What we know and don’t know

Summary

- Emerging, re-emerging and opportunistic pathogens are important threat to food safety and public health
- Bacterial pathogenesis involve ability of pathogen to invade and infect the host, survival and replication after invasion and adaptation to the new environment as well as dealing with host immunity, antibiotic resistance, and biofilm
- Horizontal gene transfer via transformation, transduction and combination as well as Mobile Genetic Elements (MGE) are particularly important for the development of pathogenic microorganisms from non-pathogenic ancestor
- Pathogenic islands, the best known Genomic islands are found in GM – and GM + bacteria and encode clusters of genes whose products contribute to virulence
- They are present in the genome of pathogenic strains of a given species but absent or only rarely present in those of non-pathogenic variants of the same or related species
- Availability of numerous complete genome sequences of bacterial pathogens and the use of genomic techniques have given us new tools to study and understand microbial pathogenesis
- Our knowledge on mechanisms of pathogenesis is increasing but much is still unknown

Final Thoughts

"Almost any bacterial species is capable of producing intestinal symptoms if swallowed in sufficient numbers"

DuPont and Pickering 1980

"Expect the unexpected"

Swerdlow and Altekruse, 1998

"The future of microbes and mankind will probably unfold as episodes of a suspense thriller that could be entitled *Our Wits Versus Their Genes*". - Joshua Lederberg. 2000.

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New Era, Old Problems: Emerging and Re-Emerging Pathogens

Meet Our Speakers

Alvin Lee, Ph.D., MASM
Illinois Institute of Technology

Brendan Niemira, Ph.D.
U.S. Department of Agriculture

Purnendu C. Vasavada, Ph.D
University of Wisconsin-River Falls

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Session Overview

- Alvin Lee – The genomic and environmental stressors that impact microbial pathogenesis
- Purnendu Vasavada – Evolution and Pathogenesis: What time has done to microorganisms - what we know and don't know.
- Brendan Niemira – Risk management options for preparedness and response to emerging pathogens
- Conclusions, Q&A

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The genomic and environmental stressors that impact microbial pathogenesis

Alvin Lee, IIT

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Evolution and Pathogenesis: What time has done to microorganisms - what we know and don’t know.

Purnendu C. Vasavada, UW River Falls

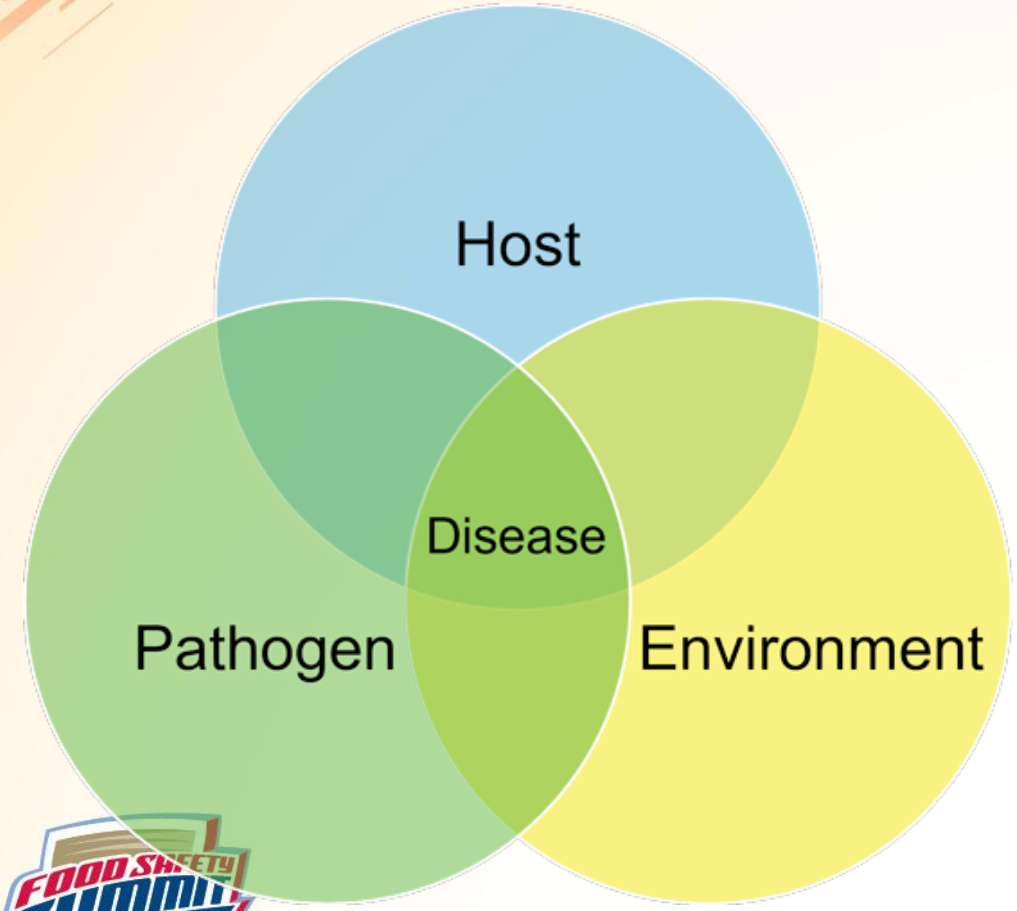
Risk management options for preparedness and response to emerging pathogens

Brendan Niemira, USDA

Risk management options

- Pathogens and food safety incidents are part of the landscape
- Risk avoidance comes from preparedness
  - Quantitative and qualitative risk assessment as part of SOP
  - Risk avoidance and mitigation strategies should be baked in
- Understand risks and potential impacts
- Risks of familiar and conventional pathogens
- Risks of unfamiliar and emerging pathogens
  - May require fresh analysis of risk matrix

Change causes change



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  - General, broadly effective
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Hazards vs. risks, with a side of analysis

- Hazard: a bad thing that could happen
- Hazard analysis:
  - A list of all* the bad things that COULD happen
  - How bad* it would be if they DID happen
- Risk: the likelihood that a hazard WILL happen
- Risk analysis: ∑ [(likelihood)(how bad it would be)]

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Risk assessment and calculation

- Models focus on primary risks, significant hazards
  - Can safely ignore risk of alien invasions, giant asteroids, etc.
- Potential food safety hazards: microbiological tests on raw ingredients are out of spec; failure of process control; break in cold chain; glass fragments in finished product; etc., etc.
- Assign numerical probabilities to each risk based on statistics, experience, record-keeping, etc.
- Derive relationships among various risks, some of which you can mitigate, some of which you can't.

Pathogen incidence: potential responses

- Avoidance
- Containment
- Eradication
- Do Nothing

Pathogen incidence: Avoidance

- Take steps to understand risks so as to avoid them
- What comes into the plant
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- Even if a limited response seems warranted, a full analysis will repay efforts by avoiding future occurrence

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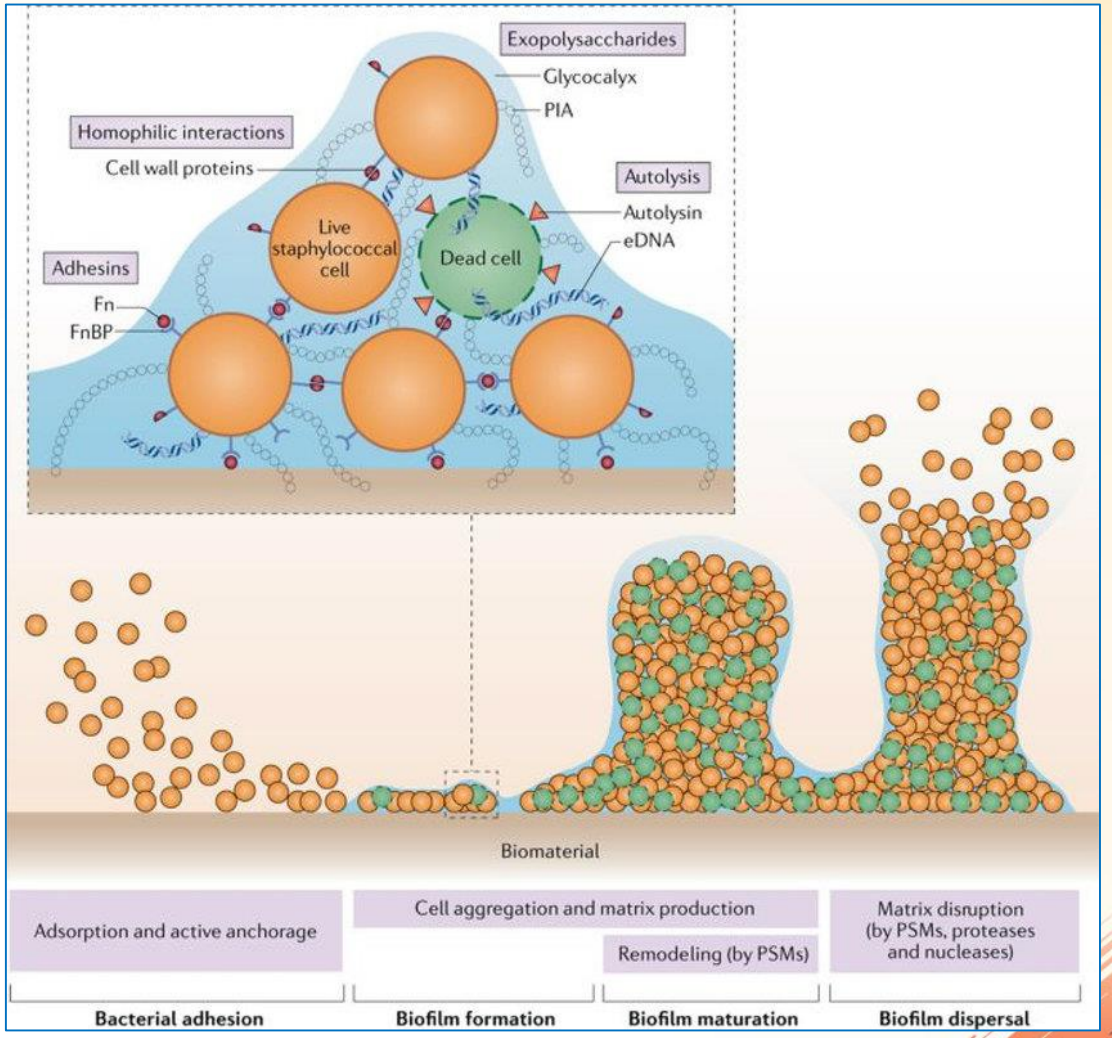
Biofilms

Biofilms – a complex challenge

- 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



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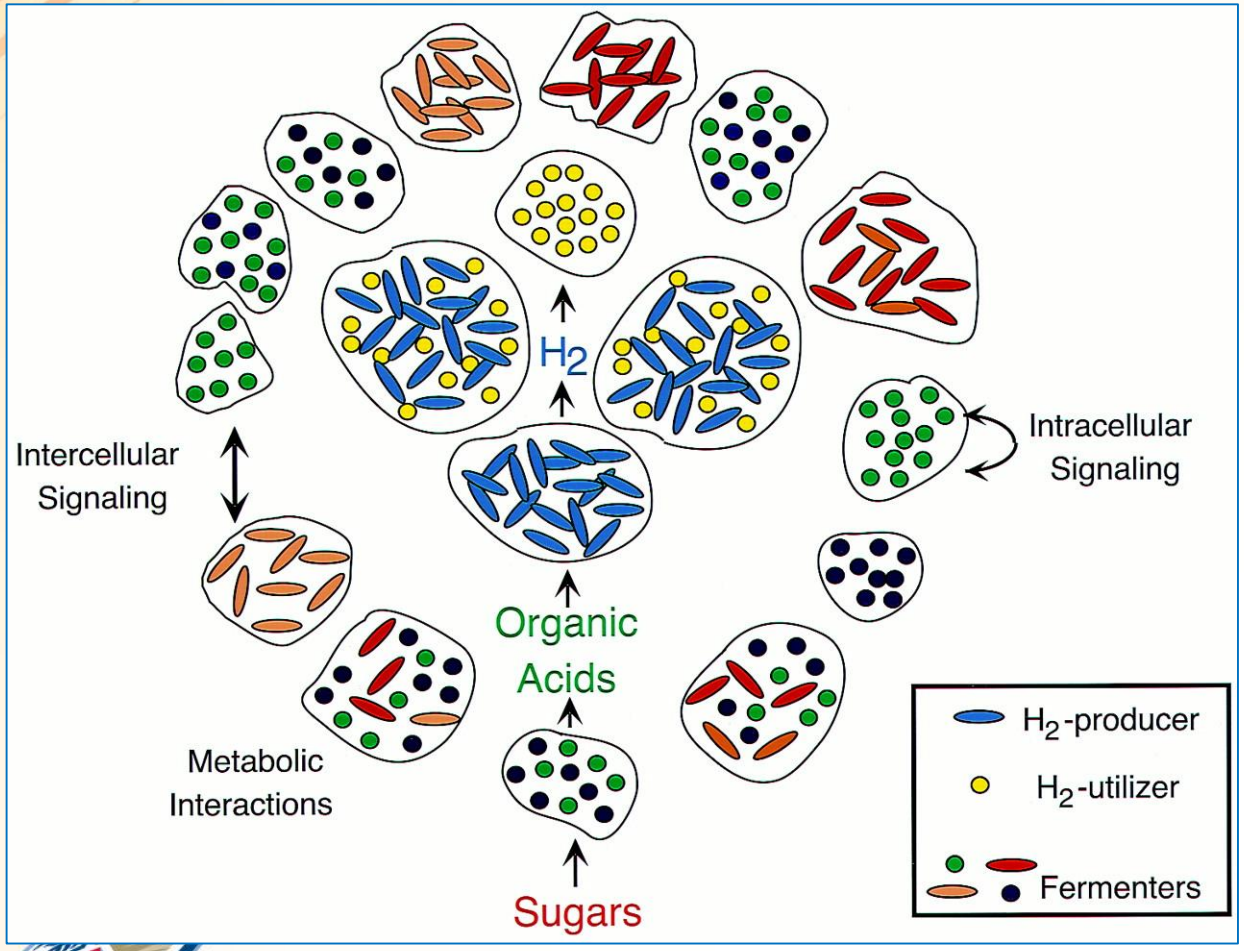

- Pathogens can participate in mostly non-pathogenic biofilms
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- Does the pathogen actively participate in biofilm formation? Or is it just along for the ride?
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Davey and O'Toole, 2000. Microb Mol Biol Rev. 64(4)

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  - 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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Evolution and Pathogenesis: What time has done to microorganisms - what we know and don't know

Purnendu C. Vasavada
Professor Emeritus of Food Science
University of Wisconsin-River Falls
River Falls, WI. 54022

Agenda

- Introduction: Emerging, Reemerging Pathogens- What is in the name?
- Mechanism of Bacterial Pathogenicity
- Evolution of microbial Pathogens
- What we know and don’t know
- Summary

Emerging, Reemerging Pathogens- What is in the name?

The Challenge of Emerging Pathogens

Infectious Diseases: Considerations for the 21st Century

Anthony S. Fauci
National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland

Clin Infect Dis. 2001;32:675–85.

The challenge of emerging and re-emerging infectious diseases

David M. Morens, Gregory K. Folkers & Anthony S. Fauci

National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services, Bethesda, Maryland 20892-2520, USA (e-mail: afauci@niaid.nih.gov)

Infectious diseases have for centuries ranked with wars and famine as major challenges to human progress and survival. They remain among the leading causes of death and disability worldwide. Against a constant background of established infections, epidemics of new and old infectious diseases periodically emerge, greatly magnifying the global burden of infections. Studies of these emerging infections reveal the evolutionary properties of pathogenic microorganisms and the dynamic relationships between microorganisms, their hosts and the environment.

***Nature,* volume 430**, pages 242–249 (2004)

Emerging, Reemerging and Opportunistic Pathogens

- Emerging pathogens are New, reemerging, or drug-resistant infections whose incidence in humans have increased within the past two decades or threatened to increase in the near future

- Reemerging Pathogens- involved in the reappearance of a known disease following a decline in incidence including newly recognized pathogens, new diseases caused by known organisms, and the extension of the geographic or host range of a pathogen

- Opportunistic Pathogens- are microbes that usually do not cause disease in healthy people, but may become virulent with immunocompromised and unhealthy individuals

Emerging, Reemerging and Opportunistic Pathogens

Early 1900

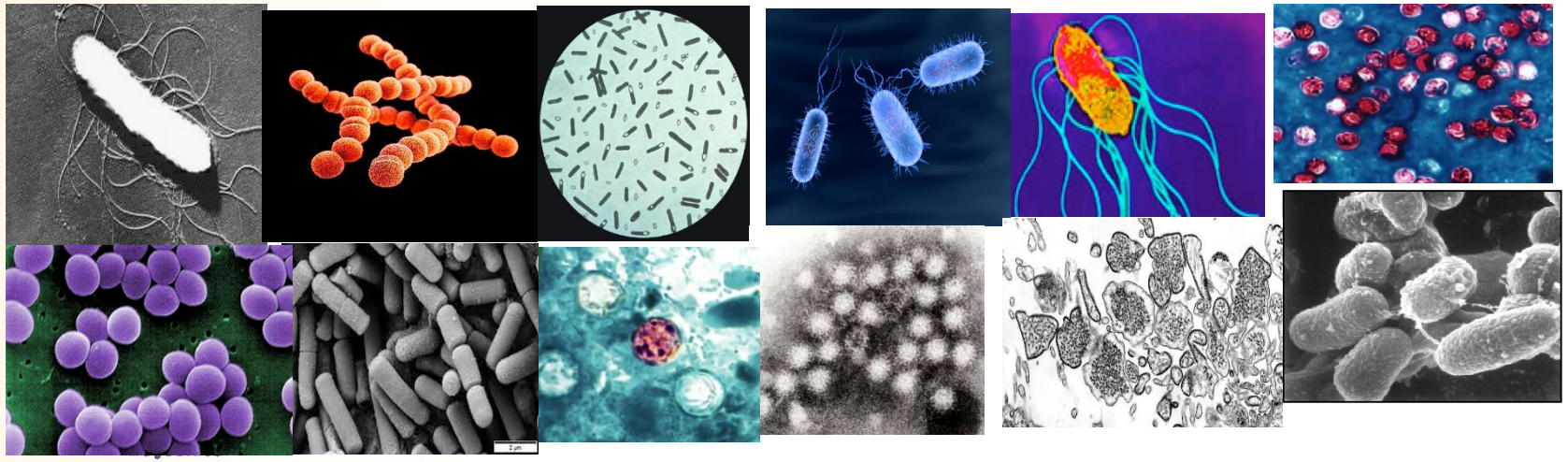
- Typhoid fever
- Tuberculosis
- Septic sore throat
- Diphtheria
- Brucellosis

1940s-1960s

- Clostridium botulinum
- Salmonella Spp.
- Staphylococcus aureus
- Streptococci

1960s - 1990s

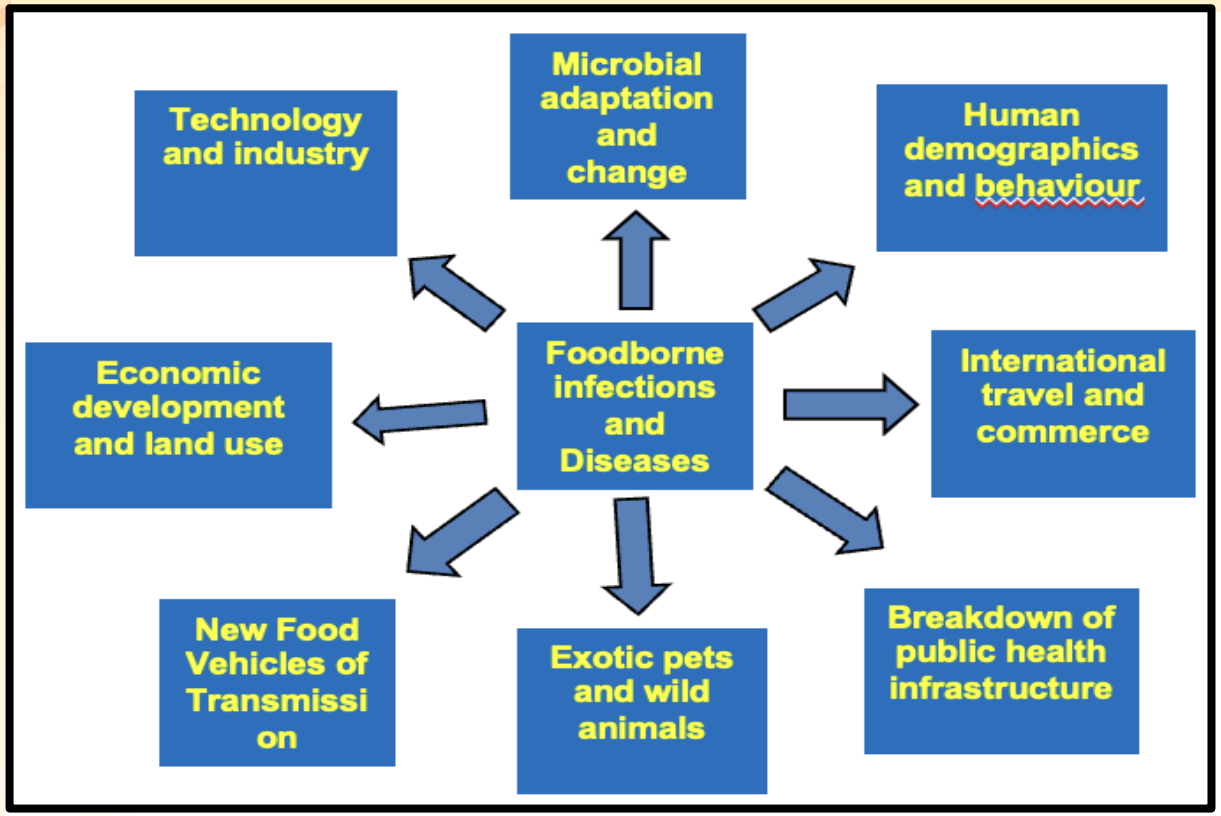
- Salmonella
- Clostridium perfringens
- Vibrio parahaemolyticus
- Bacillus cereus
- Pathogenic E. coli
- Campylobacter jejuni



2000 - s

- Salmonella Spp.
- E. coli 057:H7
- Non 0157 EHEC/STECs
- Listeria monocytogenes*
- Yersinia enterocolitica*
- Bacillus cereus*
- Staphylococcus aureus
- Cronobacter sakazakii
- Vibrio parahemolyticus
- V. vulnificus
- Cryptosporidium, Cyclospora, Toxoplasma gondii
- Norovirus, Hep A. Nipah Virus

Factors Contributing to the Emergence of Foodborne Pathogens and Diseases



- Microbial adaptation and change
- Human susceptibility to infection
- Climate and weather
- Changing ecosystems
- Human demographics and behavior
- Economic development and land use
- International travel and commerce
- Technology and industry
- Breakdown of public health measures
- Other social, political and economic factors e.g. War and famine, Lack of political will, and Intent to harm

Mechanisms of Bacterial Pathogenicity

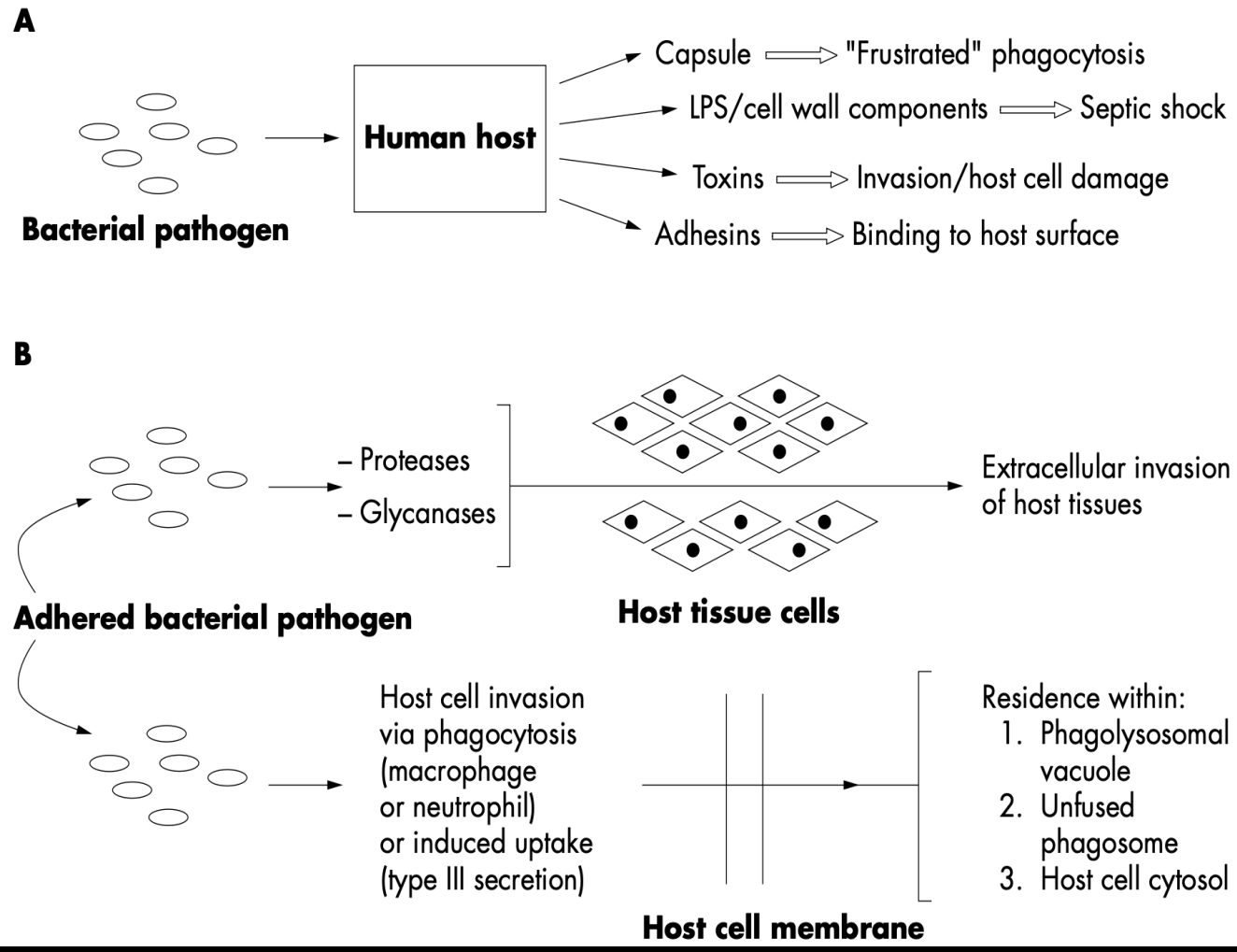
Pathogenesis and Pathogenicity

- Pathogenesis refers to the development of a disease
- Pathogenicity refers to the ability of an organism to cause disease.
- Commensals and opportunistic pathogens lack this inherent ability to cause disease
- Virulence refers to the degree of pathology caused by the organism.
- Virulence genes are involved in horizontal (lateral) transfer
- The extent of the virulence is usually correlated with the ability of the pathogen to multiply within the host and may be affected by other factors.
- Pathogenicity is used as a qualitative term, virulence is used more as a quantitative term.

Mechanisms of Bacterial Pathogenicity

- 4 Stages of Pathogenesis
- Exposure (contact)
- Adhesion (colonization)
- Invasion
- Infection

- Survival and replication after invasion
- Adaptation to the new environment
- Antibiotic resistance
- Biofilm



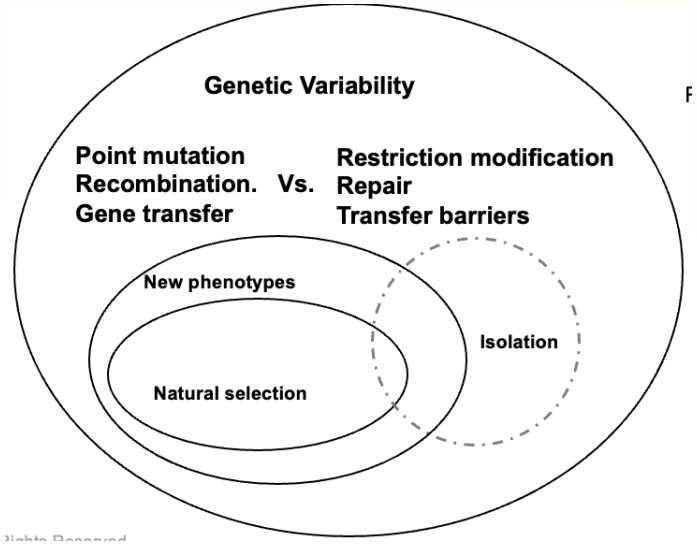
Wilson, Schurr, LeBlanc, et al.2002 Postgrad Med J: **78**:216–224

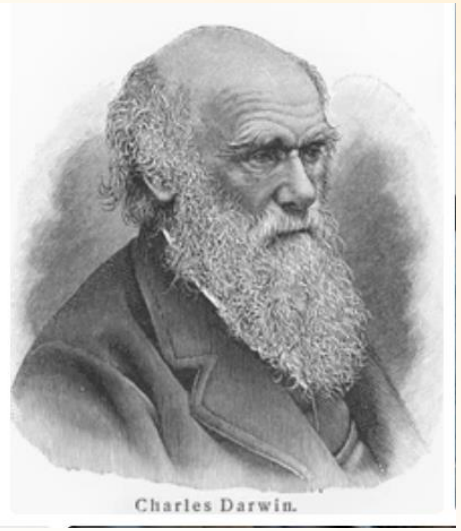
Evolution of microbial Pathogens

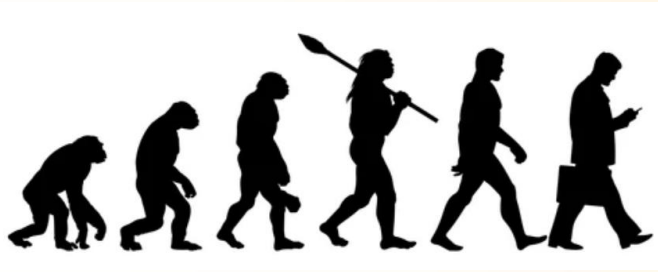
Evolution

Darwinian Principles of Evolution

- Genetic variability
- Phenotype formation
- Selection, and
- Isolation







Pathogens evolve over time following natural selection.

Morschhìuser, Kohler, Ziebuhr et al. 2000. Phil.Trans. R. Soc. Lond. B : 355, 695-704

Evolution of Microbial Pathogens

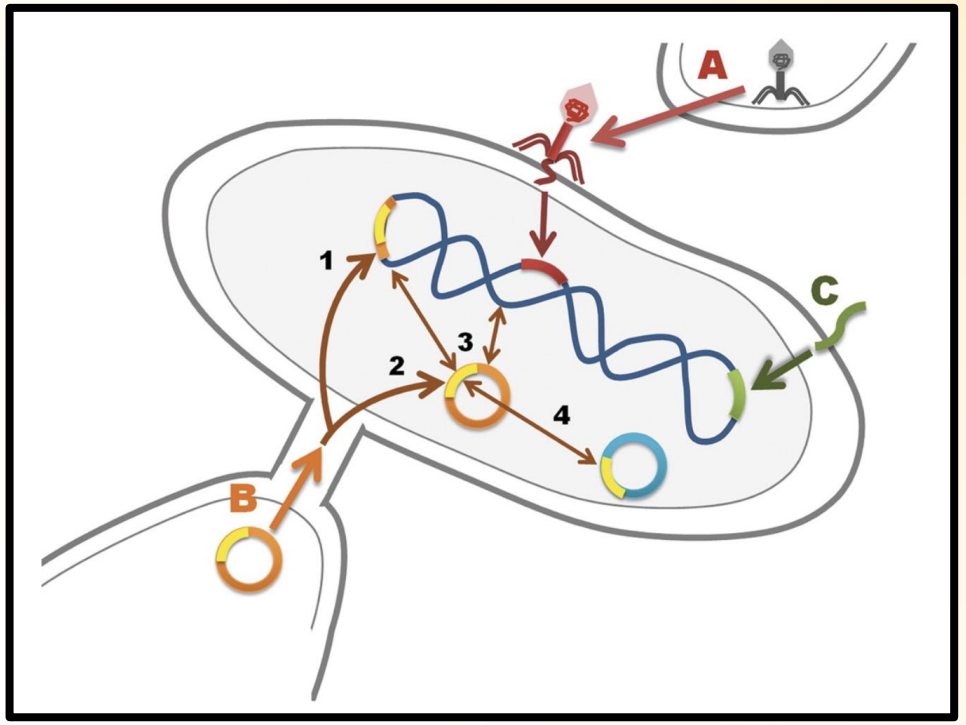
- Bacteria can evolve from non-pathogens to become pathogens through acquisition of new genetic material that enables them to colonize a host species and have detrimental effects on the host
- Bacterial evaluation occurs through mechanisms including natural selection and genetic drift and can result in adaptations to environmental change or host immunity
- The genetic makeup of bacterial genomes is subject to rapid and dramatic change through a variety of processes collectively referred to as “horizontal gene transfer” (HGT), which plays a principal part in the molecular evolution of novel bacterial pathogens
- Pathogenic bacteria can undergo further genetic modification that leads to altered virulence and changes in their genome

Genetic Mechanisms in Bacterial Evolution

- Macro evaluation- Long-term processes leading to the development of new species or subspecies
- Microevolution- Short-term developments, which occur during days or weeks
- Both processes, macro- and microevolution need horizontal gene transfer, which is particularly important for the development of pathogenic microorganisms.
- Horizontal or Lateral gene transfer (HGT) and Mobile Genetic Elements (MGE) are important in evolution of pathogen from non-pathogenic ancestor

Genetic Mechanisms in Bacterial Evolution

- Transfer of foreign DNA –
  - Horizontal or Lateral gene transfer (HGT)
    - Transformation
    - Transduction,
    - Conjugation
  - Mobile genetic elements (MGE)



Genetic Mechanisms in Bacterial Evolution

- Horizontal Gene Transfer (HGT)
- Mobile Genetic Elements (MGE) - the DNA mobilized into the host bacterium
  - Plasmids, bacteriophages, Integrative and conjugative element, prophages, and Pathogenicity islands (PAIs) play a crucial role in the evolution of pathogens.
  - Virulence genes transferred via HGT include genes for bacterial adherence to host cells, type 3 secretion systems, toxins, iron acquisition, and antimicrobial resistance

Mobile Genetic Elements in Selected Bacterial Pathogens

Organism	Mobile Element	Virulence mechanism
EHEC, EPEC, ETEC	Plasmids, Phage, Pathogenic Island	Adherence, type III secretions, stx
Salmonella enterica	Plasmid, Pathogenic Island	Invasion of nonphagocytic cells, intracellular survival and replication
Clostridium perfringens	Plasmid, Pathogenic Island	Toxins
Listeria monocytogenes	Plasmid, Pathogenic Islands	Adherence, Invasion, enzymes
Enterococcus spp.	Plasmid, Pathogenic Islands	Biofilm, toxins, pili
Staphylococcus aureus	Pathogenic Islands, Phages	Superantigen, leukocidin

Gyles and Boerlin.2014. Veterinary Pathology. Vol. 51(2) 328-340

Genomic Islands and Pathogenicity Islands

- Genomic islands— blocks of DNA containing mobile genetic elements transferred from the donor organisms to recipient.
- Widely distributed in GM + and GM – bacteria and known to encode a variety of functions
- Depending on the functions which are encoded by genomic islands, they may also be called symbiosis islands, metabolic or resistance islands and pathogenic islands

Genomic Islands and Pathogenicity Islands

- Occur as distinct units on the core chromosome with a general genetic structures characterized by a set of unifying features.
  - Present in the genomes of many bacteria but absent from the genomes of closely related strains
  - Often large (10–200 kb); however, smaller inserts (1–10 kb) can occur
  - They differ in GC content and in their codon usage from the rest of the chromosome
  - They are flanked by specific sequences (direct repeats)
  - They are usually associated with tRNA loci.

Pathogenicity Islands

- Best known Genomic islands
- Represent compact, distinct genetic units, often flanked by direct repeats
- Encode clusters of genes whose products contribute to virulence
- Different G+C content in comparison to DNA of host bacteria
- Occupy large chromosomal regions (often > 30 kb).
- Found in GM – and GM + bacteria and known to encode a variety of functions
- They are present in the genome of pathogenic strains of a given species but absent or only rarely present in those of non-pathogenic variants of the same or related species.

Functions Coded by Pathogenic Islands

Function	Organism	Increased pathogenicity
iron uptake	Salmonella enterica Klebsiella spp. Yersinia spp., Bacillus cereus	+
toxin production	Vibrio cholerae	+
Adhesins	urinary E. coli	
Methicillin resistance	Staphylococcus aureus	
type III-system	Salmonella enterica Shigella flexneri Yersinia spp.	+
type IV-system	Helicobacter pylori	+ Hacker and Carmiel (2001

What we know and don’t know

Summary

- Emerging, re-emerging and opportunistic pathogens are important threat to food safety and public health
- Bacterial pathogenesis involve ability of pathogen to invade and infect the host, survival and replication after invasion and adaptation to the new environment as well as dealing with host immunity, antibiotic resistance, and biofilm
- Horizontal gene transfer via transformation, transduction and combination as well as Mobile Genetic Elements (MGE) are particularly important for the development of pathogenic microorganisms from non-pathogenic ancestor
- Pathogenic islands, the best known Genomic islands are found in GM – and GM + bacteria and encode clusters of genes whose products contribute to virulence
- They are present in the genome of pathogenic strains of a given species but absent or only rarely present in those of non-pathogenic variants of the same or related species
- Availability of numerous complete genome sequences of bacterial pathogens and the use of genomic techniques have given us new tools to study and understand microbial pathogenesis
- Our knowledge on mechanisms of pathogenesis is increasing but much is still unknown

Final Thoughts

"Almost any bacterial species is capable of producing intestinal symptoms if swallowed in sufficient numbers"

DuPont and Pickering 1980

"Expect the unexpected"

Swerdlow and Altekruse, 1998

"The future of microbes and mankind will probably unfold as episodes of a suspense thriller that could be entitled *Our Wits Versus Their Genes*". - Joshua Lederberg. 2000.

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New Era, Old Problems: Emerging and Re-Emerging Pathogens

Meet Our Speakers

Alvin Lee, Ph.D., MASM
Illinois Institute of Technology

Brendan Niemira, Ph.D.
U.S. Department of Agriculture

Purnendu C. Vasavada, Ph.D
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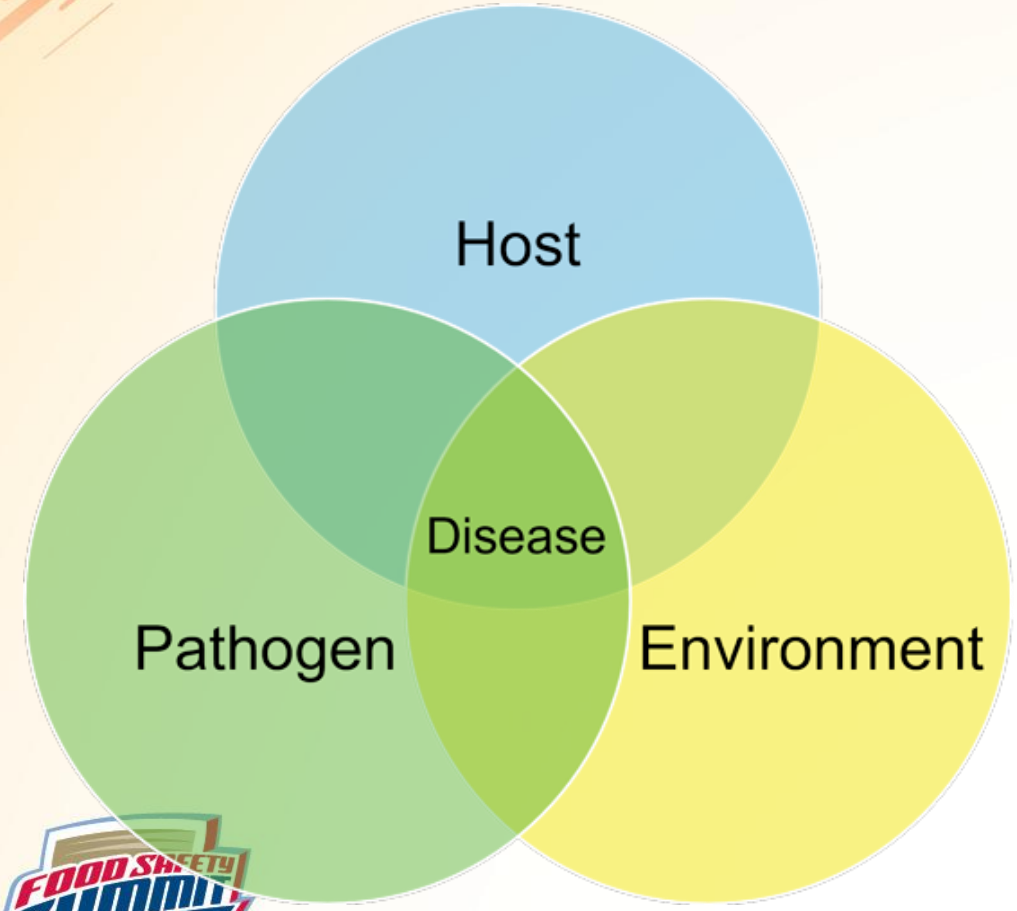

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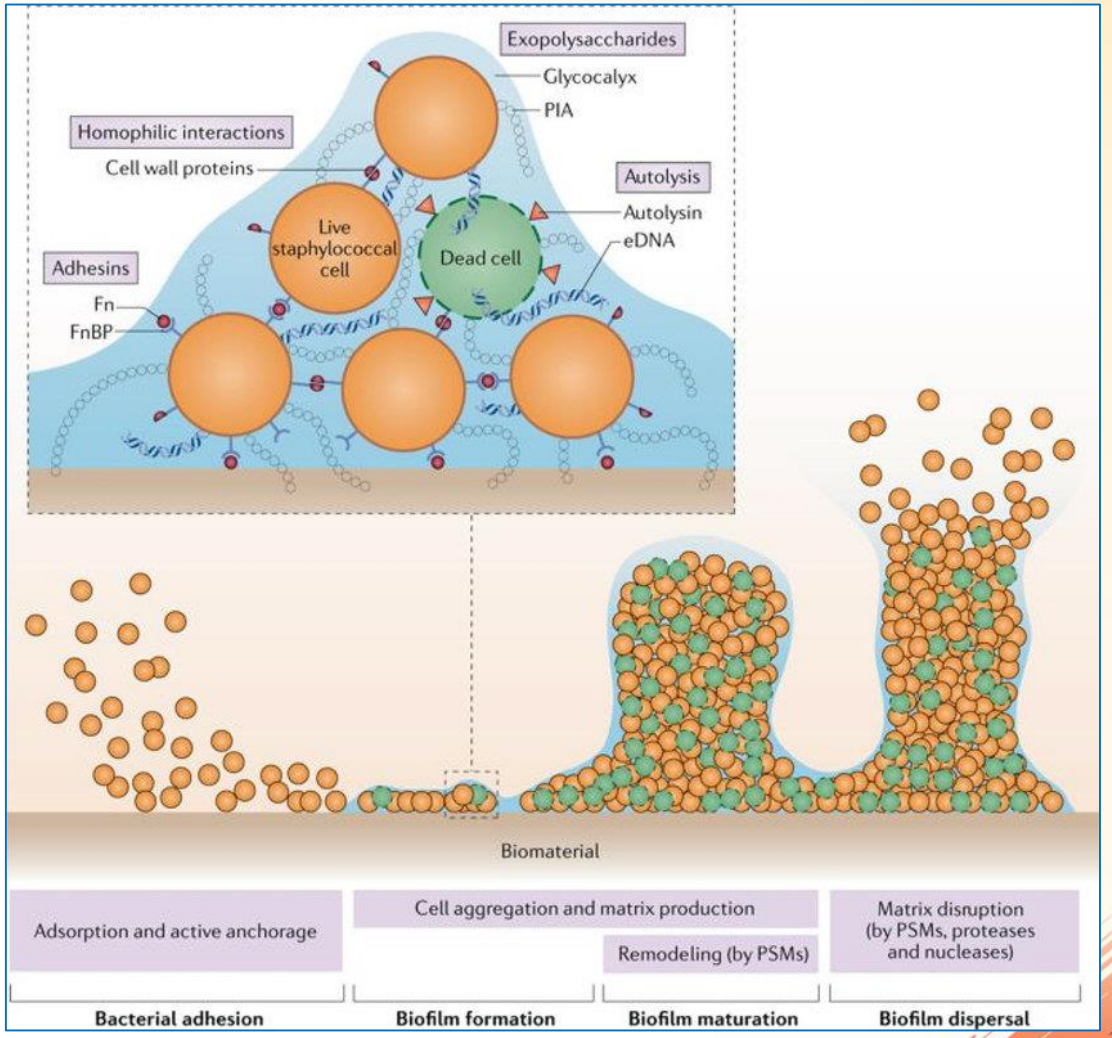

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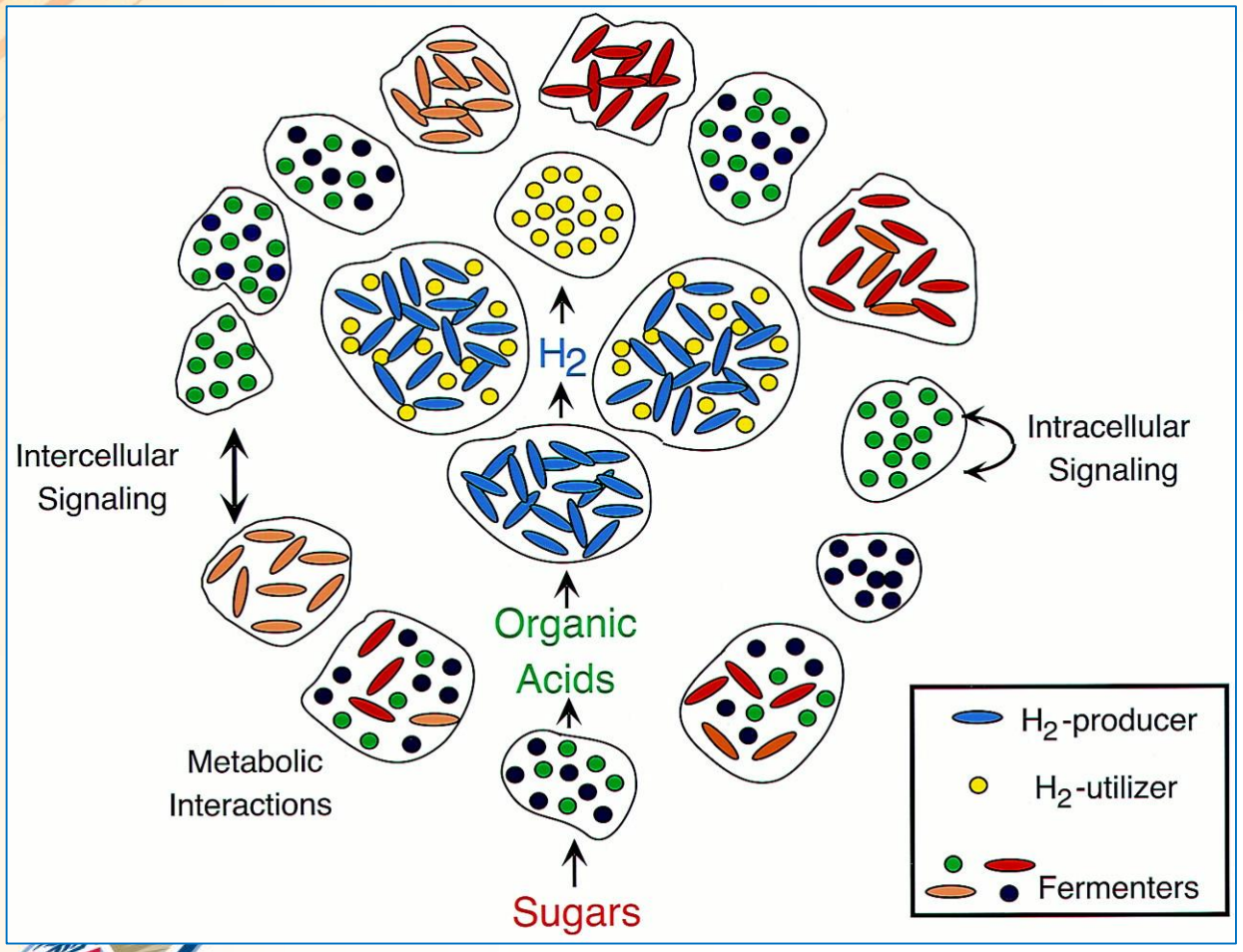

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- CDC – Current Outbreaks: www.cdc.gov/outbreaks/index.html
- CDC's "Solve the Outbreak" online game
  - "Become a disease detective" – learn about dozens of infectious organisms and diseases while investigating outbreaks, foodborne and otherwise
  - www.cdc.gov/mobile/applications/sto/web-app.html

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