Making of a Pathogen: Evolution and Development of Pathogenesis

Purnendu C. Vasavada, Ph.D.
Professor Emeritus of Food Science
University of Wisconsin-River Falls
PCV & Associates, LLC.
River Falls, WI. 54022

Presented at
IFSH Annual Meeting
Chicago, IL
September 4, 2024

Purnendu C. Vasavada

- Professor of Food Science, UW River Falls
- Food Safety and Microbiology Specialist- UW Extension
- UWRF Rapid Methods and Automation in Food Microbiology symposium and Workshop
- Professor Emeritus University of Wisconsin–River Falls
- PCV & Associates, LLC.
- FDA-ORISE Fellow
- Food Safety Preventive Controls Alliance (FSPCA)
- ToT and LI PCHF PCQI Course
- LI - International HACCP Alliance HACCP course

Evolution and Development of Pathogenesis

Agenda

- Introduction
- Emerging and Reemerging Diseases and Pathogens
- Mechanism of Bacterial Pathogenicity
- Evolution of microbial Pathogens and Development of Pathogenesis
- What we know and don't know
- Summary and Final Thoughts

The Challenge of Emerging and Reemerging Diseases and Pathogens

Dr. William H. Stewart
Surgeon General 1965-1969

Dr. Anthony Fauchi

"It is time to close the book on infectious diseases, and declare the war against pestilence won"

- *William H. Stewart (1967)*

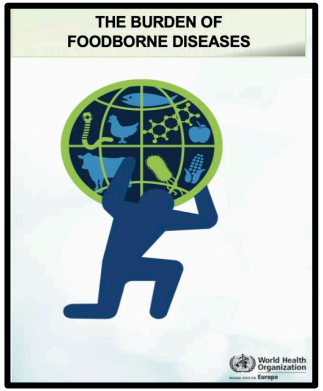
" I reflect back to December 1967, when then-Surgeon General William H. Stewart, contemplating the benefits realized from antibiotics and vaccines, declared victory against the threat of infectious diseases and suggested that our nation turn its attention and resources to the more important threat of chronic diseases".

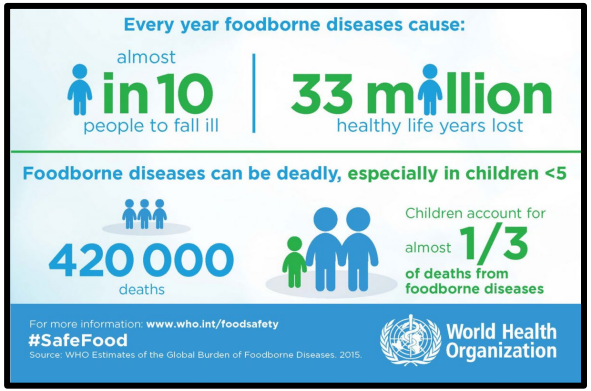
- *Anthony Fauchi (2001)*

38th annual meeting of the Infectious Diseases Society of America, held in New Orleans on 7–10 September 2000.

The Challenge of Emerging Pathogens

- Burdon of Foodborne diseases*
  - 600 million people sickened annually
  - 420,000 deaths, 1/3 of them, children
  - 33 Million healthy years lost
- Infectious diseases are the 2nd leading cause of death worldwide and 3rd leading cause of deaths in U.S.
- Approx. 1415 spp. of m.o. known to produce disease in humans
- 60.3% spp. are zoonotic
- Majority (~72%) originate in wildlife
- 175 pathogenic spp. are considered emerging





*WHO

Emerging, Reemerging and Opportunistic Pathogens

- Emerging : 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.

Lederberg et al., 1992 Lashley, 2003, 2004

Emerging, Reemerging and Opportunistic Pathogens

Early 1900

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

1940s-1960s

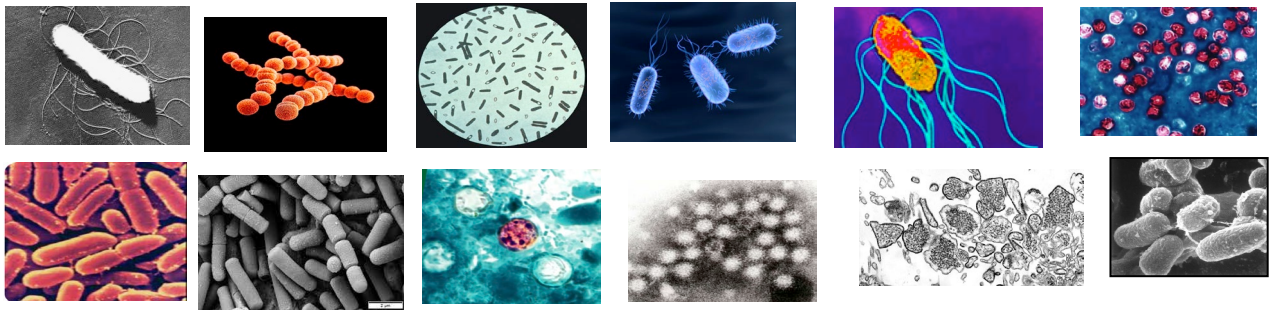
- Clostridium botulinum
- Salmonella Spp.
- Staphylococcus aureus
- Streptococci
- Clostridium perfringens

1970s - 1990s

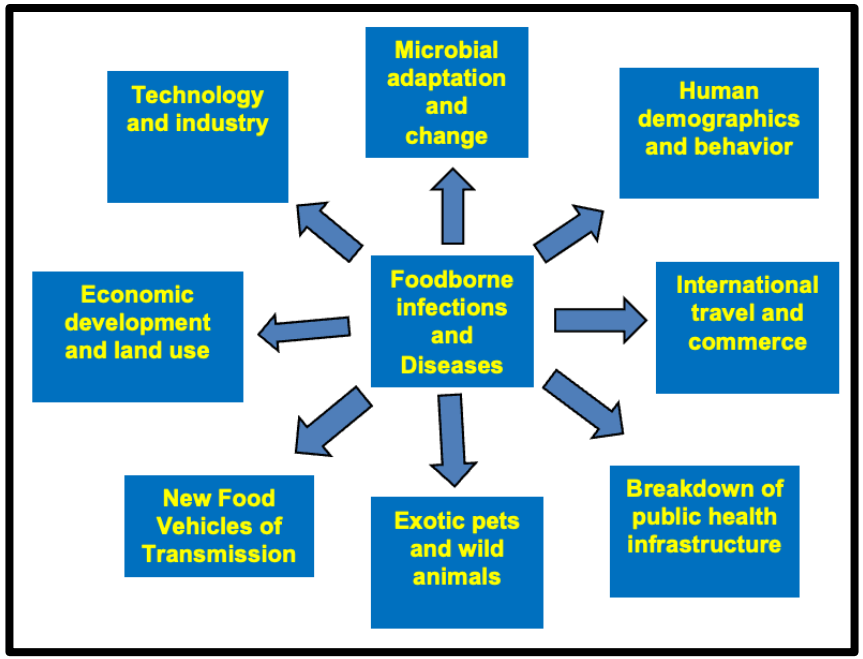
- Salmonella
- Clostridium perfringens
- Vibrio parahaemolyticus
- Bacillus cereus
- Pathogenic E. coli
- Campylobacter jejuni

2000 - s

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



Factors Contributing to the Emergence of Foodborne Pathogens and Diseases



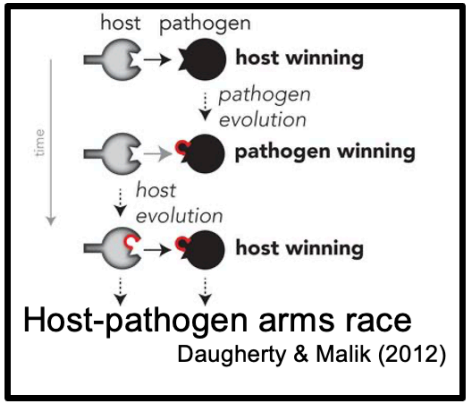
- Microbial adaptation and change
- Human demographics and behavior
- Human susceptibility to infection
- International travel and commerce
- New Vehicles of transmission
- Climate and weather
- Changing ecosystems
- Economic development and land use
- Technology and industry
- Breakdown of public health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

Mechanism 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.

Making of a Pathogen: Host-Pathogen Arm Race



- Conflicting drives between host and pathogen lead to an evolutionary "arms-race," where an asymmetric "attack-defense" strategy comes into play.

  Dawkins and Krebs (1979)

- The host-microbe relationship is a dynamic equilibrium.
- Physiological or genetic changes in either partner may prompt commensal microbes to invade the tissue of their host.

  J. Lederberg (2000)

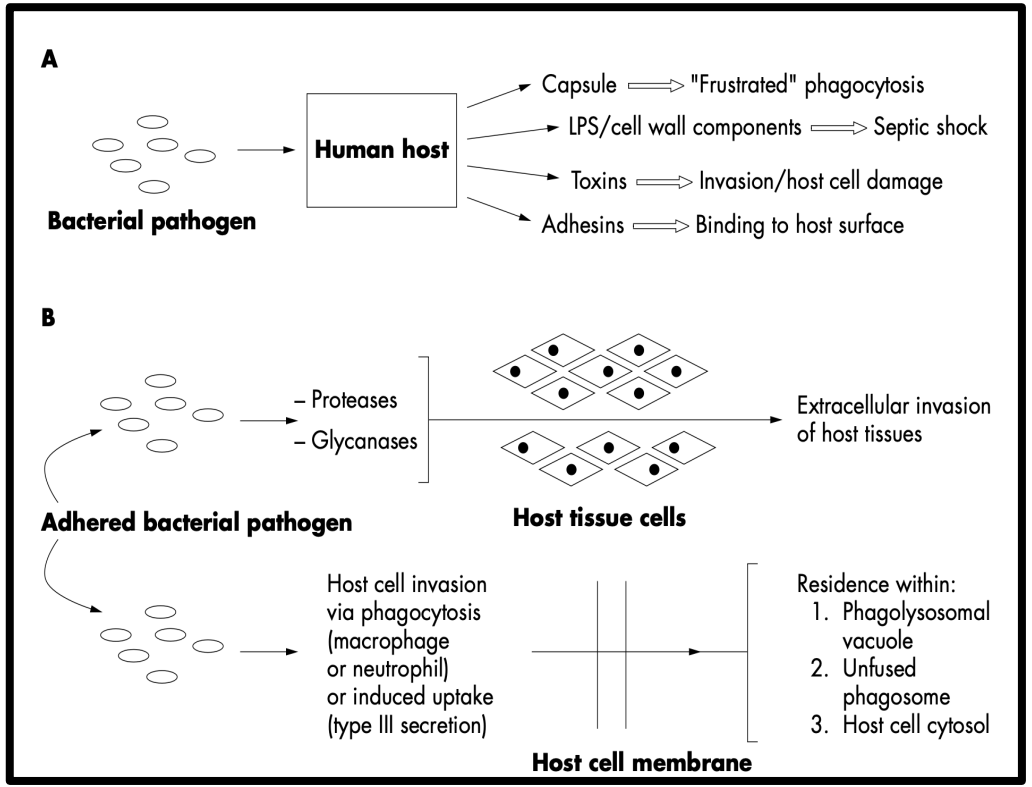
- Pathogen Emergence (PE) is a multifactorial, non- linear phenomenon that culminates with the ability of a bacterium to effectively colonize and harm the human host.

Mechanisms of Bacterial Pathogenicity

Stages of Pathogenesis

- Exposure (contact)
- Adhesion (colonization)
- Invasion
- Infection

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



Wilson, et al. 2002

Pathogenic mechanisms to evade or overcome selective pressure within human host

Selective Pressure	Pathogenic mechanisms to evade or overcome selective pressure
Physical barriers in host (i.e. mucosal epithelium)	Mucinases, Enetrotoxins, exfoliative toxins, Transcytosis through M cells
Host complement	Complement inhibitor protein, C3 protease
Sequestration of host resources (e.g. iron)	Enterobactin/aerobactin systems
Host B and T cell lymphocytes	Cytotoxins, T3SS- mediated apoptosis
Antibiotics, antimicrobial peptides	Efflux pumps, Mutation in antimicrobial targets, Enzymes to inactivate antibiotics (e.g. β lactamase)
Bacterial colicins	Colicin immunity proteins
Bacterial T6SSs	T6S immunity proteins

Bliven and Maurelli. 2016. Microbiol Spectrum 4(1):VMBF- 0017-2015.

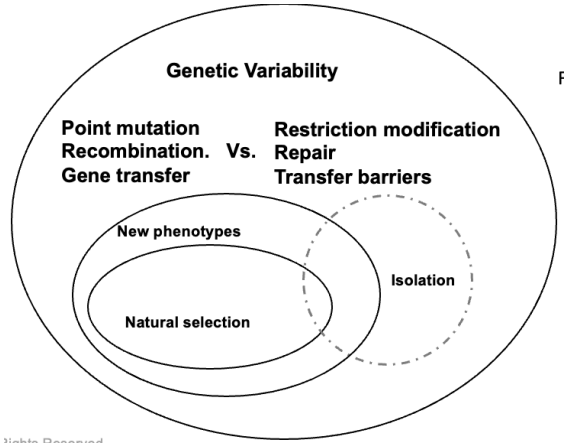
Making of Pathogen:
Evolution of Microbial Pathogens

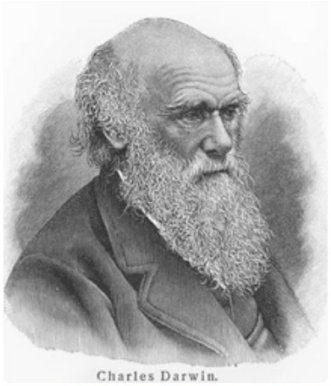
Making of a Pathogen

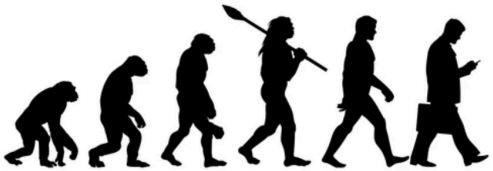
Evolution is the way living organisms change over time, driven by natural selection.

Darwinian Principles of Evolution

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







Pathogens evolve over time following natural selection.

Evolution of microbial pathogens

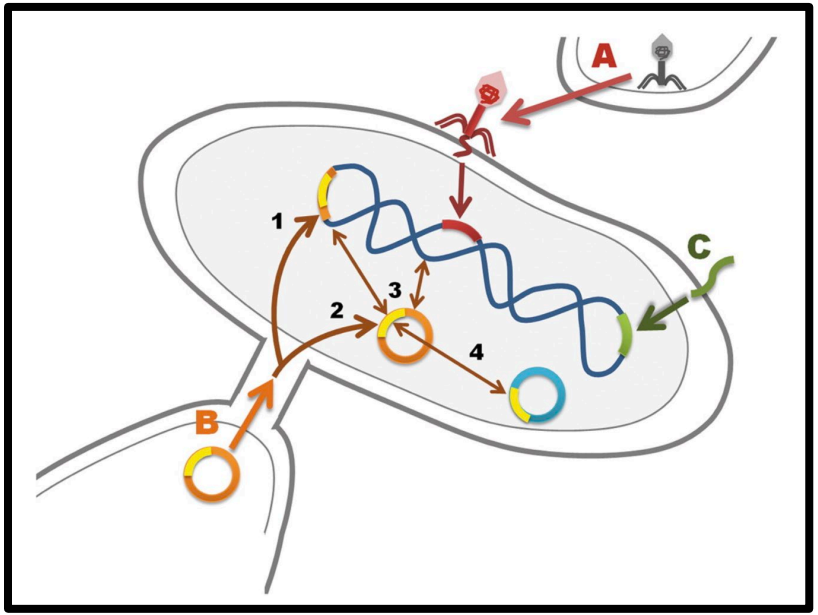
- Bacteria can evolve from non-pathogens to become pathogens through acquisition of new genetic material that enables them to colonize a host species and have detrimental effects on the host
- Bacterial evaluation occurs through mechanisms including natural selection and genetic drift and can result 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

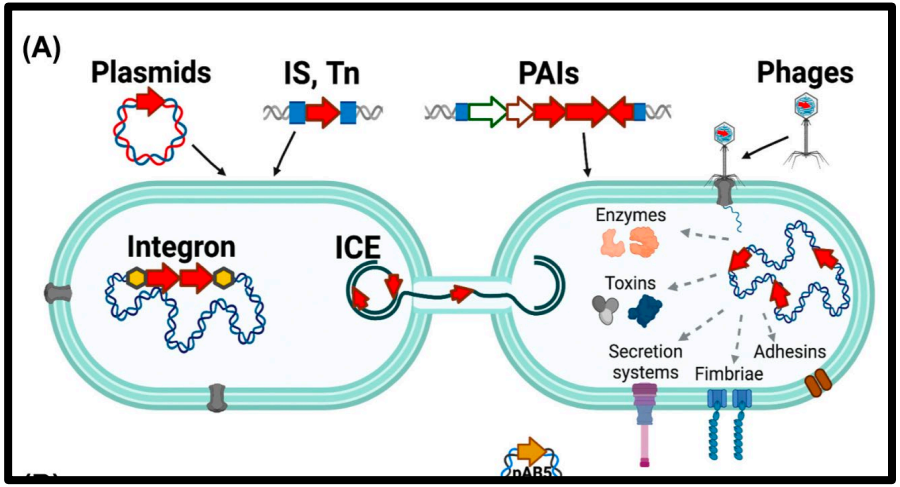
Genetic mechanisms in Bacterial Evolution

- Transfer of foreign DNA - Horizontal Gene Transfer (HGT) and Mobile Genetic Elements (MGE)
  - Transformation
  - Transduction,
  - Conjugation
- Virulence genes transferred via HGT include genes for bacterial adherence to host cells, type 3 secretion systems, toxins, iron acquisition, and antimicrobial resistance



Horizontal gene transfer (HGT) mechanisms and mobile genetic elements (MGEs) associated with pathogen emergence

- Horizontal gene transfer (HGT) is a fundamental driver of PE process
- Genes encoding pathogenic traits (red arrows) can be acquired by bacteria on various elements such as plasmids, insertion sequences (ISs), transposons (Tns), pathogenicity islands (PAIs), integrative and conjugative elements (ICEs), or bacteriophages (phages)



- These genes can encode virulence factors such as toxins, adhesins, or secretion systems, or help modulate the expression of virulence traits
- Integrons present in the chromosome enable successful integration and expression of exogenous virulence genes

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 non-phagocytic 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
Yersinia spp.	Pathogenic Islands, plasmids, Integrative and conjugative element	Siderophore, type III secretion system and effectors
Clostridium botulinum	Phage	Botulinum toxin

Gyles and Boerlin.2014

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

- Horizontal gene transfer via transformation, transduction and conjugation as well as Mobile Genetic Elements (MGE) are particularly important for the development of pathogenic microorganisms from non-pathogenic ancestor
- 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 unknown**

Summary and Final Thoughts

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
- Pathogenic microorganisms emerge from non-pathogenic ancestor by acquiring virulence genes via Horizontal gene transfer via transformation, transduction and conjugation as well as Mobile Genetic Elements (MGE) such as plasmids,phages, Integrative and conjugative elements and Pathogenic Islands.
- 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

Summary

- Making of a pathogen involve evolution process following Darwinian Principles of Evolution.
- Involves common mechanisms related to the acquisition of large blocks of virulence genes from a common microbial ancestor, which can be disseminated to other bacteria via horizontal transfer.
- Horizontal gene transfer via transformation, transduction and conjugation as well as Mobile Genetic Elements (MGE) are particularly important for the development of pathogenic microorganisms from non-pathogenic ancestor
- Evolution of Food Safety and Health: From Harvest to Table requires multifaceted approach including:
  - Advanced detection technologies and Early detection through enhanced surveillance and reporting
  - Strict regulatory standards and compliance
  - Consumer education, cooperation and communication

Final Thought

"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.

THANK YOU !!

Dr. Purnendu C. Vasavada
Purnendu.C.Vasavada@uwrf.edu

Any Questions?