Chapter 4: Introduction to host-bacteria interaction

aa 2020-21

100% Human?

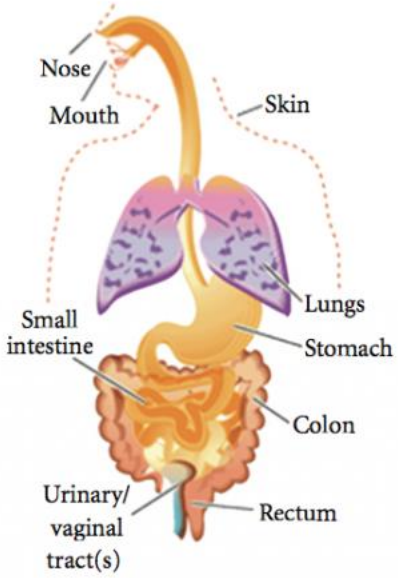
Bacteria are consistently associated with the body of animals.

Bacteria-human host is an ecosystem comprising 10^{14} microbs and only 10^{13} mammalian cells!

The bacteria and other microbes that are consistently associated with an host are called the **microbiota** (**microflora**) of the animal.

We are composed of several species:	• Eucaryotic • Bacterial • Archaea
As adults our microbial census exceeds the total number of our own human cells	• By about 10 fold
The largest collection of microbes resides within the intestine	• With 10^{13-14} cells!!!! • Several hundreds of species • «The GUT MICROBIOTA»

100 % Human ?



Human body 10^{13} cells 23,000 genes ⟷ Normal flora 10^{14} microbial cells on the human body. 3.3 million genes

Amount of bacteria per gram of cellular component

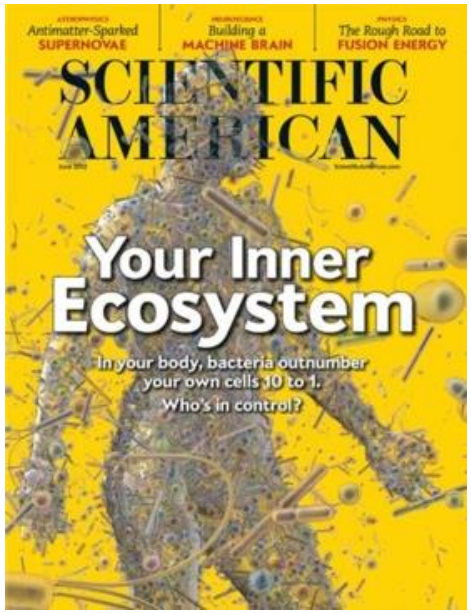
- Stomach – 10^{1} to 10^{2} cells
- Duodenum – 10^{3} cells
- Jejunum – 10^{4} cells
- Ileum 10^{4} to 10^{7} cells
- Proximal colon 10^{10} to 10^{11} cells
- Transverse colon 10^{11} to 10^{12} cells
- Distal colon $>10^{12}$ cells

In a healthy animal, the internal tissues, e.g. blood, brain, muscle, etc., are normally free of microorganisms. However, the surface tissues, i.e., skin and mucous membranes, are constantly in contact with environmental organisms and become readily colonized by various microbial species.

The Nature of Bacterial Host-Parasite Relationship in Humans

These bacteria have a full range of **symbiotic interactions** with their animal hosts:

- **mutualism:** both members of the association benefit
- **commensalism:** there is no apparent benefit or harm to either member of the association
- **parasitism:** one member grows, feeds and is sheltered on or in a different organism while contributing nothing to the survival of its host.



Interactions sometimes are not fully understood (neither for *E. coli*).

The host obtains from the normal microbiota:

1. Certain nutritional and digestive benefits (digestion complex sugars, synthesis of short chain FA, B12 and K vitamins detoxification of xenobiotics)
2. Stimulation, development and activity of immune system.
3. Protection against colonization and infection by pathogenic microbes.

Normal microbiota obtains from the host a warm, moist, nutrient-rich environment.

Dysbiosis: any perturbation of the normal microbiota content that could disrupt the symbiotic relationship between the host and associated microbes,

The human microbiota

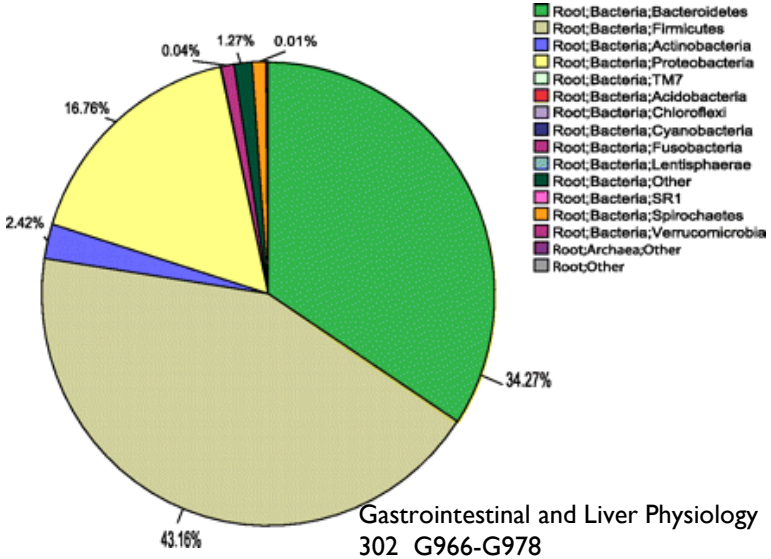
Microbial population consists of hundreds species. Bacteria are the most numerous but few eukaryotic fungi and protists are also present. Methods to characterize the human microbiota:

1) Lab cultivation

2) Nucleic acid-based approaches. PCR to amplify 16S RNA probes to detect the diversity of bacteria. Example: in dental plaque only 1-5% of the total species found have ever been cultivated. Similar observations have been made with the intestinal microbiota.

3) Massively parallel methods of DNA sequencing (metagenomics).

Intestine **microbiome**: about **60000 different genes** representative of 1000 bacterial species have been sequenced. 3-4 phyla are dominant: Firmicutes, Bacteroidetes Proteobacteria and Actinobacteria on 14-15 total phyla detected.



Gastrointestinal and Liver Physiology 2012 Vol. 302 G966-G978

Pie chart pyrosequencing data analyzed at the phylum level. Uncommon phyla that are a very small fraction of the total are not visible in the chart but they are present in the legend.

Structure, function and diversity of the healthy human microbiome

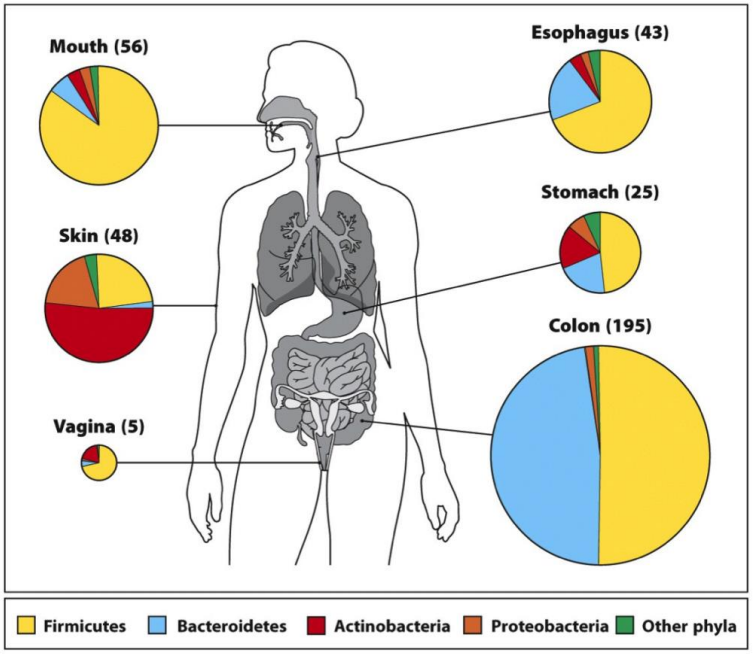
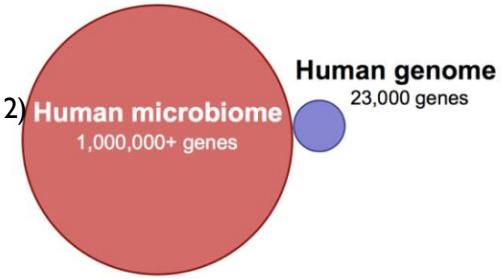


Figure 12.3 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

Human Microbiome Project (HMP) aimed to decrypt the whole genomes of all micro-organisms that live in our body (our **microbiota**).

Nature 486, 207–214 (14 June 2012)

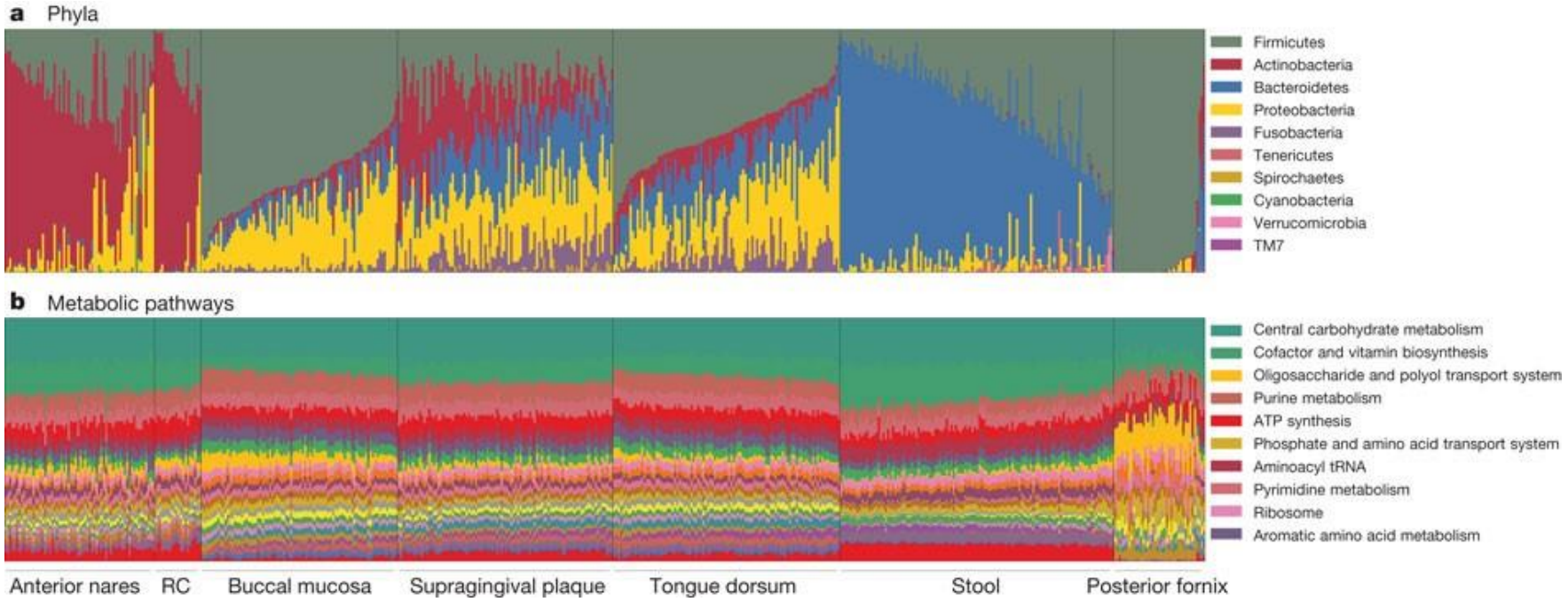


A total of 4,788 specimens from 242 healthy adults were available for this study

- diversity and abundance of each habitat's signature microbes to vary widely even among healthy subjects, with strong niche specialization both within and among individuals.
- Diet, environment, host genetics and early microbial exposure have all been implicated in individual diversity
- Metagenomic carriage of metabolic pathways (metabolome) was stable among individuals despite variation in community structure

Carriage of microbial taxa varies while metabolic pathways remain stable within a healthy population

- Diet, environment, host genetics and early microbial exposure have all been implicated in individual diversity
- Metagenomic carriage of metabolic pathways (metabolome) was stable among individuals despite variation in community structure

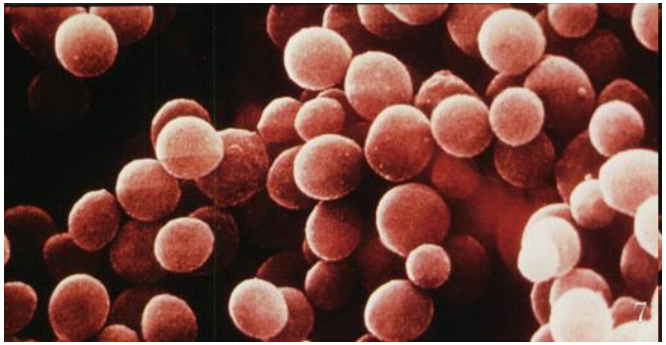


bars indicate relative abundances colored by microbial phyla from binned OTUs (**a**) and metabolic modules (**b**).

Pathogens and pathogenesis

These harmless relationships may break down and can lead to a situation in which the prokaryotes gain at the expense of the eukaryotes: they become parassites.

A **pathogen** is a microorganism (bacterium or virus, fungus or protist) that is able to damage its host:
e.g. the interruption of normal tissue structure and/or function of the host that applies at the cellular (necrosis, apoptosis, synaptic blockage), tissue (granulomatous inflammation, fibrosis), and organ (ductal obstruction) levels producing a disease.



S. aureus, likely the most prevalent pathogen of humans, may cause up to one third of all bacterial diseases

Pathogenicity is the ability to produce damage in a host organism under certain conditions.
Virulence a term which refers to the degree of pathogenicity of the microbe, that is, the comparative ability to cause damage,

Virulence factors: specific components of a pathogen that contributes to virulence

Primary and secondary pathogens

Pathogens can be classified as either primary or secondary (also known as opportunistic) pathogens (accordingly to https://www.cdc.gov/).

Primary pathogens infect a normally healthy body. They normally do not associate with their host except in the case of disease. When this occurs, the result is a parasitic relationship in which the prokaryote exogenous pathogen causes damage to its host.

Several members of the normal microbiota are potential pathogens: **opportunistic pathogens (secondary pathogens).** They cause disease in their host when they have an opportunity to do it.

Primary pathogens

- *Clostridium tetani* *
- *Legionella pneumophila**
- *Bacillus Anthracis**
- *Yersinia spp.**
- *Vibrio cholerae**
- *Salmonella typhi*
- *Brucella spp.**
- *Mycobacterium tuberculosis*
- *Corynebacterium diphtheriae*
- *Neisseria Gonorrhoeae*
- *Shigella spp.*
- *Listeria monocytogenes**
- *Bordetella pertussis*

*Accidental pathogens: non associated with humans but with animals or soil or water.

opportunistic pathogens

- *Staphylococcus aureus*
- *Enterococcus faecalis*
- *Streptococcus pneumoniae*
- *Neisseria meningitidis*
- *Enterobacteriaceae*
- *E. coli (some strains)*
- *Klebsiella pneumoniae*
- *Haemophilus influenzae*
- *Pseudomonas aeruginosa**

Opportunities:

- Weakness in the host's anatomical barriers: damage of the epithelium, presence of foreign body.
- Suppression of immune system (drugs or radiation), co-infections of an exogenous pathogen.
- Alterations of microbiota (e.g. by antibiotics).

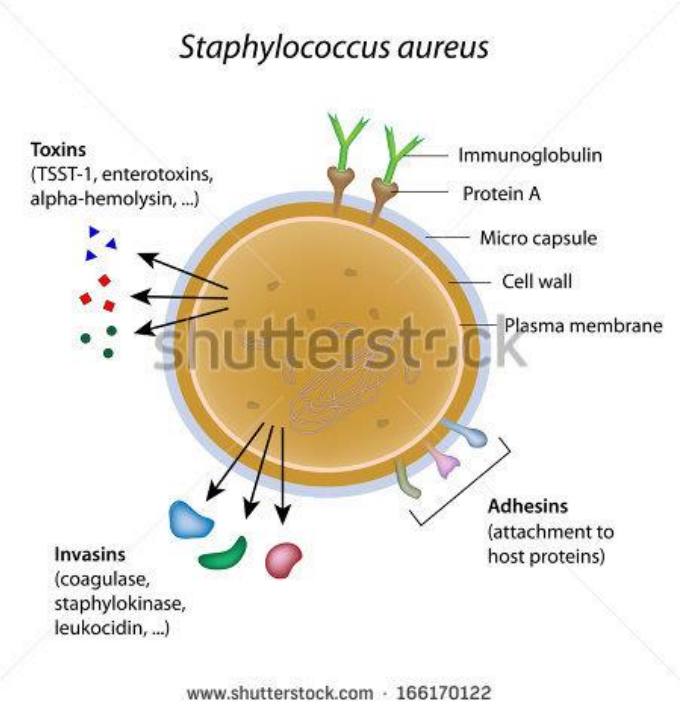
Virulence factors and multifactorial nature of virulence

Genes that contribute to the ability of an organism to become virulent are **virulence genes** and their product are **virulence factors.**

Few pathogens have a main single gene of virulence: E.g. *Clostridium tetani* and *Corynebacterium diphtheriae*, are able to produce disease, the symptoms of which depend on a single genetic trait: the ability to produce a toxin.

Most pathogens possess **a large repertoire virulence genes**. They are able to produce a more complete range of diseases that affect different tissues in their host.

Bacterial virulence factors could be classified into different groups:



1. Factors are related to the **invasiveness**: adhesins, invasins (exoenzymes), impedins (capsule).

2. Factors related to **toxigenesis:** aggressins (toxins), modulins (effector proteins).

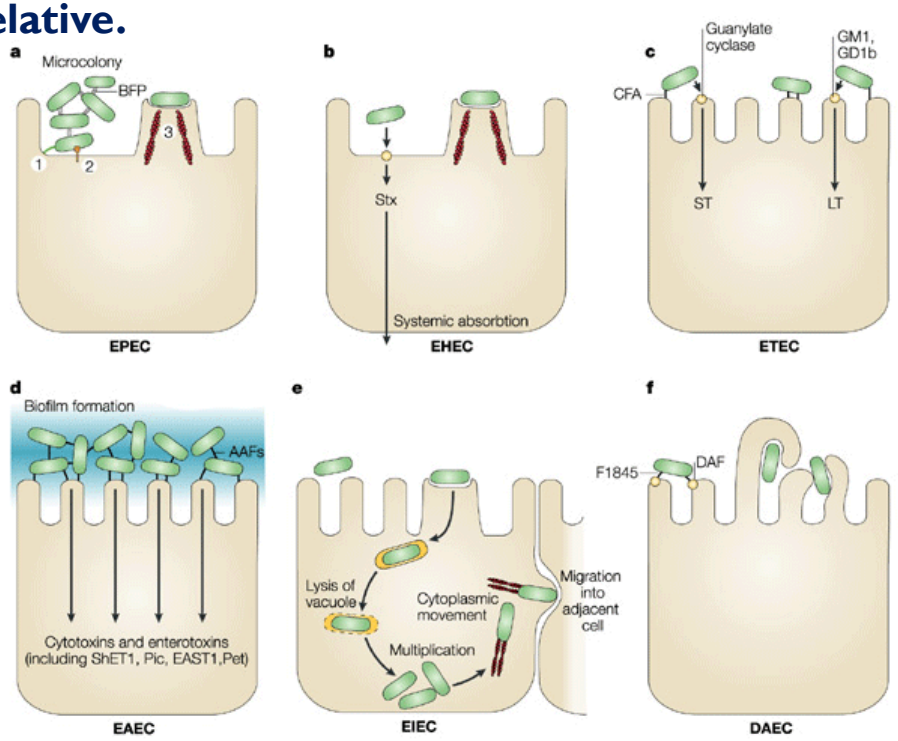
Different types (virotypes) of Escherichia coli

A relatively small number of genes causes the significant differences between a virulent pathogenic bacterium and its closest **non-pathogenic relative.**

As a pathogen, *E. coli* is best known for its ability to cause intestinal diseases (diarrheagenic virotypes).
Different classes (**virotypes, or pathotypes**) of diarrheagenic *E. coli* are now recognized:

enteropathogenic *E. coli* (EPEC)
enterohemorrhagic *E. coli* (EHEC)
enterotoxigenic *E. coli* (ETEC)
enteroaggregative *E. coli* (EAEC)
enteroinvasive *E. coli* (EIEC)
Diffusely adherent E. coli (DAEC)

Uropathogenic *E. coli* (UPEC): non-diarrheagenic E. coli causing 90% of the urinary tract infections (UTI)

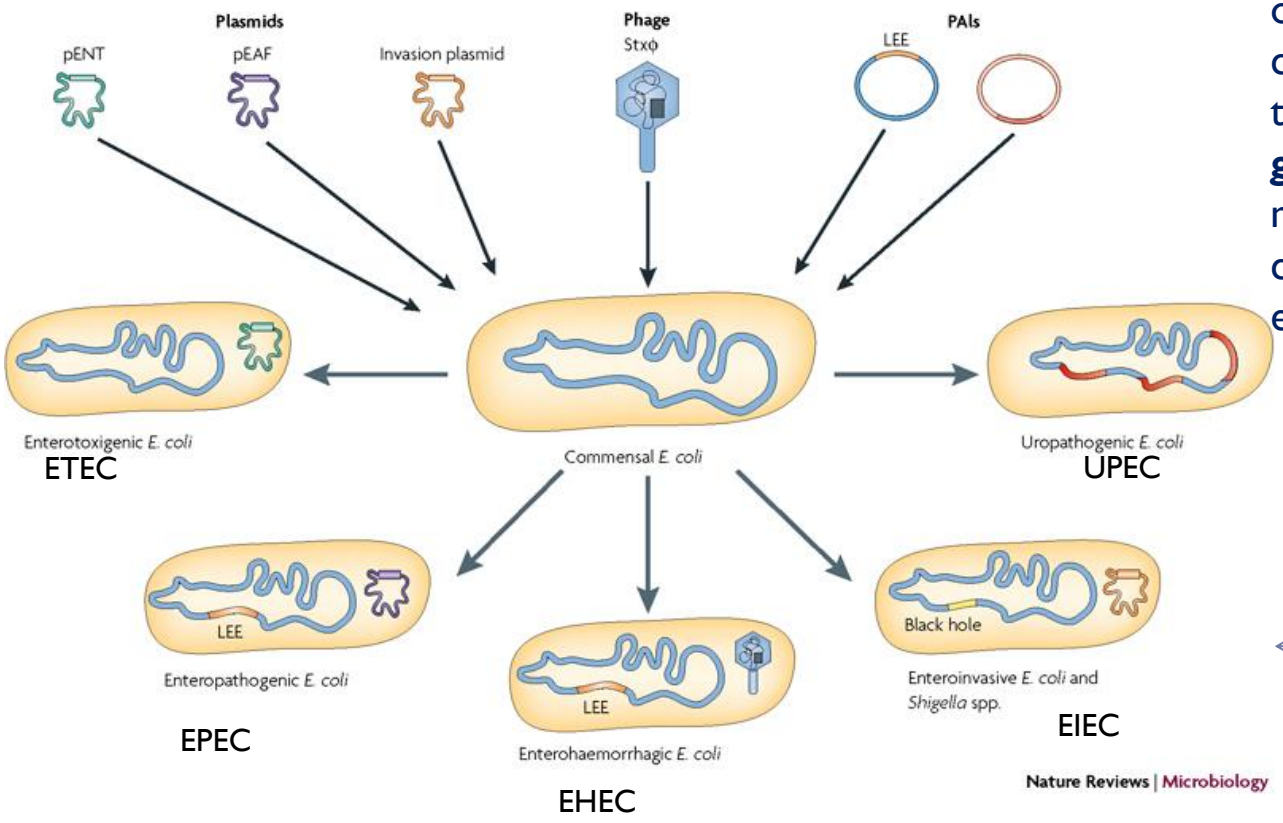


Gut epithelia damaged by different diarrheagenic *E. coli* virotypes.

These *E. coli* virotypes differ regarding their preferential host colonization sites, virulence mechanisms, and the resulting clinical symptoms and consequences.

Acquisition of virulence genes

Virulence genes are frequently clustered together, either in groups on the bacterial chromosome called ***pathogenicity islands*** or on extrachromosomal ***virulence plasmids.*** These genes may also be carried on mobile ***bacteriophages*** (bacterial viruses).



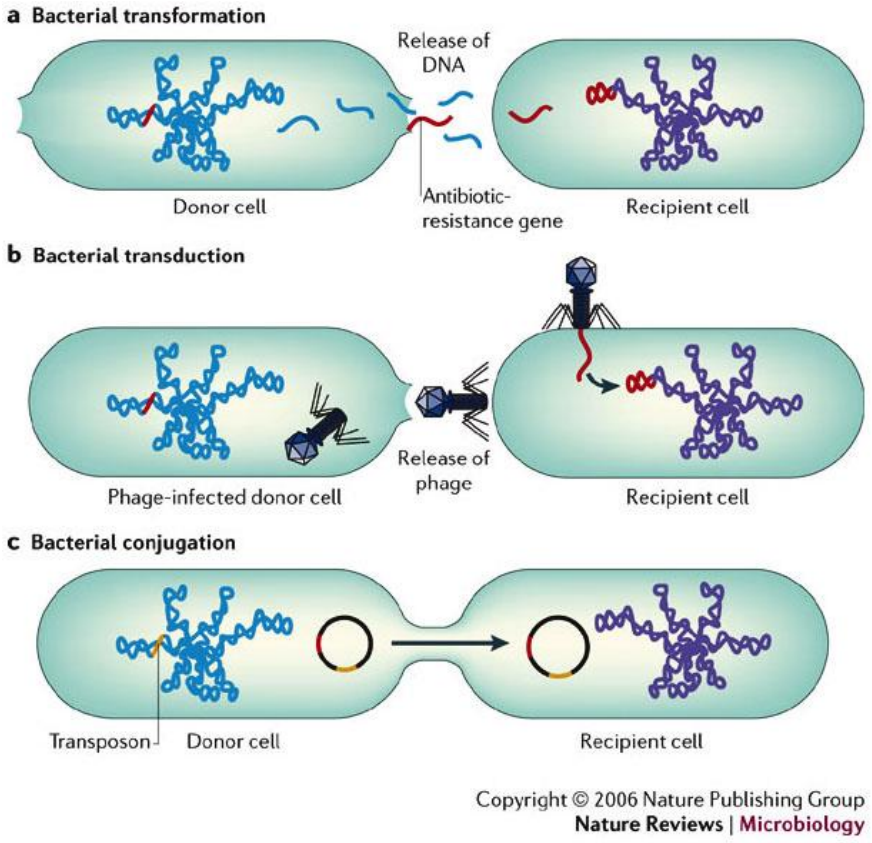
Acquisition of large pieces of DNA and other large chromosomal changes is the results of ***horizontal gene transfer (HGT)*** mechanisms that have contributed to bacterial evolution

Genetic differences between pathogenic and nonpathogenic *E. coli*

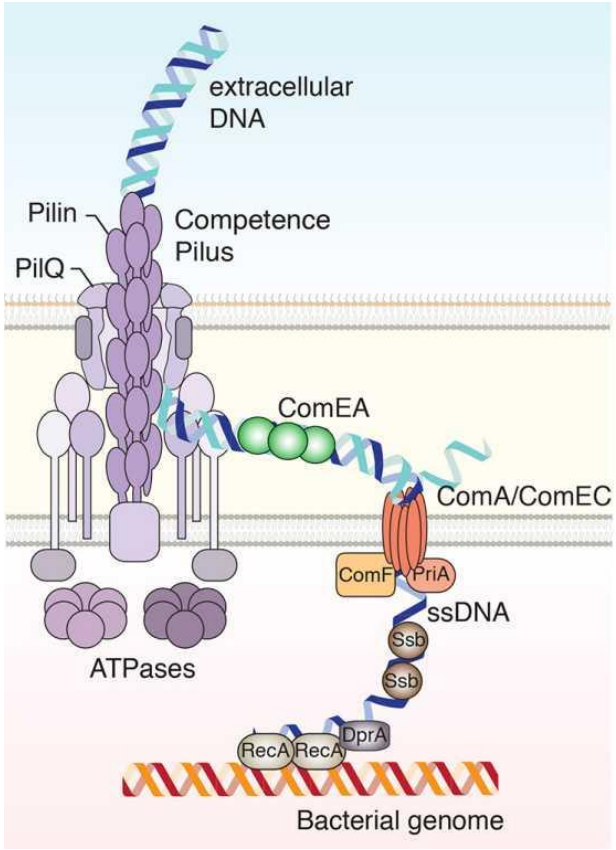
Horizontal gene transfer (HGT)

Horizontal gene transfer: movement of genetic material between bacteria other than by descent in which information travels through the generations as the cell divides. HGT mechanisms allow the exchange and acquisition of news genes representing a potent strategy for the bacteria to adapt to new environments

There are at least three mechanisms of HGT, equivalent to the three processes of genetic exchange in bacteria. These are **transformation conjugation** and **transduction.**



DNA uptake and transformation by competent Gram-negative bacteria



Natural transformation involves the uptake of extracellular DNA, its transport into the cytoplasm and its integration into the bacterial genome by homologous recombination.
Transformation is a highly regulated process. DNA uptake sequences serve as recognition sites for binding and uptake

- dsDNA is bound at the cell surface.
- DNA is pulled through the a secretin pore by retraction of a Type IV-like pilus. One strand is translocated intact into the cytoplasm by a complex protein; the other is degraded.
- The new strand recombines with a homologous sequence in the chromosome, displacing the resident strand.

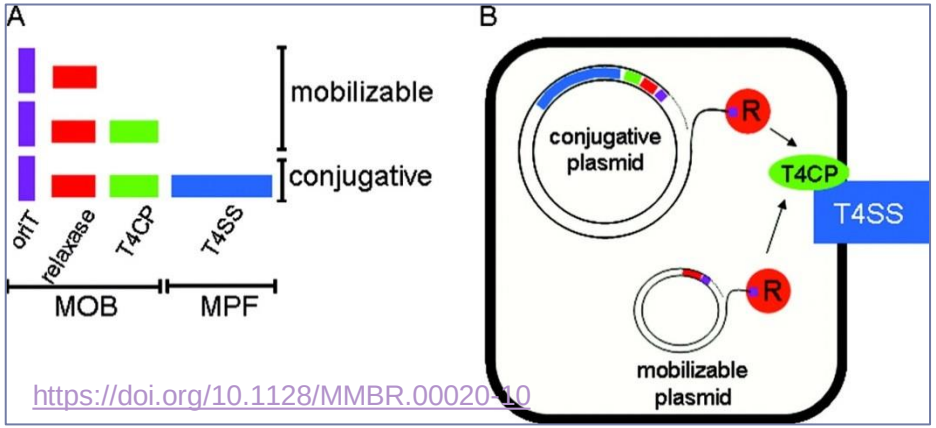
https://hospenthalgroup.ethz.ch/research/projects.html

Mobile genetic elements employed in conjugation: conjugative plasmids and transposones

DNA from plasmids and transposones are transferred by conjugation. Interspecies transfer is likely possible.(emerging of antibiotic resistance).

Natural occurring plasmids are **self-transmissible:** they possess conjugation genes known as **tra** genes, relaxase and *oriT* (sequences for initiation of DNA transfer) enable the bacterium to conjugate with another bacterial cell.

Other plasmids are **mobilizable plasmids:** they have *oriT* but lack the *tra* genes, it needs help to move its DNA.

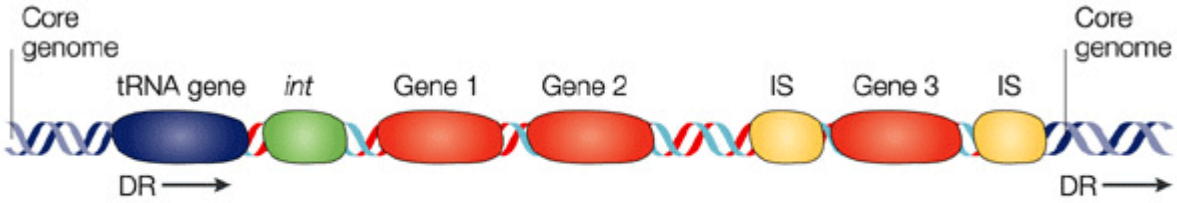


Self-transmissible or conjugative plasmids code for the four components of a conjugative apparatus : oriT (violet), a relaxase (red) a typel V coupling protein (T4CP) (green) and a type IV secretion system (T4SS) (blue).

Complex transposons (Tn3, Tn5 Tn10 etc) consist of two IS and other selectable genes in the middle. Some of them are **conjugative transposons,** have **tra** genes and promote transfer of their DNA and can promote transfer of their own DNA.

Genomic islands, DNA segments differing between closely related bacterial strains

Genomic islands (GEI): discrete DNA segments differing between closely related bacterial strains which are horizontally acquired DNA regions and that are chromosomally inserted. GEIs show a large variety of sizes and abundance in bacterial genomes. Different GEI families have been recognized on the basis of predicted sequence and functional homologies.



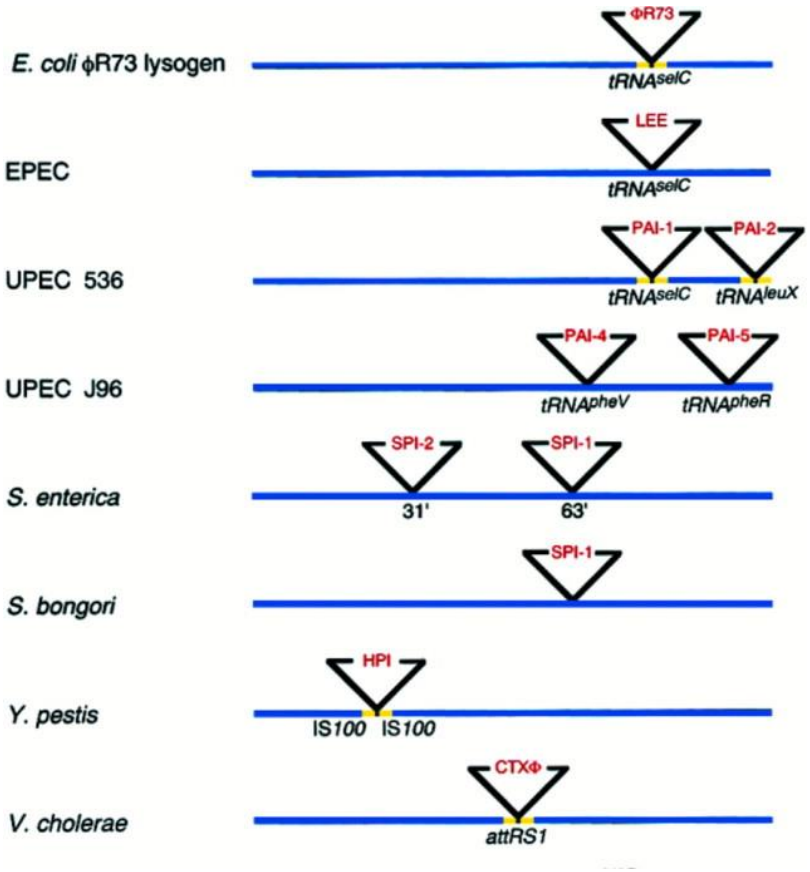
Nature Reviews Microbiology 2, 414-424 (May 2004)

Nature Reviews | Microbiology

A typical GEI is flanked by direct repeat (DR) structures and carries genes encoding traits that may increase bacterial adaptability or fitness under certain growth conditions including such traits as symbiosis, metabolic capability, antibiotic resistance, and virulence.

GEIs carry multiple functional and fragmented insertion sequence (IS) elements and other mobility-related genes, (integrase, int gene), involved in insertion and deletion of the DNA.

Pathogenicity islands (PAIs) insertion in different pathogens



Volume 87, Issue 5, p791–794, 29 November 1996

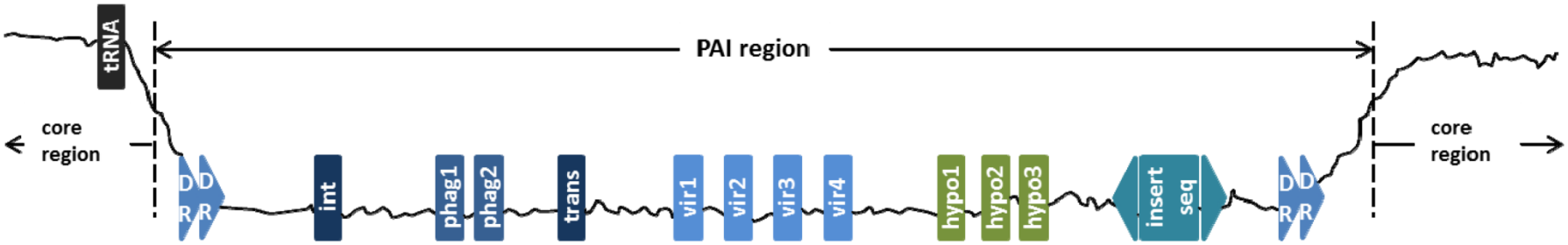
PAIs are distinct regions of DNA that are present in the genome of pathogenic bacteria but absent in non-pathogenic strains of the same or related species. PAI have a length of 10-200 kb, and these insertions may constitute 10-15 % of the genome.

PAIs are mostly inserted in the backbone genome of the host strain in specific sites: tRNA genes as phage attachment site or IS.

Comparison of a large number of genome sequences has revealed the importance of PAIs in the diversification of strains within a single species.

Characteristics of pathogenicity islands (PAI)

The PAI region has biased sequence composition (G+C % content) and often a different codon usage (used to identify new PAI).



They have boundaries determined by direct repeats DRs.

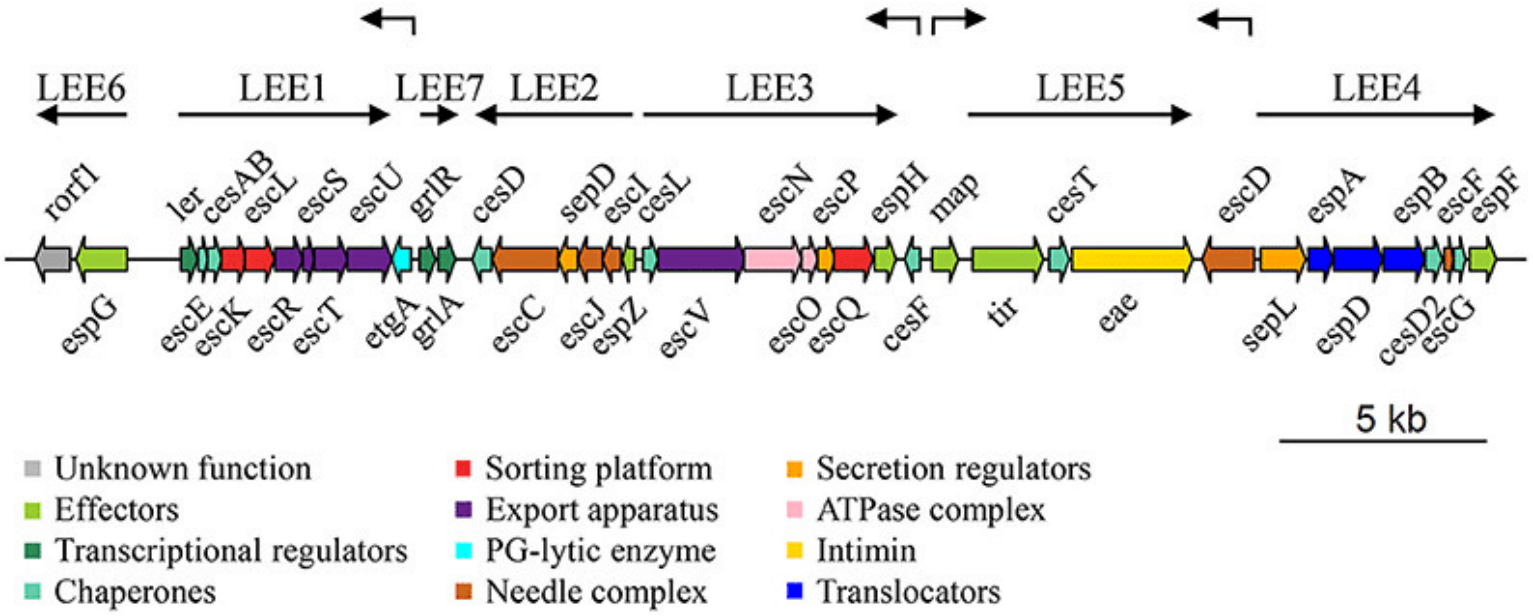
PAIs contain **mobility genes**, complete or defective IS elements (some mobile genetic elements seem to be potentially mobilizable), genes linked to virulence (vir) phage-related genes (phag) and other protein-coding genes.

They could the originated by lysogenic phages integrated into the bacterial chromosome (prophages) that have lost by mutation the ability to undergo a lytic phase. Defective prophages (dormant) can still express many of their virulence genes. They have high potential for generating new pathogens in relatively short period of time.

The genomic organization of a PAI

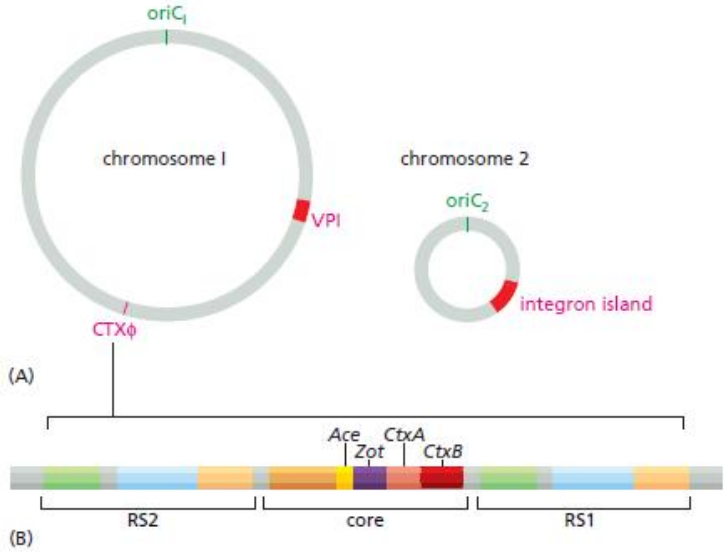
PAIs may include genes that confer a variety of new functions: new iron uptake systems, different adhesins, different toxins, second-messenger pathway toxins, secreted lipases and proteases, type I, III, IV, and V protein secretion systems, antibiotic resistance. Example:

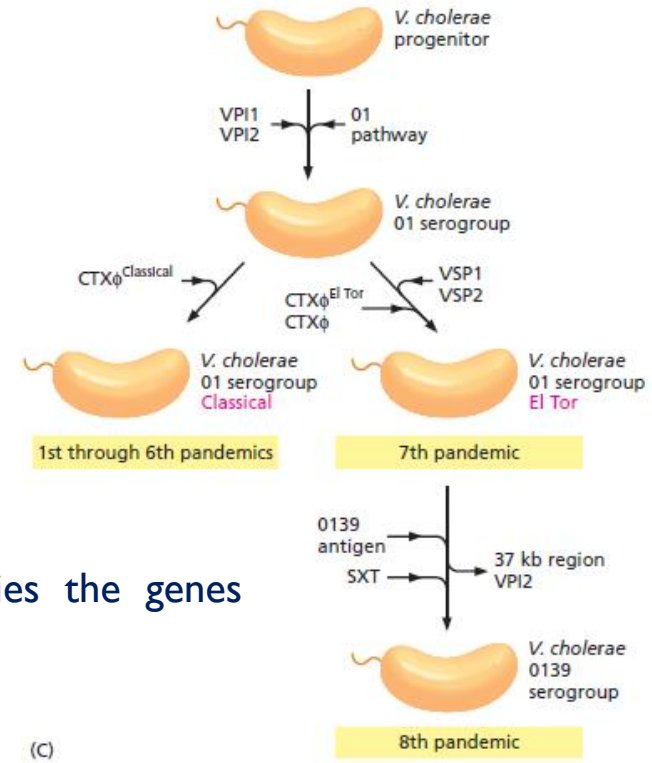
The Locus of Enterocyte Effacement (**LEE**) has been described in the *E. coli* EPEC strain, causative agent of infant diarrhoea and in E. coli EHEC.
LEE contains **≈40 ORFs** and is organized into polycistronic operons.



Evolution of a pathogen: acquisition of virulence genes

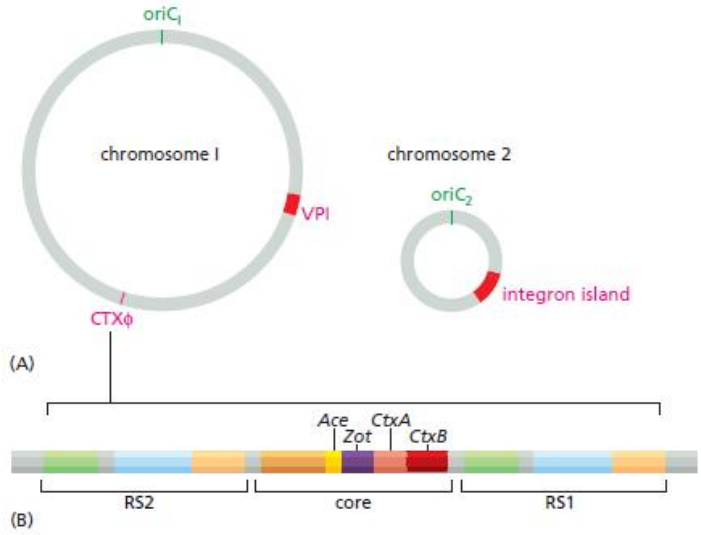
Acquisition of genes and gene clusters can drive the rapid evolution of pathogens and turn non pathogens into pathogens. *Vibrio cholerae: a* Gram-negative bacterium that causes the epidemic diarrheal disease cholera.

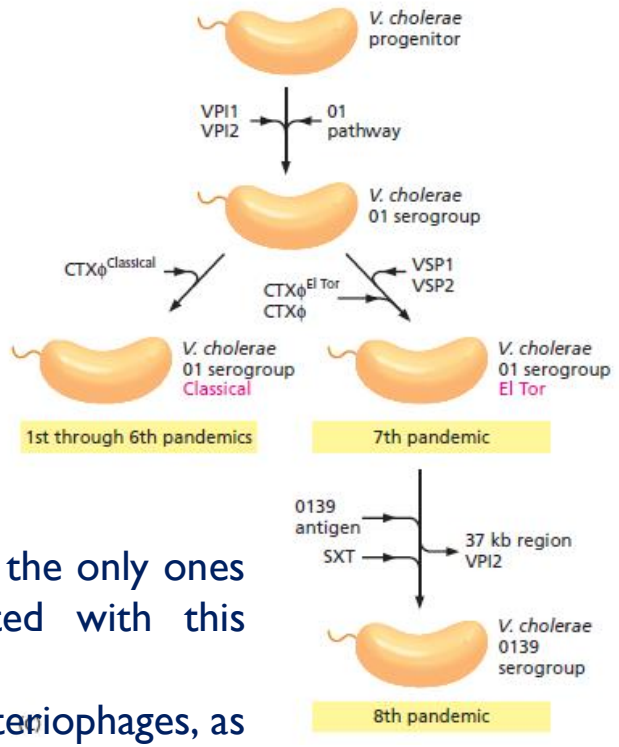




CTXΦ: an integrated bacteriophage genome that carries the genes encoding cholera toxin(CtxA and CtxB).
O1 and O139 = primary carbohydrate surface antigen
VPI, VSP (pathogenicity islands)

Comparative-genomics-based model for the evolution of pathogenic V. cholerae strains





Of all the *Vibrio cholerae* strains found in lakes in the wild, the only ones that cause pandemic human disease are those infected with this **bacterial virus** (pandemics 1-6) : classical strains.

A new strain "El Tor", appeared when it acquired two bacteriophages, as well as at least two new pathogenicity islands not found in Classical strains.

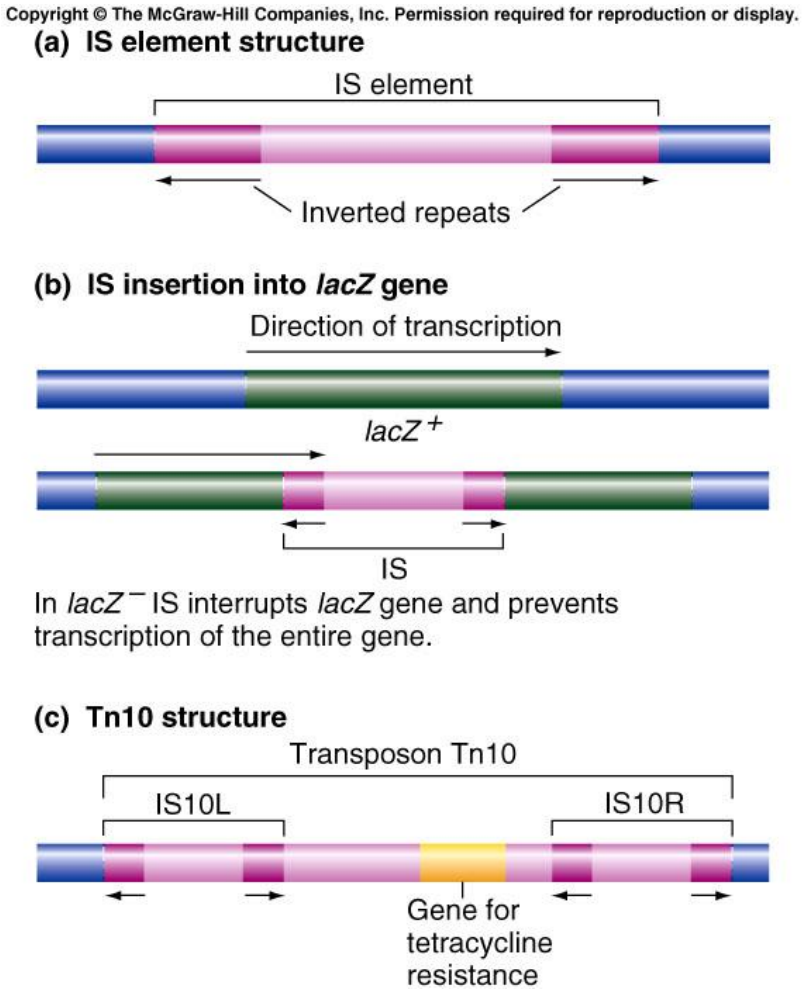
In 1991, an **eighth pandemic began**, even people who had suffered cholera previously were not immune, as the new strain had a different type of O antigen, rendering the anti-O1 antibodies present in the blood of survivors of previous cholera epidemics ineffective against the new strain.

Slides aggiuntive per chi non conosce questi argomenti (non in programma d’esame)

General structure of a IS element and of a transposon

DNA transposons: DNA element that can move (transpose) from one place in DNA to another.

Smallest one: Insertion sequence (IS) contains a **transposase gene** and inverted-repeat sequences at their ends used to target IS sites in the target DNA. More complex transposons (Tn3, Tn5 Tn10 etc) consist of two IS and other selectable genes in the middle. Some of them are **conjugative transposons,** have **tra** genes and promote transfer of their DNA and can promote transfer of their own DNA.



Mechanism of DNA transposition by the Tn5 transposon

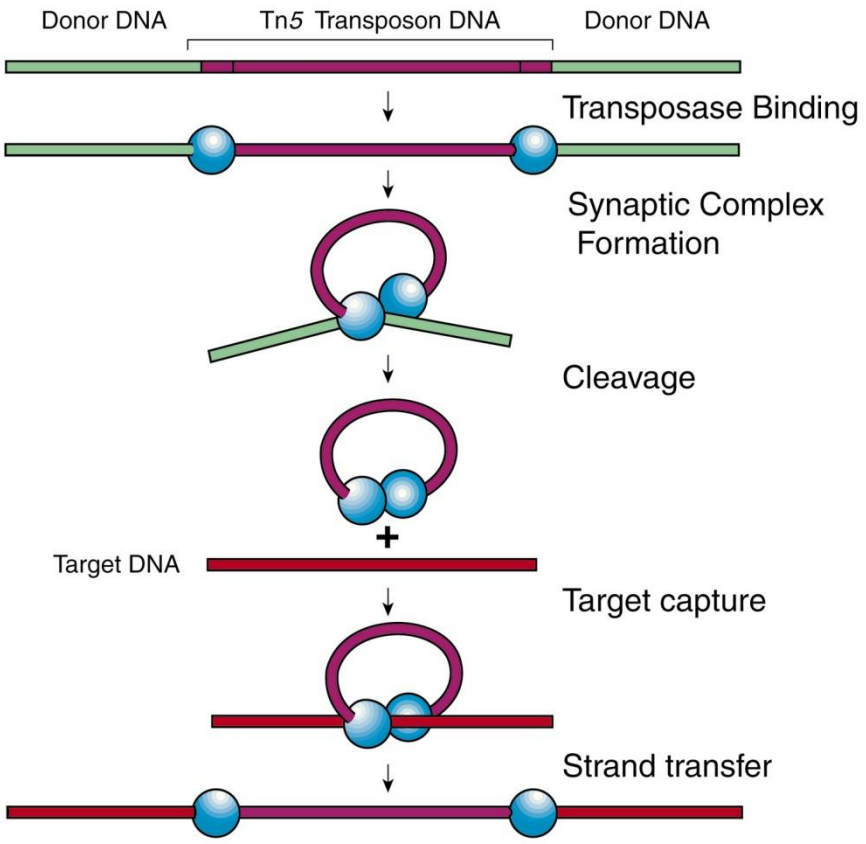
Model of cut-and-paste transposition.

Transposase catalyzes the excision of the element and its insertion at a new target site. Individual molecules of transposase (blue spheres) bind to specific sites at the ends of the transposon.

Looping of the transposon DNA results in formation of a synaptic complex that brings the two ends of the transposable element close together.

The Tn5 transposase cuts the transposon DNA away from the flanking "donor" DNA (green).

The Tn5 transposase/DNA complex can move about freely until it encounters and binds to the "target" DNA (red), the transposase catalyzes insertion of the transposon DNA into the target DNA



Schematic illustration of the mechanism of transposition catalyzed by the Tn5 transposase

Phage Transduction

DNA exchange between cells from bacteriophages are mediated by **DNA transduction.** Some lysogenic bacteriophages carry genes for toxins or other virulence genes.
Generalized transduction: phage can accidentally transfer pieces of bacterial DNA during the lytic phase. New sequences are incorporated by recombination.
Specialized transduction: it occours in phage that undergo both lytic and lysogenic phases in their life cycles.
Phage moves their own phage genes but sometimes can also package segments of DNA that flank the phage attachment site.

Sometimes integrated lysogenic phages (prophages) may mutate and lose the ability to undergo a lytic phase