

Mechanisms of Infectious Disease • Fall 2008

Lecture 2

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Genetic Basis of Variation in Bacteria

- I. Organization of genetic material in bacteria
 - a. chromosomes
 - b. plasmids
- II. Genetic variation: Source
 - a. point mutations
 - b. DNA rearrangements
- III. Genetic variation: Transmission
 - a. transformation
 - b. transduction
 - c. conjugation
- IV. Genetic variation: Implications for pathogenesis

Genetic Basis of Variation in Bacteria

I. Organization of genetic material in bacteria

- a. chromosomes
- b. plasmids

II. Genetic variation: Source

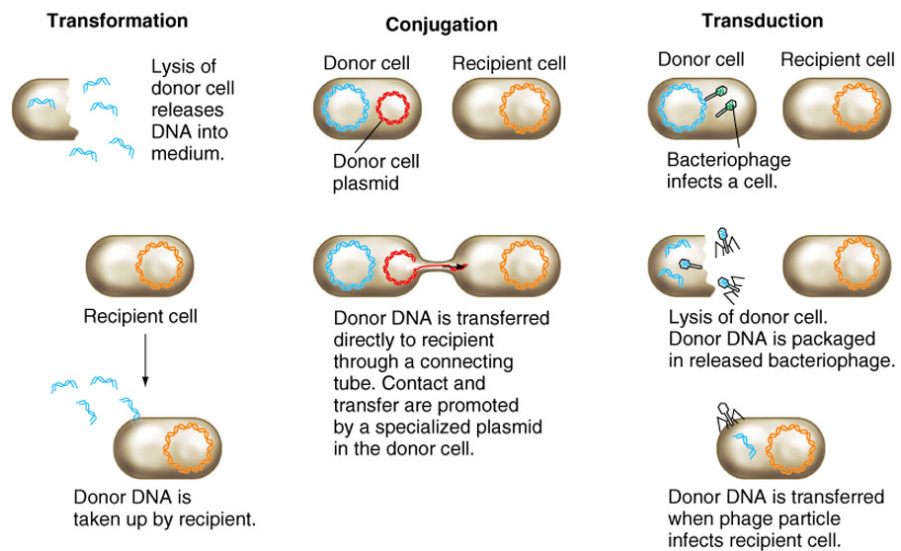
- a. point mutations
- b. DNA rearrangements

III. Genetic variation: Transmission

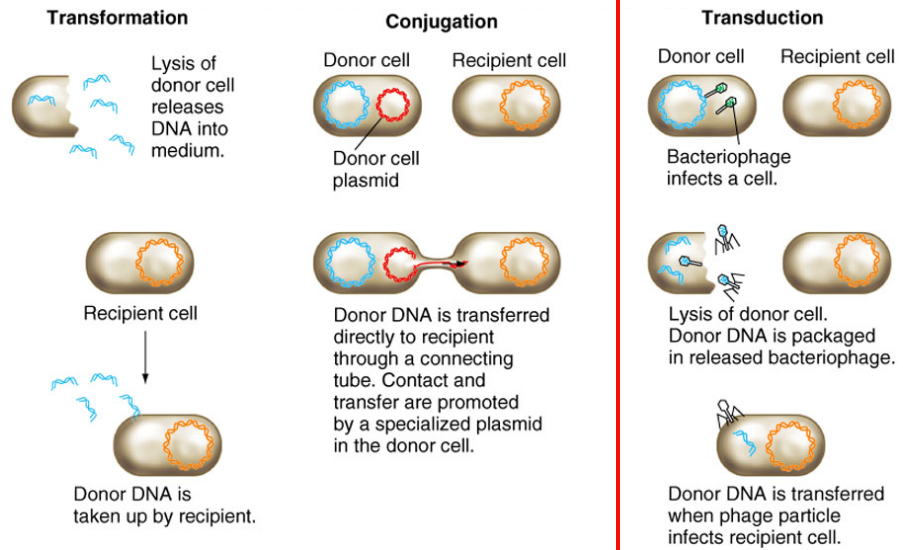
- a. transformation
- b. transduction
- c. conjugation

IV. Genetic variation: Implications for pathogenesis

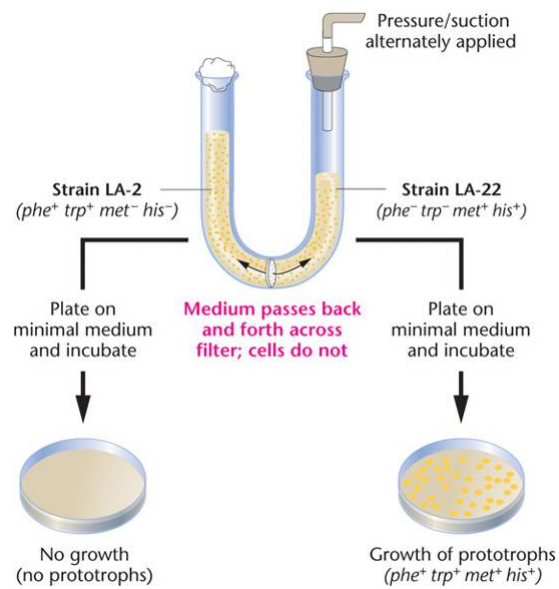
Transmission of genetic variation



Transmission of genetic variation: **transduction**



Transmission of genetic variation: **transduction**

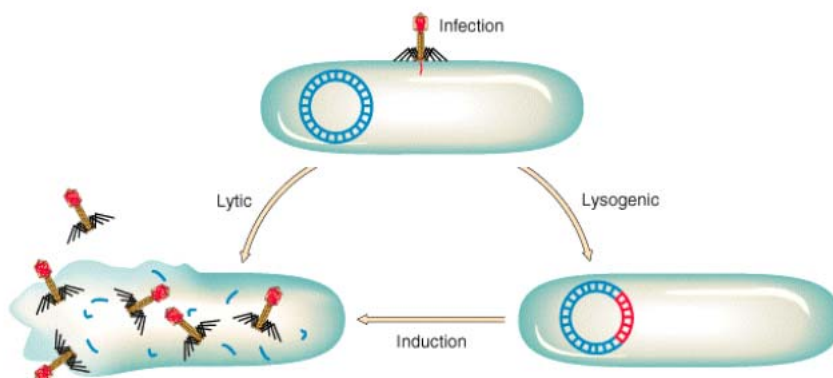


Transmission of genetic variation: **transduction**

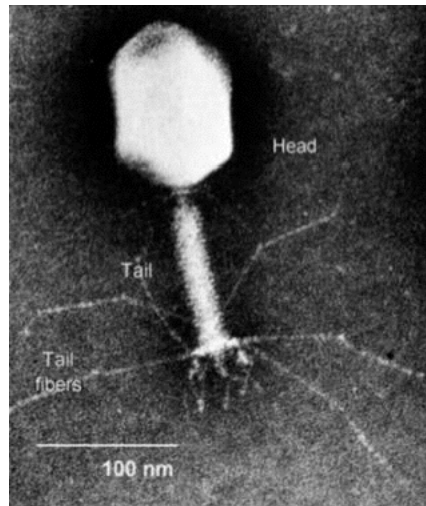
How did Zinder and Lederberg prove that the phenotype was the result of transduction?

- presence of DNAase rules out transformation
- filter prevented contact so no conjugation
- reducing filter pore size to below size of phage inhibited

Transmission of genetic variation: **transduction**



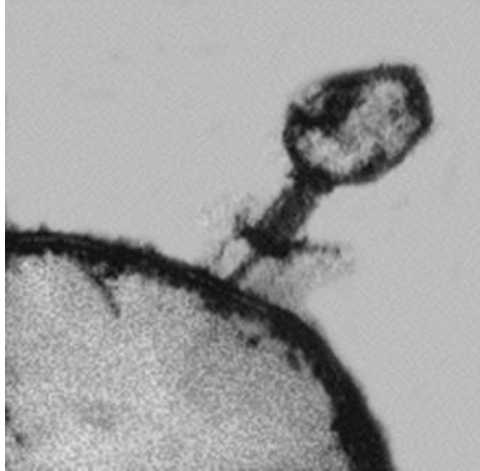
Transmission of genetic variation: **transduction**



Transmission of genetic variation: **transduction**



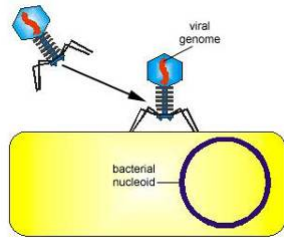
Transmission of genetic variation: **transduction**



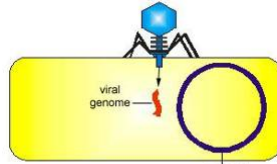
Transmission of genetic variation: **transduction**

- There are two types of transduction:
 - **generalized transduction**: A DNA fragment is transferred from one bacterium to another by a [lytic bacteriophage](#) that is now carrying donor bacterial DNA due to an error in maturation during the lytic life cycle.
 - **specialized transduction**: A DNA fragment is transferred from one bacterium to another by a [temperate bacteriophage](#) that is now carrying donor bacterial DNA due to an error in spontaneous induction during the lysogenic life cycle

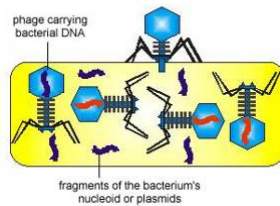
Transmission of genetic variation: **generalized transduction**



1. A **lytic bacteriophage** adsorbs to a susceptible bacterium.

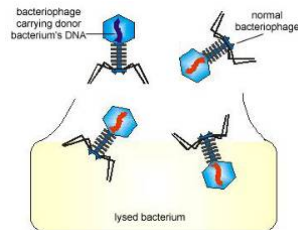


2. The bacteriophage genome enters the bacterium. The genome directs the bacterium's metabolic machinery to manufacture bacteriophage components and enzymes

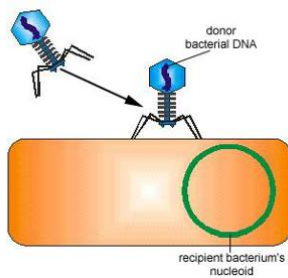


3. Occasionally, a bacteriophage head or capsid assembles around a fragment of donor bacterium's nucleoid or around a plasmid instead of a phage genome by mistake.

Transmission of genetic variation: **generalized transduction**

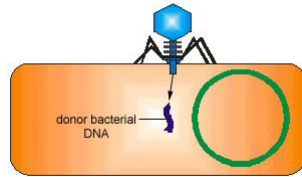


4. The bacteriophages are released.

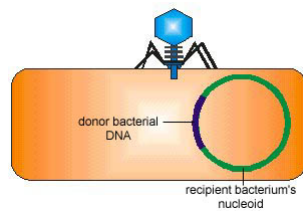


5. The bacteriophage carrying the donor bacterium's DNA adsorbs to a recipient bacterium

Transmission of genetic variation: **generalized transduction**



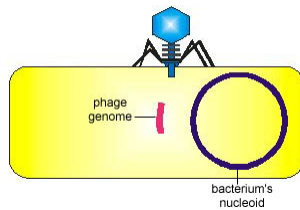
6. The bacteriophage inserts the donor bacterium's DNA it is carrying into the recipient bacterium .



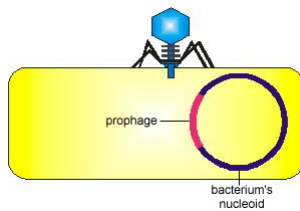
7. The donor bacterium's DNA is exchanged for some of the recipient's DNA.

<http://www.cat.cc.md.us/courses/bio141/lecguide/unit4/genetics/recombination/transduction/transduction.html>

Transmission of genetic variation: **specialized transduction**

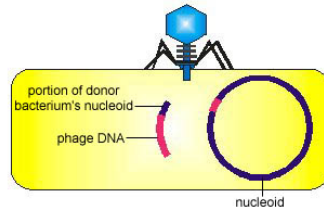


1. A temperate bacteriophage adsorbs to a susceptible bacterium and injects its genome .

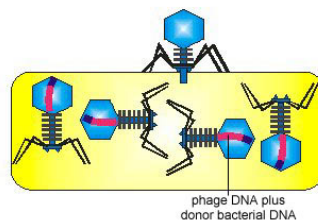


2. The bacteriophage inserts its genome into the bacterium's nucleoid to become a prophage.

Transmission of genetic variation: **specialized transduction**

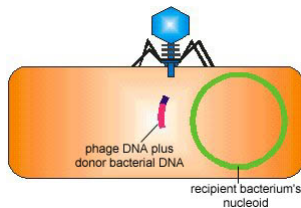


3. Occasionally during spontaneous induction, a small piece of the donor bacterium's DNA is picked up as part of the phage's genome in place of some of the phage DNA which remains in the bacterium's nucleoid.

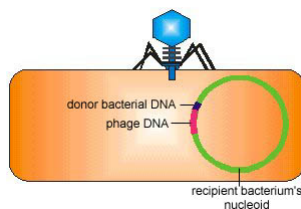


4. As the bacteriophage replicates, the segment of bacterial DNA replicates as part of the phage's genome. Every phage now carries that segment of bacterial DNA.

Transmission of genetic variation: **specialized transduction**



5. The bacteriophage adsorbs to a recipient bacterium and injects its genome.

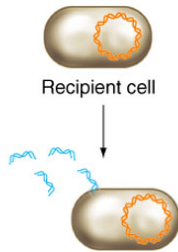
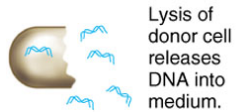


6. The bacteriophage genome carrying the donor bacterial DNA inserts into the recipient bacterium's nucleoid.

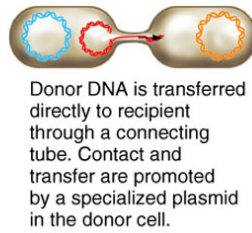
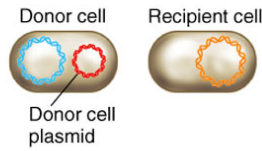
<http://www.cat.ec.edu/courses/bio141/lecguide/unit4/genetics/recombination/transduction/spectran.html>

Transmission of genetic variation: mechanisms

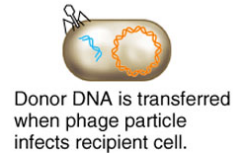
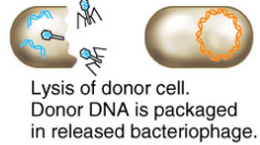
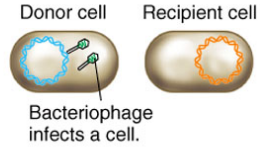
Transformation



Conjugation

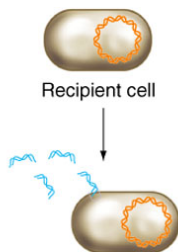
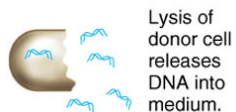


Transduction

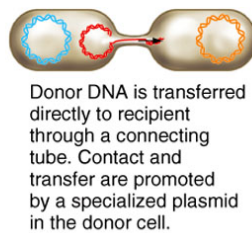
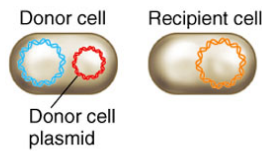


Transmission of genetic variation: conjugation

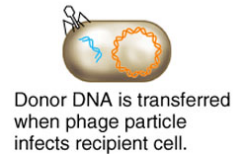
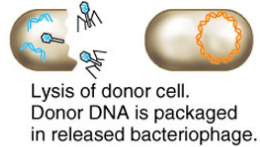
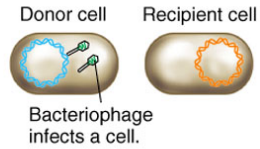
Transformation



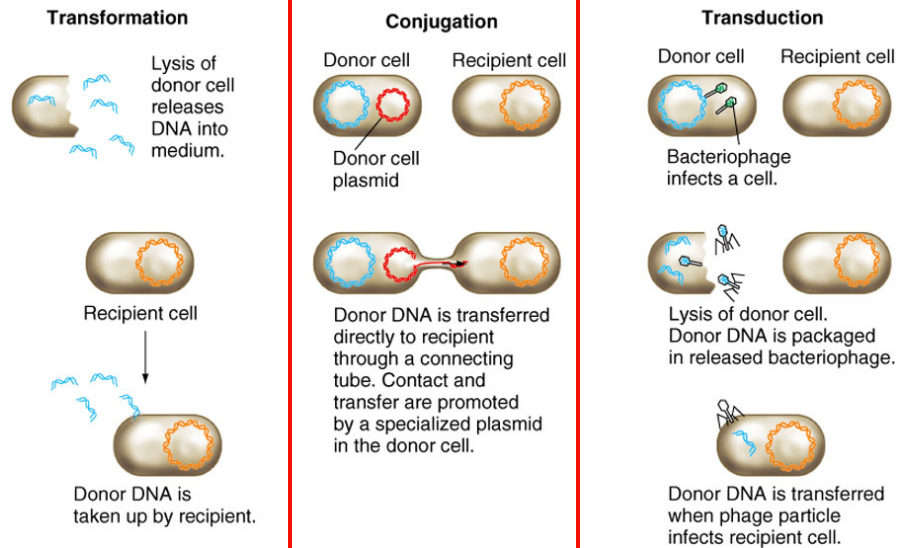
Conjugation



Transduction

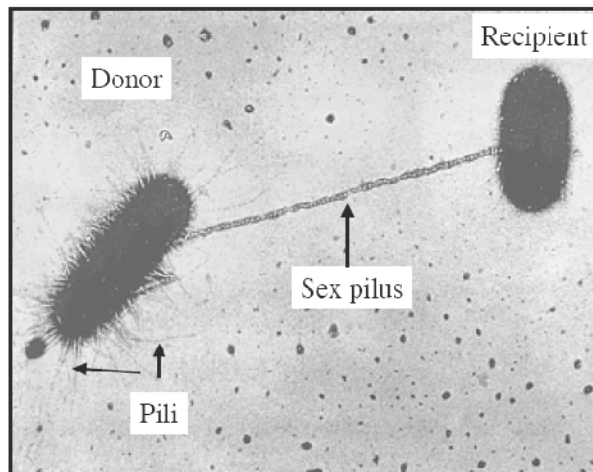


Transmission of genetic variation: conjugation

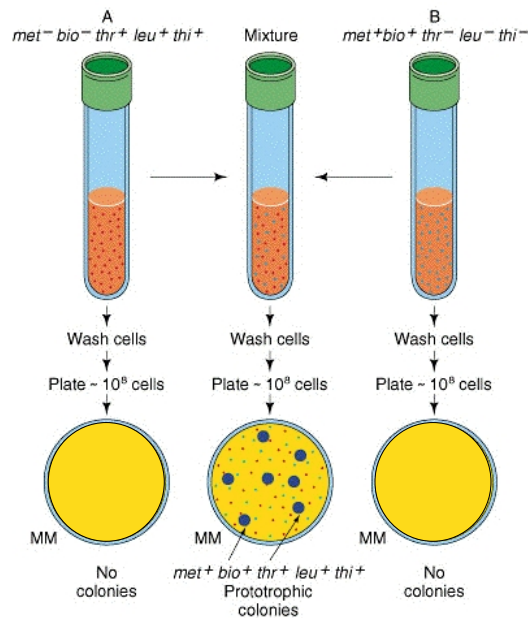


Transmission of genetic variation: conjugation

Bacterial Conjugation is genetic recombination in which there is a transfer of DNA from a living donor bacterium to a recipient bacterium. Often involves a sex pilus.

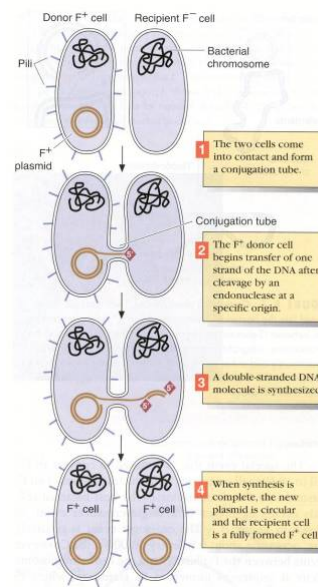


Transmission of genetic variation: conjugation



Transmission of genetic variation: conjugation

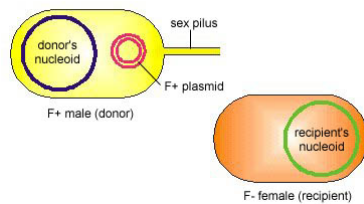
- Direct transfer of DNA from one strain to another.
 - Best studied in *E. coli*, and approximately a third of freshly isolated *E. coli* have plasmids.
- Conjugative plasmids have been found in approximately 30 genera of bacteria, mostly gram-negative. Antibiotic-resistance plasmids RP4 & R68.45 can propagate and promote conjugation in virtually any gram-negative bacterium.
- Some gram-positive conjugate such as *Streptococci*, *Staphylococcus*, *Streptomyces*, *Clostridium*, and *Bacillus*.



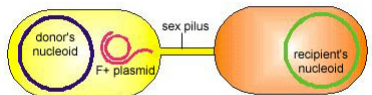
Transmission of genetic variation: F+ conjugation

F+ Conjugation: Genetic recombination in which there is a transfer of a large (95kb) plasmid F+ plasmid (coding only for a sex pilus) but not chromosomal DNA from a male donor bacterium to a female recipient bacterium. Involves a sex (conjugation) pilus. Other plasmids present in the cytoplasm of the bacterium, such as those coding for antibiotic resistance, may also be transferred during this process. F can be transferred from *E. coli* to *Salmonella*, *Shigella*, and *Proteus*.

Transmission of genetic variation: F+ conjugation

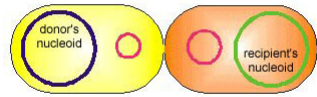


1. The F+ male has an F+ plasmid coding for a sex pilus and can serve as a genetic donor

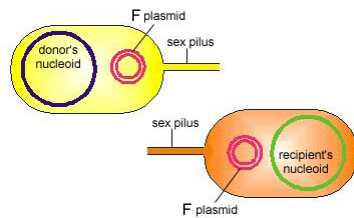


2. The sex pilus adheres to an F- female (recipient). One strand of the F+ plasmid breaks

Transmission of genetic variation: F+ conjugation



3. The sex pilus retracts and a bridge is created between the two bacteria. One strand of the F+ plasmid enters the recipient bacterium

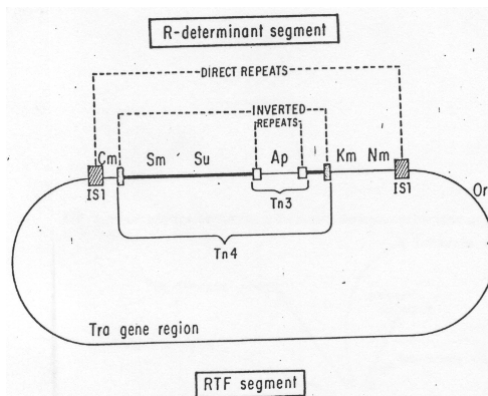


4. Both bacteria make a complementary strand of the F+ plasmid and both are now F+ males capable of producing a sex pilus. There was no transfer of donor chromosomal DNA although other plasmids the donor bacterium carries may also be transferred during F+ conjugation.

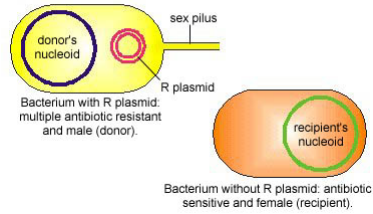
<http://www.cat.cc.md.us/courses/bio141/lecguide/unit4/genetics/recombination/conjugation/f.htm>

Transmission of genetic variation: R-plasmid conjugation

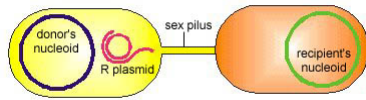
- R factors - Drug-resistance plasmids first isolated in late 1950's in *Shigella* during an outbreak of dysentery. The first plasmid isolated carried resistant determinants to four different antibiotics: chloramphenicol, tetracycline, streptomycin, and sulfonamides. Later the same plasmid was found in *E. coli*.
- In patients given oral tetracycline, the predominant fecal *E. coli* isolates carry tetracycline-resistance R plasmids within one week.



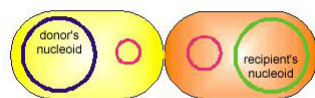
Transmission of genetic variation: R-plasmid conjugation



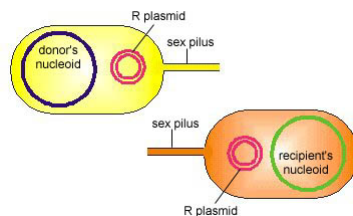
1. The bacterium with an R-plasmid is multiple antibiotic resistant and can produce a sex pilus (serve as a genetic donor).



2. The sex pilus adheres to an F- female (recipient). One strand of the R-plasmid breaks.



3. The sex pilus retracts and a bridge is created between the two bacteria. One strand of the R-plasmid enters the recipient bacterium.



4. Both bacteria make a complementary strand of the R-plasmid and both are now multiple antibiotic resistant and capable of producing a sex pilus.

<http://www.cat.cc.md.us/courses/bio141/lecguide/unit4/genetics/recombination/conjugation/r.html>

Transmission of genetic variation: R-plasmid conjugation

Properties of some R plasmids

Plasmid	Origin	Resistances	Size (kb)
RP1	England	CbKmTc	36
R527	Spain	CbCmGmKmSmSuTcHg	49
pMG5	Japan	AkKmSuTmBorHgPmrTer	280
pMG90	France	CbCmGmKmSmSuTcTmBorHg	150
Rms149	Germany	CbGmSmSuTra	36
pMG38	USA	CbGmKmSuTcTmHg	53
FP110	Australia	CmaPaeFp110	60
pMG25	South Africa	CbCmGmKmSmSuTmBor	66
pMG69	Ireland	CbGmKmSmSuTcTmTra	47

Transmission of genetic variation: R-plasmid conjugation

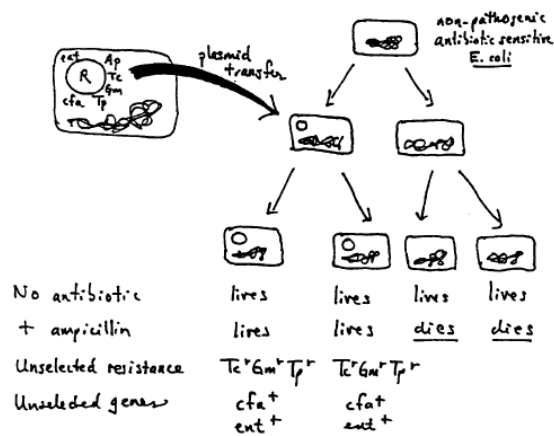
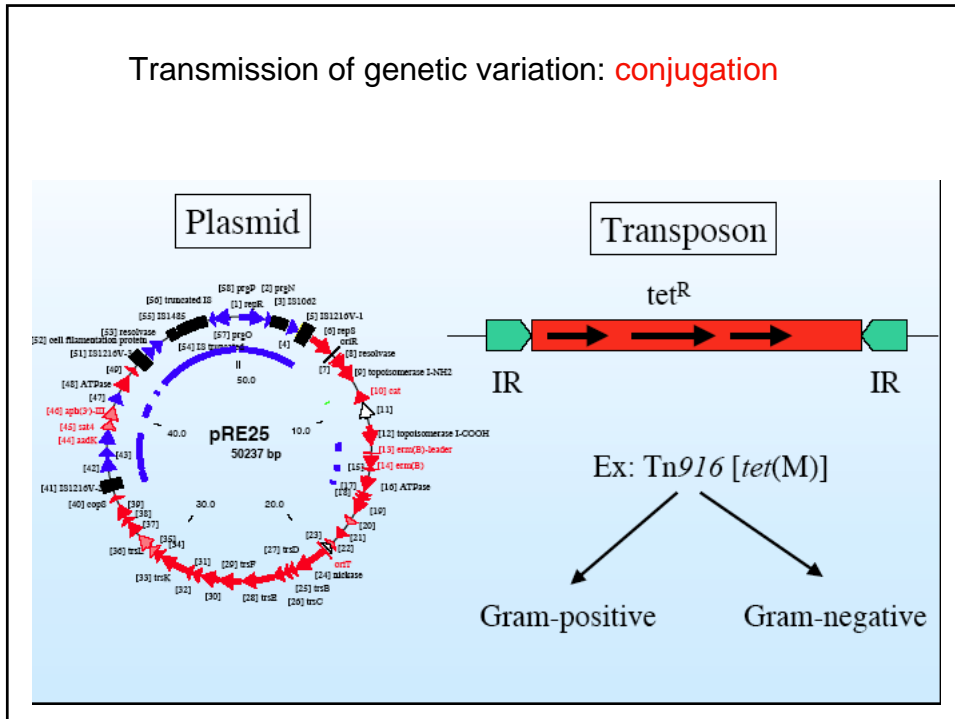


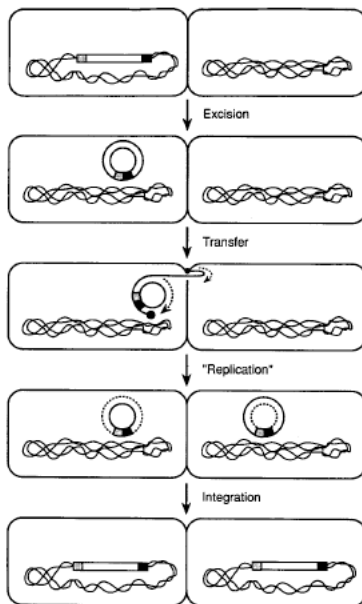
Fig. 6. Indirect selection for multiple resistance.

Transmission of genetic variation: **conjugation**



Transmission of genetic variation: **conjugative transposition**

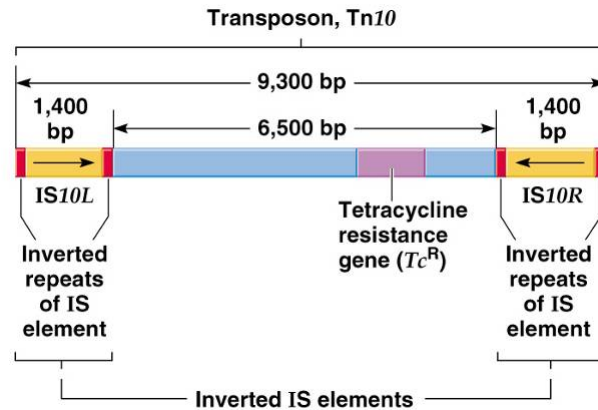
B. Intercellular Transposition



Transmission of genetic variation: conjugative transposition

Composite transposons (Tn):

- Carry genes (e.g., a gene for antibiotic resistance) flanked on both sides by IS elements.
- Tn10 is 9.3 kb and includes 6.5 kb of central DNA (includes a gene for tetracycline resistance) and 1.4 kb inverted IS elements.
- IS elements supply transposase and ITR recognition signals.



Genetic variation: Implications for pathogenesis and antibiotic resistance

I. Transduction

- a. *Vibrio cholera*
- b. *Corynebacterium diphtheriae*
- c. *Neisseria meningitidis*

II. Transformation

- a. *Neisseria gonorrhoeae* pilin variation

III. Conjugation

- a. *Bacillus* spp.

Genetic variation: Implications for pathogenesis and antibiotic resistance

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Genetic variation: Implications for pathogenesis and antibiotic resistance

I. Transduction

- a. *Vibrio cholera*
- b. *Corynebacterium diphtheriae*
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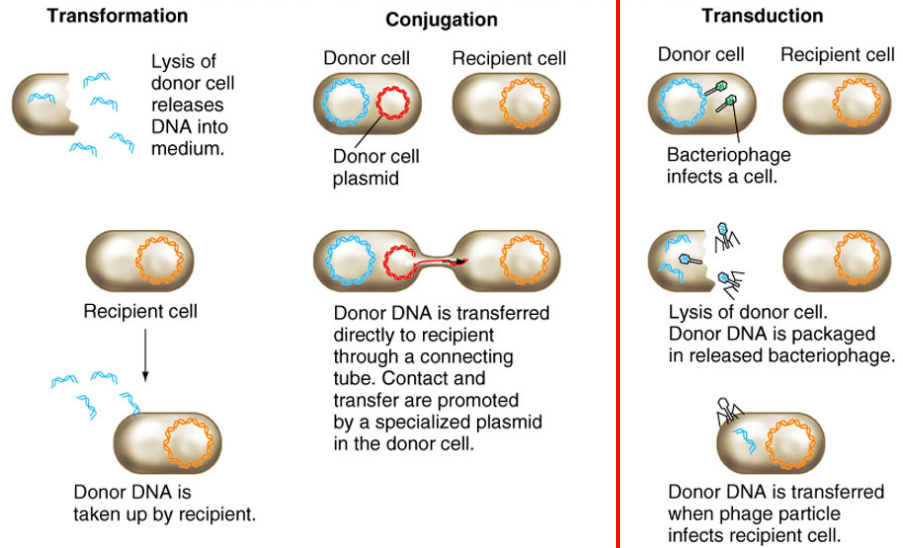
II. Transformation

- a. *Neisseria gonorrhoeae* pilin variation

III. Conjugation

- a. *Bacillus spp.*

Transmission of genetic variation



Transduction: Examples of Virulence Factors Carried by Phage

Bacterium	Phage	Gene Product	Phenotype
<i>Vibrio cholerae</i>	CTX phage	cholerae toxin	cholera
<i>Escherichia coli</i>	lambda phage	shigalike toxin	hemorrhagic diarrhea
<i>Clostridium botulinum</i>	clostridial phages	botulinum toxin	botulism (food poisoning)
<i>Corynebacterium diphtheriae</i>	corynephage beta	diphtheria toxin	diphtheria
<i>Streptococcus pyogenes</i>	T12	erythrogenic toxins	scarlet fever

Transduction: *Corynebacterium diphtheriae*

STUDIES ON THE VIRULENCE OF BACTERIOPHAGE-INFECTED STRAINS OF CORYNEBACTERIUM DIPHTHERIAE¹

VICTOR J. FREEMAN

*Department of Public Health and Preventive Medicine, University of Washington,
School of Medicine, Seattle, Washington*

Received for publication February 26, 1951

The relationship of naturally occurring avirulent strains to virulent strains of *Corynebacterium diphtheriae* is an unanswered question in the epidemiology of diphtheria and in the evolution of the diphtheria bacillus. The detailed investigations reported here have revealed that avirulent strains of *C. diphtheriae* infected with bacteriophage have yielded virulent *C. diphtheriae* strains.

Transduction: *Corynebacterium diphtheriae*

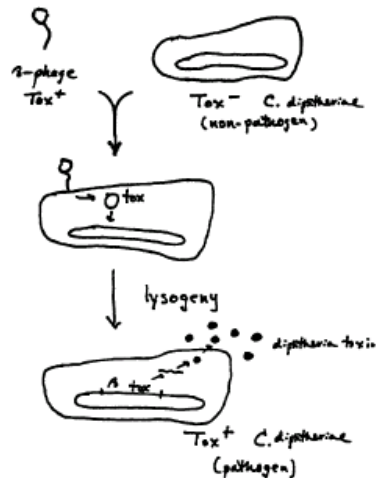


Figure 8. Lysogenic conversion in *C. diphtheriae*

Transduction: *Corynebacterium diphtheriae*

TABLE 3
Subcutaneous tests of bacteriophage lysates in guinea pigs*

STRAIN NO.	CULTURE PLUS SALINE	CULTURE PLUS PHAGE A	CULTURE PLUS PHAGE B	CULTURE PLUS PHAGE B AND ANTITOXIN
444	0/3†	0/1	4/4	0/2
1174	0/1	0/1	2/2	0/1
1180	0/1	0/1	2/2	0/1
770	0/1	0/1	2/2	0/1
411	0/1	0/1	0/1	0/1
Total	0/7	0/5	10/11	0/6

* All cultures and culture lysates were washed off agar media with 0.85 per cent saline and inoculated in 1.0-ml doses.

† The numerator represents the number of guinea pigs that died; the denominator, the total number tested.

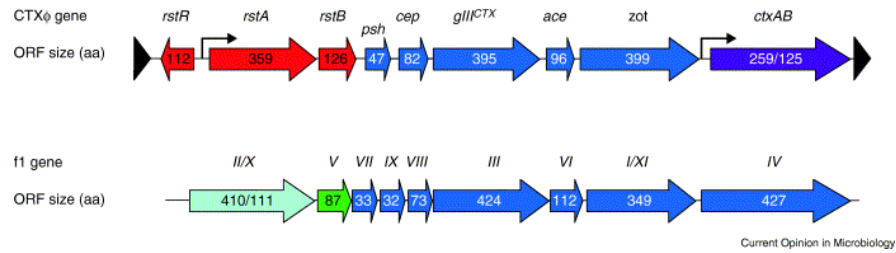
Transduction: *Vibrio cholera*

Lysogenic Conversion by a Filamentous Phage Encoding Cholera Toxin

Matthew K. Waldor* and John J. Mekalanos

Vibrio cholerae, the causative agent of cholera, requires two coordinately regulated factors for full virulence: cholera toxin (CT), a potent enterotoxin, and toxin-coregulated pili (TCP), surface organelles required for intestinal colonization. The structural genes for CT are shown here to be encoded by a filamentous bacteriophage (designated CTX Φ), which is related to coliphage M13. The CTX Φ genome chromosomally integrated or replicated as a plasmid. CTX Φ used TCP as its receptor and infected *V. cholerae* cells within the gastrointestinal tracts of mice more efficiently than under laboratory conditions. Thus, the emergence of toxigenic *V. cholerae* involves horizontal gene transfer that may depend on in vivo gene expression.

Transduction: *Vibrio cholera*



Transduction: *Neisseria meningitidis*

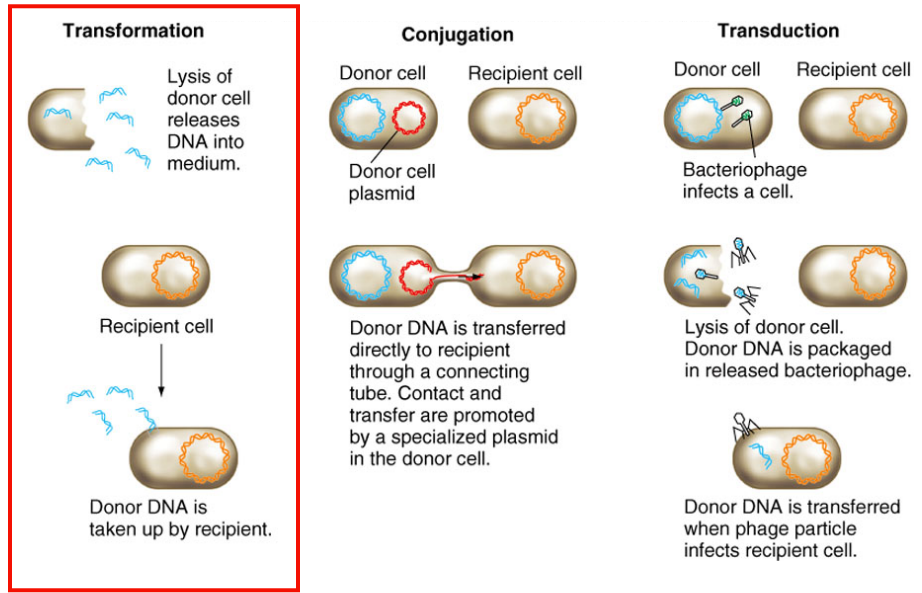
A chromosomally integrated bacteriophage in invasive meningococci

Emmanuelle Bille,¹ Jean-Ralph Zahar,¹ Agnes Perrin,¹ Sandrine Morelle,¹ Paula Kriz,² Keith A. Jolley,³ Martin C.J. Maiden,³ Catherine Dervin,⁴ Xavier Nassif,¹ and Colin R. Tinsley^{1,4}

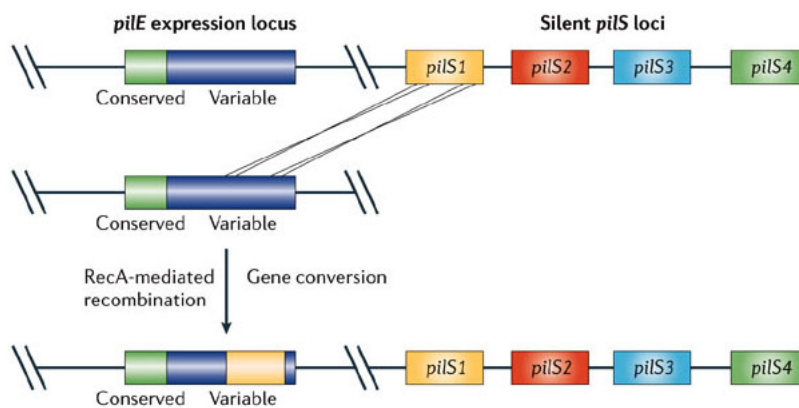
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Cerebrospinal meningitis is a feared disease that can cause the death of a previously healthy individual within hours. Paradoxically, the causative agent, *Neisseria meningitidis*, is a common inhabitant of the human nasopharynx, and as such, may be considered a normal, commensal organism. Only in a small proportion of colonized people do the bacteria invade the bloodstream, from where they can cross the blood-brain barrier to cause meningitis. Furthermore, most meningococcal disease is caused by bacteria belonging to only a few of the phylogenetic groups among the large number that constitute the population structure of this genetically variable organism. However, the genetic basis for the differences in pathogenic potential remains elusive. By performing whole genome comparisons of a large collection of meningococcal isolates of defined pathogenic potential we brought to light a meningococcal prophage present in disease-causing bacteria. The phage, of the filamentous family, excises from the chromosome and is secreted from the bacteria via the type IV pilin secretin. Therefore, this element, by spreading among the population, may promote the development of new epidemic clones of *N. meningitidis* that are capable of breaking the normal commensal relationship with humans and causing invasive disease.

Transmission of genetic variation

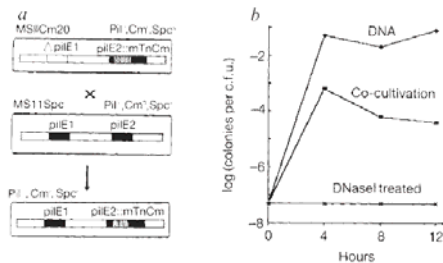


Transformation: *Neisseria gonorrhoeae* pilin variation



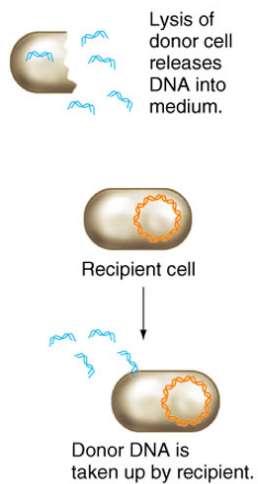
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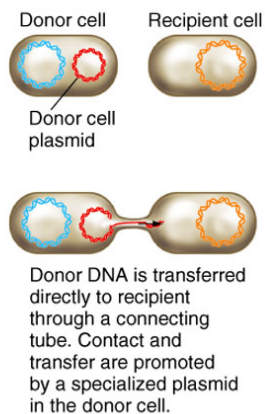


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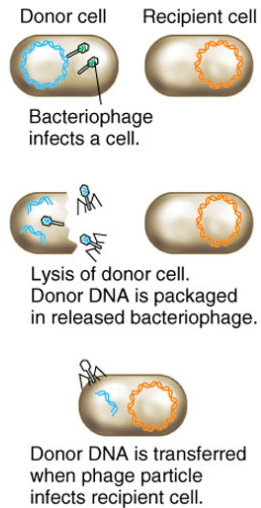
Transformation



Conjugation



Transduction



Conjugation: *Bacillus spp.*

Bacillus anthracis, *Bacillus cereus*, and *Bacillus thuringiensis*—
One Species on the Basis of Genetic Evidence

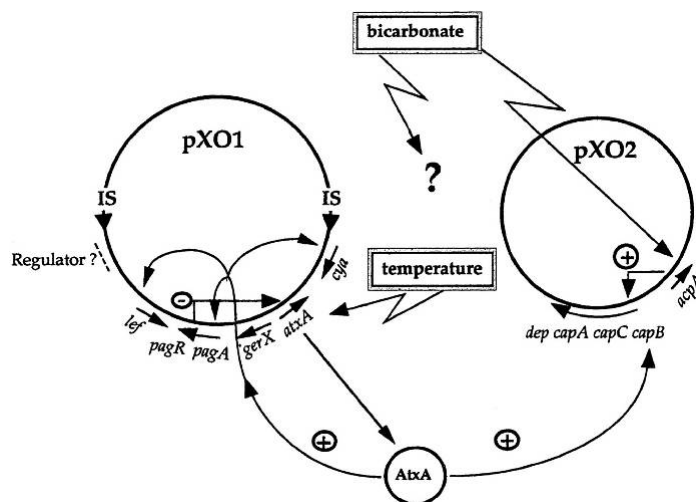
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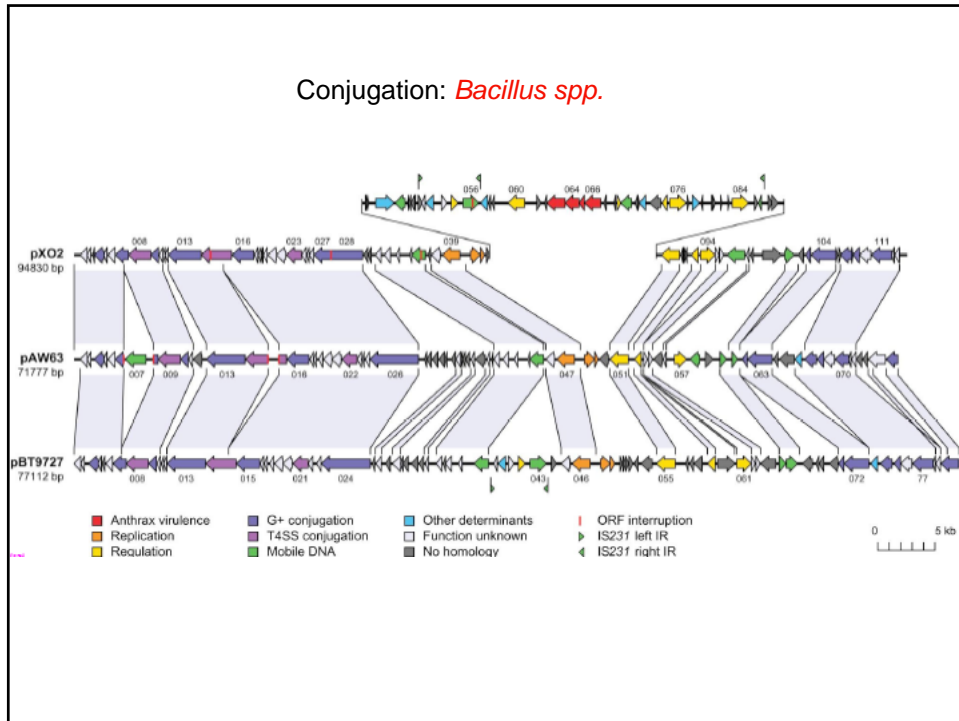
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Bacillus anthracis, *Bacillus cereus*, and *Bacillus thuringiensis* are members of the *Bacillus cereus* group of bacteria, demonstrating widely different phenotypes and pathological effects. *B. anthracis* causes the acute fatal disease anthrax and is a potential biological weapon due to its high toxicity. *B. thuringiensis* produces intracellular protein crystals toxic to a wide number of insect larvae and is the most commonly used biological pesticide worldwide. *B. cereus* is a probably ubiquitous soil bacterium and an opportunistic pathogen that is a common cause of food poisoning. In contrast to the differences in phenotypes, we show by multilocus enzyme electrophoresis and by sequence analysis of nine chromosomal genes that *B. anthracis* should be considered a lineage of *B. cereus*. This determination is not only a formal matter of taxonomy but may also have consequences with respect to virulence and the potential of horizontal gene transfer within the *B. cereus* group.

Conjugation: *Bacillus spp.*



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Conjugation: *Bacillus spp.*

Identification of anthrax toxin genes in a *Bacillus cereus* associated with an illness resembling inhalation anthrax

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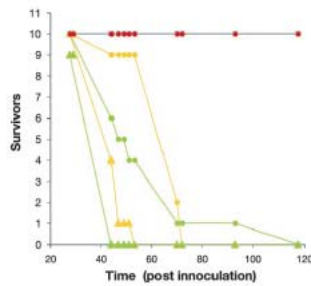


Fig. 4. Survival of A/J mice i.p. challenged with *B. cereus* G9241 (green), *B. anthracis* Sterne (yellow), and *B. cereus* ATCC10987 (red). High-spore dose (1×10^8) and low-spore dose (1×10^6) are represented by triangles and circles, respectively. The experiment was monitored for 14 days, after which the mice inoculated with *B. cereus* ATCC10987 were still alive.

Genetic Basis of Variation in Bacteria

I. Organization of genetic material in bacteria

- a. chromosomes
- b. plasmids

II. Genetic variation: Source

- a. point mutation
- b. DNA rearrangements

III. Genetic variation: Transmission

- a. transformation
- b. transduction
- c. conjugation

IV. Genetic variation: Implications for pathogenesis

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