Mechanisms of Infectious Disease • Fall 2008

Lecture 1

Jonathan Dworkin, PhD

Department of Microbiology

jonathan.dworkin@columbia.edu

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 mutation
- 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

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

Genetic Basis of Variation in Bacteria

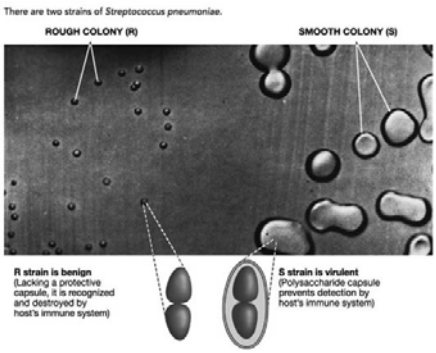
I. Organization of genetic material in bacteria
- a. chromosomes
- b. plasmids

II. Genetic variation: Source
- a. point mutations
- b. DNA rearrangements

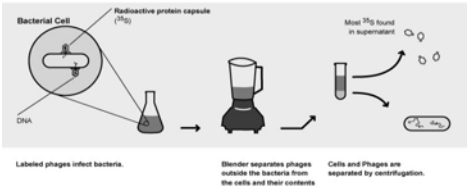
III. Genetic variation: Transmission
- a. transformation
- b. transduction
- c. conjugation

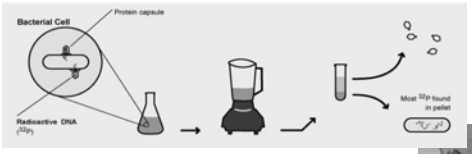
IV. Genetic variation: Implications for pathogenesis

Genetic basis of variation: Griffiths (1928)

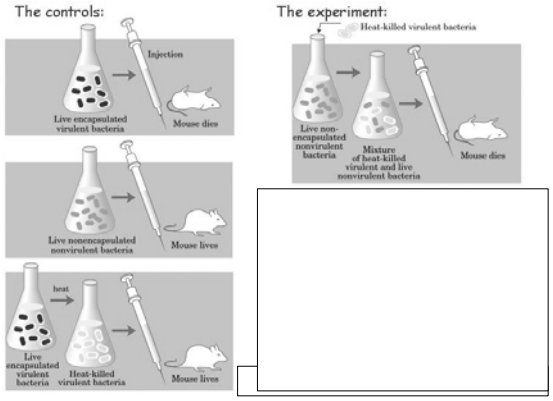


Genetic basis of variation: Hershey and Chase (1952)





Genetic basis of variation: Griffiths (1928)



Genetic Basis of Variation in Bacteria

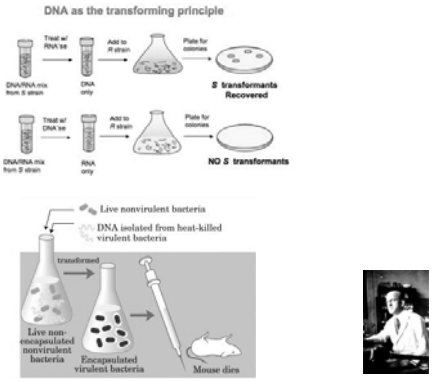
I. Organization of genetic material in bacteria
   a. chromosomes
   b. plasmids

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

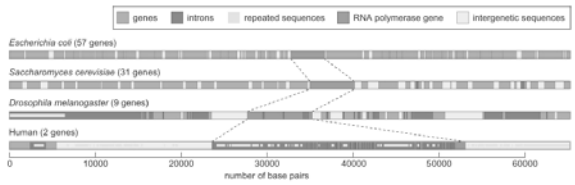
Genetic basis of variation: Avery et al. (1944)



Organization of genetic material in bacteria: chromosomes

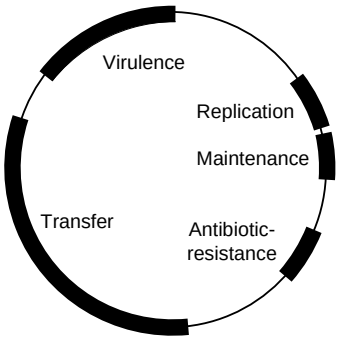
- **Most** bacteria contain a single chromosome (+ extrachromosomal elements)
- **Some** bacteria have been found also to contain 2-3 replicons which can be considered either megaplasmids or minichromosomes e.g. 3.0 Mb and 0.9 Mb replicons in *Rhodobacter sphaeroides*
- A **few** bacterial genera contain >1 chromosome e.g. 2.1 Mb and 1.2 Mb chromosomes in *Brucella*
- **Some** bacteria harbour large replicons essential for survival in a specific ecological niche but not under laboratory conditions e.g. 1.4 Mb and 1.7 Mb replicons in *Rhizobium meliloti* are required for plant symbiosis

Organization of genetic material in bacteria: chromosomes

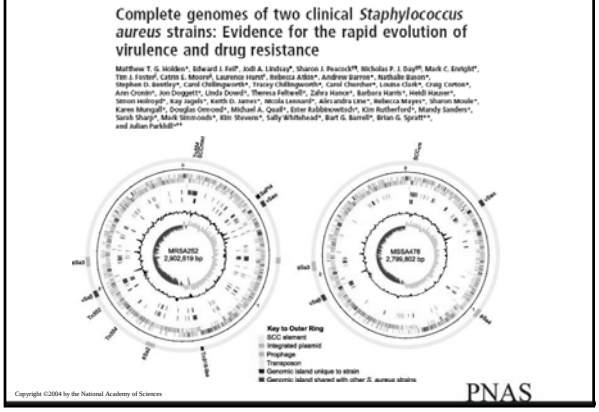


Copyright © 2004 Pearson Education, Inc., publishing as Benjamin Cummings

Organization of genetic material in bacteria: plasmids



Organization of genetic material in bacteria: chromosomes



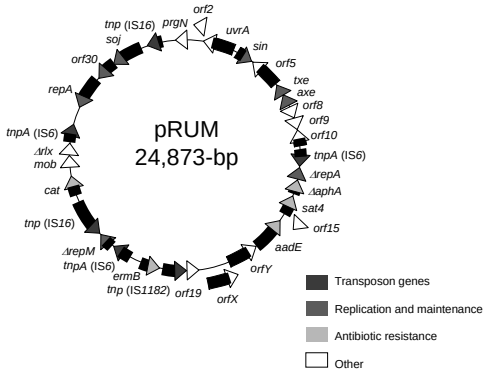
Complete genomes of two clinical *Staphylococcus aureus* strains: Evidence for the rapid evolution of virulence and drug resistance

Matthew T. G. Holden*, Edward J. Feil*, Jodi A. Lindsay*, Sharon J. Peacock¶, Nicholas P. J. Day¶, Mark C. Enright*, Tim J. Foster†, Catrin E. Moore¶, Laurence Hurst‡, Rebecca Atkin*, Andrew Barron*, Nathalie Bason*, Stephen D. Bentley*, Carol Chillingworth*, Tracey Chillingworth*, Carol Churcher*, Louise Clark*, Craig Corton*, Ann Cronin*, Jon Doggett*, Linda Dowd*, Theresa Feltwell*, Zahra Hance*, Barbara Harris*, Heidi Hauser*, Simon Holroyd*, Kay Jagels*, Keith D. James*, Nicola Lennard*, Alexandra Line*, Rebecca Moyes*, Sharon Moule*, Karen Murphy*, Douglas Oliver‖, Michael A. Quail*, Ester Rabbinowitsch*, Kim Rutherford*, Mandy Sanders*, Sarah Sharp*, Mark Simmonds*, Kim Stevens*, Sally Whitehead*, Bart G. Barrell*, Brian G. Spratt**†, and Julian Parkhill*,**†

Copyright ©2004 by the National Academy of Sciences

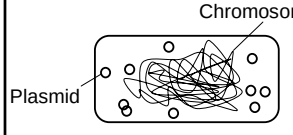
PNAS

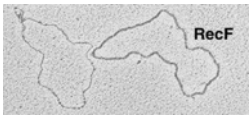
Organization of genetic material in bacteria: plasmids



Organization of genetic material in bacteria: plasmids

- Extrachromosomal
- Circular or linear
- 2 kb to hundreds of kb in size
- Non-essential
- May carry 'supplemental' genetic information or may be cryptic
- Employ host functions for most of DNA metabolism





http://www.biochem.wisc.edu/inman/empics/dna-prot.htm

Organization of genetic material in bacteria: plasmids

Examples of naturally-occuring plasmids and relevant features

Plasmid	Host	Plasmid size (kb)	Relevant feature
pT181	*Staphylococcus aureus*	4.4	Tetracycline resistance
ColE1	*Escherichia coli*	6.6	Colicin production and immunity
pGKL2	*Kluyveromyces lactis*[5]	13.5	Killer plasmid
pAMβ1	*Enterococcus faecalis*	26.0	Erythromycin resistance
pSK41	*Staphylococcus aureus*	46.4	Multidrug resistance
pBM4000	*Bacillus megaterium*	53.0	rRNA operon
pI258	*Staphylococcus aureus*	28.0	Metal ion resistance
pSLT	*Salmonella enterica* subsp. *typhimurium*	93.9	Virulence determinants
pMT1	*Yersinia pestis*	101.0	Virulence determinants
pADP-1	*Pseudomonas* sp.	108.8	Atrazine (herbicide) catabolism
pWW0	*Pseudomonas putida*	117.0	Aromatic hydrocarbon degradation
pBtoxis	*Bacillus thuringiensis* subsp. *israelensis*	137.0	Mosquito larval toxicity
pX01	*Bacillus anthracis*	181.7	Exotoxin production
pSOL1	*Clostridium acetobutylicum*	192.0	Solvent production
pSymB	*Sinorhizobium meliloti*	1683.3	Functions associated with plant symbiosis

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

Sources of genetic variation: point mutations

Mutation phenotypes

- Silent mutation (synonymous), no change in amino acid
  AGG > AGA , both codons specify Arginine
- Missense mutation (replacement; nonsynonymous), change in amino acid
  - Nonsynonymous missense (or radical replacement)
    UUU (Phe) > UCU (Ser): Phe is hydrophobic and Ser is polar
- Nonsence mutation, premature termination of translation
  CAG (Gln) > UAG (Stop)
- Frameshift, addition or deletion of base pairs, not in a multiple of three, within the coding region of a gene.

Sources of genetic variation: point mutations

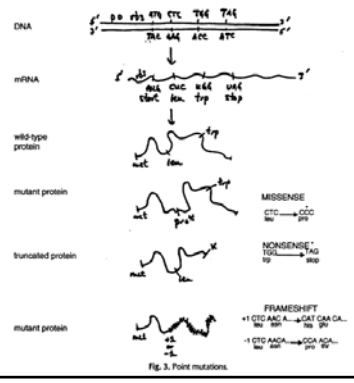
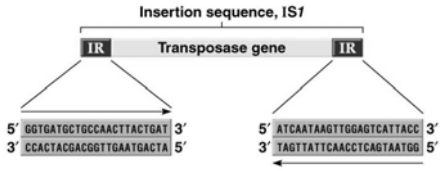


Fig. 3. Point mutations.

Sources of genetic variation: DNA rearrangements

Insertion sequence (IS) elements:

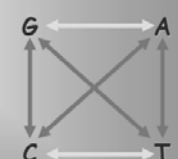
1. **Simplest type of transposable element found in bacterial chromosomes and plasmids.**
2. **Encode only genes for mobilization and insertion.**
3. **Range in size from 768 bp to 5 kb.**
4. **IS1 first identified in *E. coli*'s glactose operon is 768 bp long and is present with 4-19 copies in the *E. coli* chromosome.**
5. **Ends of all known IS elements show inverted terminal repeats (ITRs).**



Sources of genetic variation: point mutations

Point Mutations

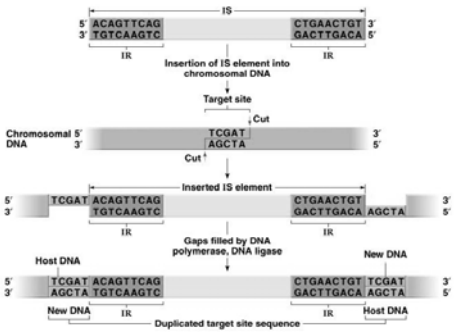
- Transitions
  - Purine ⟺ Purine
  - Pyrimide ⟺ Pyrimide
    A ⟺ G
    C ⟺ T
- Transversions
  - Purine Pyrimide
    A ⟺ C
    A ⟺ T
    G ⟺ C
    G ⟺ T



There are four types of transitions.
There are eight types of transversions

Sources of genetic variation: DNA rearrangements

Integration of IS element in chromosomal DNA.



Sources of genetic variation: DNA rearrangements

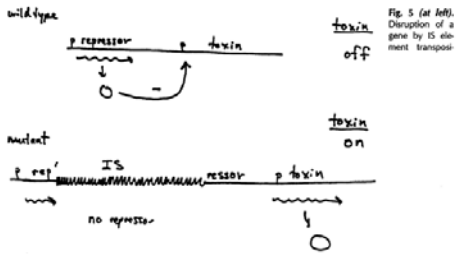


Fig. 5 (at left). Disruption of a gene by IS element transposition.

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

Sources of genetic variation: DNA rearrangements

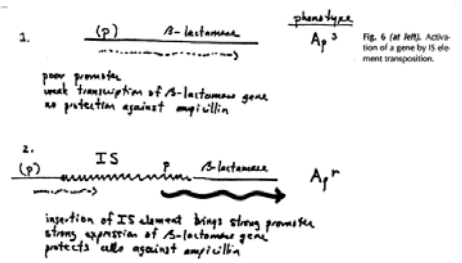


Fig. 6 (at left). Activation of a gene by IS element transposition.

Transmission of genetic variation

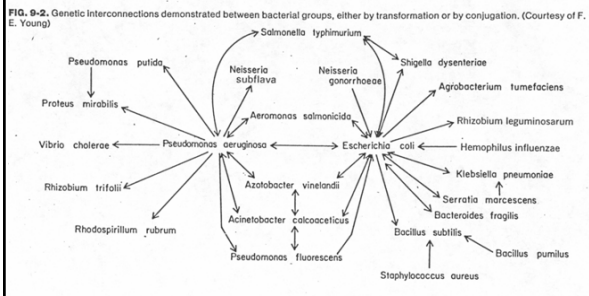
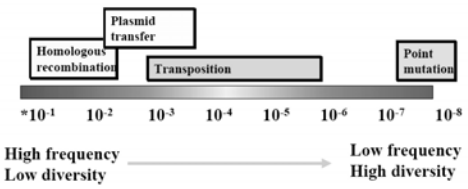


FIG. 9-2. Genetic interconnections demonstrated between bacterial groups, either by transformation or by conjugation. (Courtesy of F. E. Young)

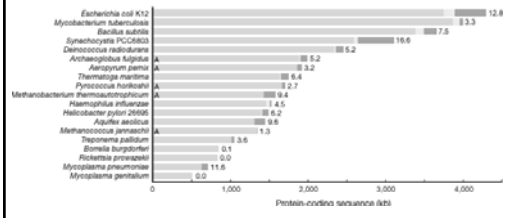
Sources of genetic variation: frequency of occurrence



High frequency
Low diversity

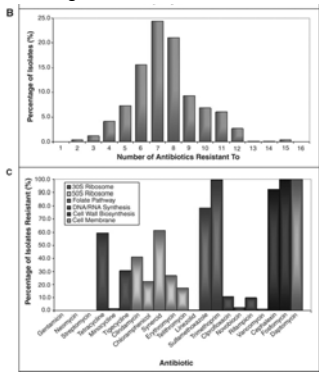
Low frequency
High diversity

* As frequency per cell per generation



Lengths of bars denote the amount of protein-coding DNA. For each bar, the native DNA is blue; foreign DNA identifiable as mobile elements, including transposons and bacteriophages, is yellow, and other foreign DNA is red. The percentage of foreign DNA is noted to the right of each bar. 'A' denotes an Archaeal genome.

Transmission of genetic variation: antibiotic resistance



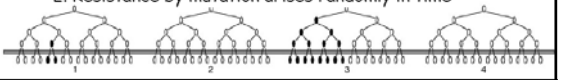
V. M. D'Costa et al. Science (2006)

Fig. 1. Antibiotic resistance profiling of 480 soil-derived bacterial isolates

Transmission of genetic variation: Luria-Delbruck test

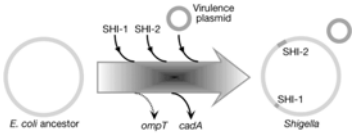
1. Resistance by mutation is a physiological response

2. Resistance by mutation arises randomly in time



Time of exposure to selective agent

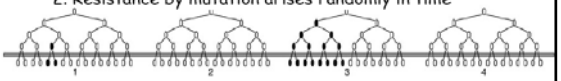
Transmission of genetic variation: pathogenesis

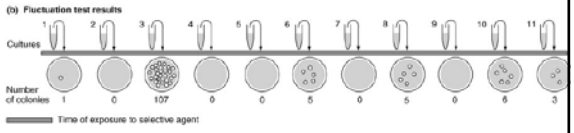


Transmission of genetic variation: Luria-Delbruck test

1. Resistance by mutation is a physiological response

2. Resistance by mutation arises randomly in time



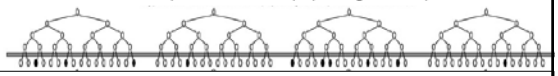


Time of exposure to selective agent

Results fit with expectations if random mutation occur at random.

Transmission of genetic variation: Luria-Delbruck test

1. Resistance by mutation is a physiological response



Time of exposure to selective agent

Linear transmission of genetic variation

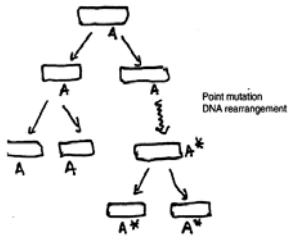


Fig. 1. Clonal variation.

Horizontal transmission of genetic variation

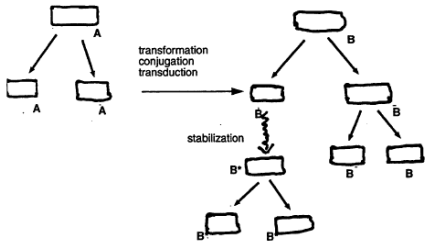
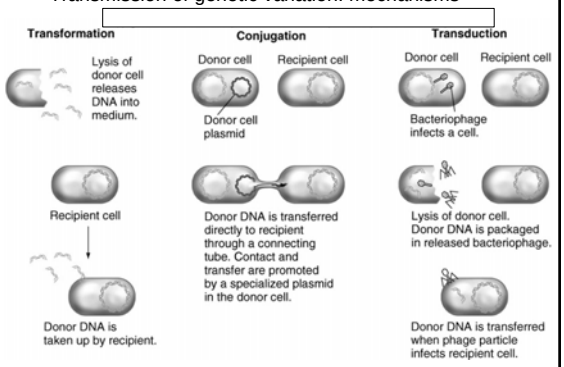


Fig. 2 *(at left).* Variation by transfer of genetic information.

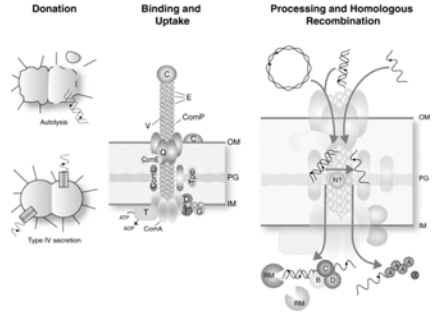
Transmission of genetic variation: transformation

- Gene transfer resulting from the uptake of DNA from a donor.
- Factors affecting transformation
  - DNA size and state
    - Sensitive to nucleases
  - Competence of the recipient (*Bacillus, Haemophilus, Neisseria, Streptococcus*)
    - Competence factor
    - Induced competence

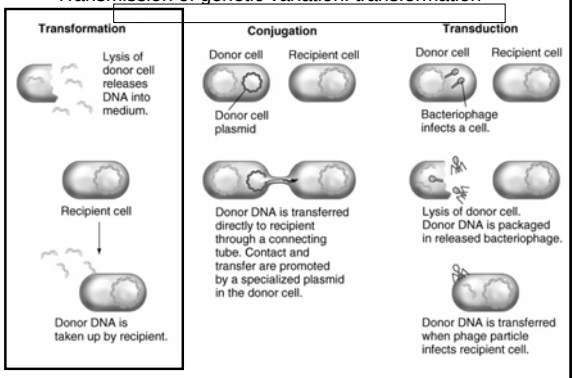
Transmission of genetic variation: mechanisms



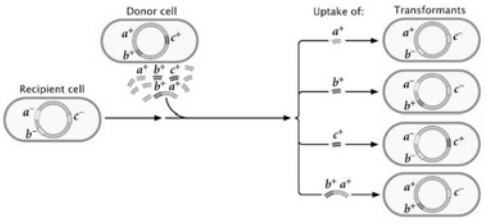
Transmission of genetic variation: transformation



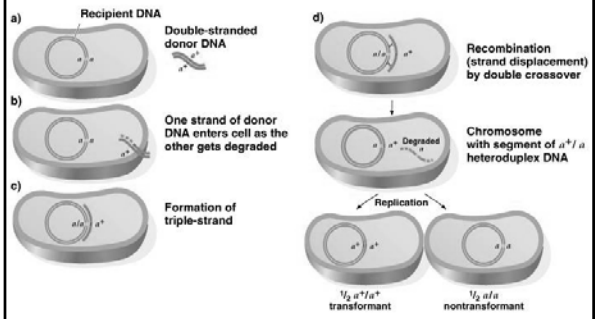
Transmission of genetic variation: transformation



Transmission of genetic variation: transformation



Transmission of genetic variation: transformation



Genetic Basis of Variation in Bacteria

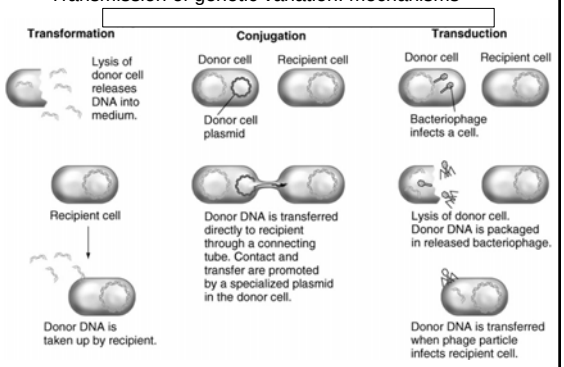
I. Organization of genetic material in bacteria
- a. chromosomes
- b. plasmids

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: mechanisms



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 mutation
- b. DNA rearrangements

III. Genetic variation: Transmission
- a. transformation
- b. transduction
- c. conjugation

IV. Genetic variation: Implications for pathogenesis