MICROBIAL GENETICS – PLASMIDS/MOBILE GENETIC ELEMENTS

PLASMIDS

Characteristics, Resistance Factors, Resistance Transfer Factors
Drug Resistance Mechanisms
Penicillin Resistance, Penicillinase, Beta-lactamase
Multiple Drug Resistance
Transformation by Plasmids

MOBILE GENETIC ELEMENTS

Insertion Sequences
Transposons
Integrons
Superintegrons
Conjugative Transposons
Genomic Islands
The Busy Genome
Plasmids: Small, circular, double-strand DNA molecules, ~5-50 genes, cytoplasmic location, not essential (normally) replication genes & sites, 1 - 20 copies each/cell, several different plasmids/cell.

Resistance factors - plasmids which carry genes which encode proteins which make the bacterial host resistant to antibiotic - called drug resistance.

Resistance transfer factors - all above plus ability to transfer plasmid in mating [conjugation].

Other plasmid genes: hydrocarbon catabolism, toxin production, mineral uptake.
MECHANISMS OF DRUG RESISTANCE

1. MUTATION RESULTS IN ALTERED BACTERIAL PROTEIN. IT NO LONGER RECOGNIZES ANTIBIOTIC BUT CONTINUES TO PERFORM NORMAL FUNCTION IN BACTERIAL GROWTH

   EX: STREPTOMYCIN

2. BACTERIA ACQUIRE NEW GENE WHICH CODES FOR ENZYME WHICH DESTROYS ANTIBIOTIC

   EX: PENICILLINASE DESTROYS PENICILLIN

3. BACTERIA ACQUIRE NEW GENE WHICH CODES FOR ENZYME WHICH PUMPS ANTIBIOTIC BACK OUTSIDE THE CELL

   EX: TETRACYCLINE RESISTANCE
PENICILLIN CLEAVAGE (INACTIVATION) BY PENICILLINASE

**β-LACTAM RING**

**PENICILLINASE ([β-LACTAMASE])**

**INACTIVE**
MULTIPLE DRUG RESISTANCE:
JAPAN, 1957, SHIGELLA DYSENTERIAE,
DYSENTERY,
RESISTANT TO: SULFONAMIDES,
STREPTOMYCIN,
CHLORAMPHENICOL,
NEOMYCIN

JAPAN: 1957 - 1 CASE;
1964 - 50%

LONDON: 1962 - 3%;
1964 - 61%

NEISSERIA GONORRHOEOAE
1945 - PENICILLIN-SENSITIVE
~1975 - PENICILLIN-RESISTANT, BUT
SPECTINOMYCIN-SENSITIVE
~1985-1990 - SPECTINOMYCIN-RESISTANT,
CEFTRIAXONE-SENSITIVE
Bacterium resistant to penicillin

Gene for penicillinase

Phenotype: organism grows on medium containing penicillin
TRANSFORMATION BY PLASMIDS

Penicillin resistance plasmid

Penicillin-sensitive cells

Mix, some plasmids enter cells

Penicillin-resistant transformant

Select: Plate on medium + penicillin plasmid only \(\rightarrow\) no colonies

Recipient cells only \(\rightarrow\) no colonies

Recipient cells + plasmids \(\rightarrow\) colonies penicillin-R
~10⁶ PEN-S CELLS [RECIPIENT]

~1 mg PLASMID [PENICILLIN-R]

~10 RECIPIENT CELLS PLUS ~1 mg PLASMID

PEN-R TRANSFORMANTS
MOBILE DNA

INSERTION SEQUENCES (IS)
SHORT (<2.5 KB), LINEAR
ONE GENE - TRANSPOSASE
TRANSPOSES TO MULTIPLE
SITES IN DNA

NO PHENOTYPE
(UNLESS GENE DISRUPTED)
TRANSPOSONS

5-10 KB, LINEAR
TRANSPON 내 TO MULTIPLE SITES
CARRY ANTIBIOTIC (DRUG)
RESISTANCE GENES
BRACKETED BY 2 INVERTED
INSERTION SEQUENCES
TRANSFERS DRUG RESISTANCE

TN5

TRANSPOSASE ACTS ON:
OE + OE → Tn5 MOVES
IE + OE → IS 50R MOVES
INTEGRONS - GENE CAPTURE ELEMENTS

- Code for information necessary to insert host gene into itself
  - Integrase to move gene
  - Attachment site to put the gene into

- Have a promoter to express captured gene

- Acquire various host genes

- Exist in many forms - differ in number and identity of captured genes
- Integron

- Integrase

- Promoter for integrase

- Promoter for inserted gene

- ATT site for incoming genes

- Integron with inserted host gene

- Gene for drug resistance or toxin

- Integron with multiple insertions of host genes

- Host gene 1

- Host gene 2
• Super Integron - Vibrio cholerae chromosome

• 179 inserted gene

• How are integrons involved in mobility of DNA?

Because integrons are located on transposons + on conjugative plasmids

They catalyze movement of host genes into themselves + therefore into transposons + conjugative plasmids
CONJUGATIVE TRANSPOSONS

- Move from first host (donor) genome → second host (recipient) genome
- Cell-cell contact
- Some move to new sites
- Some move to identical sites

- Simple - single structural unit
  - or
- Complex - two or more units - each capable of independent movement

- 20-100 KB genes for:
  - Integration + excision
  - Conjugation
  - Antibiotic resistance

- Transfer via circular intermediate + then integrate
**Excision (in donor cell)**

- Single strand transferred to recipient
- Circularizes in recipient
- Copied into double strand
- Integrates into recipient chromosome
GENOMIC ISLANDS

LINEAR, 10-200 KB, IN CHROMOSOMES

INTEGRATION/TRANSFER ENZYMES

INTEGRATE IN 3’ ENDS OF tRNA GENES
(SIMILAR IN SEQUENCE TO PROPHAGE ATTACHMENT SITES)

BOUNDARIES ARE DIRECT REPEATING SEQUENCES

DIFFER FROM HOST CHROMOSOME IN G & C CONTENT OF DNA - INDICATES ORIGINATION IN ANOTHER GENUS

PERMIT EVOLUTION IN QUANTUM LEAPS
BRING IN GENES WHICH HELP SURVIVAL (OF BACTERIA)

PATHOGENICITY ISLANDS - TOXIN GENES
VIBRIO CHOLERAE

ADHESINS (PILI)
UROPATHOGENIC
E. COLI

IRON UPTAKE
YERSINIA PESTIS
CAUSES PLAGUE
FLEA VECTOR
FROM RATS

ECOLOGICAL ISLANDS - PHENOL CATABOLISM
PSEUDOMONAS PUTIDA

SYMBIOSIS ISLANDS - NITROGEN FIXATION
MESORHIZOBIUM LOTI

APROPHYTIC ISLANDS - IRON UPTAKE
E COLI GROWING IN INTESTINE
UROPATHOGENIC E. COLI -

MOST COMMON CAUSE OF BLADDER & KIDNEY INFECTIONS

E. COLI (06: K15: H31)

FOUR PATHOGENICITY ISLANDS

PAI - I  CODES FOR HEMOLYSIN

PAI - II  CODES FOR HEMOLYSIN, ADHESIN

PAI - III CODES FOR ADHESIN, IRON UPTAKE

PAI - IV  IRON UPTAKE
The Busy Genome:
Elements of Horizontal Exchange

GENOMIC ISLANDS

PROPHAGES

MINIMAL SPECIES GENOMIC BACKBONE

CONJUGATIVE TRANSPOSONS (gram +ve)

SUPER INTEGRONS (mainly γ protobacteria)

INSERTION SEQUENCES

UNIT TRANSPOSONS + INTEGRONS

COMPOUND TRANSPOSONS

e.g. Echerichia coli
Common: 4.1Mb
K12 islands: 0.53Mb
0157:H7 islands: 1.34Mb

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