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MCB 202 ASSIGNMENT.

**MICROBIAL VARIATION AND HEREDITY IN BACTERIA.**

Variation refers to change in an organism relative to its parents or former state. Any change in the genotype of a bacterium or its phenotype is known as variation. The diversity of form and function among micro-organisms is unparalleled among other groups of organisms. Micro-organisms exhibit an enormous capacity to evolve new potentialities. Genotypic variation can occur as a result of changes in the genes by way of mutation, loss or acquisition of new genetic elements. Phenotypic variations are seen temporarily when bacteria are grown under certain environmental conditions.

Bacreia may reproduce asexually. Cells enlarge, copy their DNA, and divide into two new cells. The only source of variation therefore is mutation, and each mutation must accumulate with other mutations, one after another, before a new combination of genes is possible. For a given population, there are three sources of variation; mutation, recombination and immigration of genes. However recombination by itself does not produce variation unless alleles are segregating already at different loci; otherwise there is nothing to recombine.

Bacteria have been on this planet for approximately 3.5 billion years, and are classified by their shape. Bacterial genetics studies the mechanisms of their heritable information, their chromosomes, plasmids, transpoons and phages.

ANTIGENECITY OF BACTERIA.

Antigenic variation or antigenic alteration refers to the mechanism by which an infectious agent such as a protozoan, bacterium or virus alters the proteins or carbohydrates on the surface and thus avoids a host immune response. Antigenic variation in bacteria is best demonstrated by species of the genus *neisseria, streptococcus and mycoplasma.*

ANTIMICROBIAL RESISTANCE OF BACTERIA.

Antimicrobial resistance is the ability of a microbe to resist the effects of medication that once could successfully treat the microbe. The term antibiotic resistance is a subset of antimicrobial resistance as it applies only to bacteria becoming resistant to antibiotics. Resistance arises through one of three mechanisms; natural resistance in certain types of bacteria, genetic mutation or by one species acquiring resistance from another.

PLASMIDS.

A plasmid is a small circular piece of DNA that is different than the chromosomal DNA, which is all the genetic material found in an organism’s chromosomes. It replicates independently of chromosomal DNA. Plasmids are mainly found in bacteria, but they can also be found in archaea and multicellular organisms.

Plasmids have many different functions. They may contain genes that enhance the survival of an organism, either by killing other organisms or by defending the host cell by producing toxins. Some plasmids facilitates the process of replication in bacteria. Five main types of plasmids are; fertility F-plasmids, resistance plasmids, virulence plasmids, degradative plasmids, col plasmids.

Conjugative and non-conjugative plasmids: bacteria reproduce by sexual conjugation, which is pretransfer of genetic material from one bacterial cell to another, either through direct contact or a bridge between the two cells. Some plasmids conatain genes called transfer genes that facilitate the beginning of conjugation. Non-conjugative plasmids cannot start the conjugative process, and can only be transferred through sexual conjugation with the help of conjugative plasmids.

Incompactability: another plasmid classification is by uincompactability group. In a bacterium, different plasmids can only co-occur if they are compatable with each other. An incompactable plasmid will be expelled from the bacterial cell. Plasmids are compactable if they have the same reproduction strategy in the cell; this allows the plasmid to inhabit a certain territory without other plasmids interfering.

BACTERIOPHAGE

A bacteriophage is a virus that infects and replicates within a bacteria and archaea. Bacteriophages are composed of proteins that encapsulate a DNA or RNA genome, and may have structures that are either simple or elaborate. Bacteriophages replicate within the bacterium following the injection of their genome into the cytoplasm.

Virulent phage is a bacteriophage that causes the destruction of the host bacterium by lysis. Temporate phage is a bacteriophage that can choose between the lytic and lysogenic pathways of development. Prophage is a bacteriophage genome inserted and integrated into the circular bacterial DNA chromosome or exists as an extrachromosomal plasmid. Lysogenic phages incorporate their nucleic acid into the chromosome of the host cell and replicate with it as a unit without destroying the cell. Under certain conditions lysogenic phage can be induced to follow a lytic cycle. A lysogen or lysogenic bacterium is a bacterial cell which can produce a bacteriophage.

TRANSPOSABLE ELEMENT.

A transposable element is a segment of DNA that can become integrated at different sites along a chromosome, plasmid and phage. The following are transposable elements found in bacteria.

1. Insertion sequences
2. Transposons
3. Contergration model of transposition
4. Conjugative transposons
5. Integrons
6. Transposon mutagenesis
7. Transposing bacteriophage mu

MUTATION AND SELECTION.

Perhaps the most obvious way genetic diversity can be created is by mutations (Latin mutare, to change). Several types of mutations exist. Some arise from the alteration of single pairs of nucleotides and from the addition or deletion of one nucleotide pair in the coding regions of a gene. Such small changes in DNA are called point mutations because they affect only one base pair in a given location. Larger mutations are less common. These include large insertions, deletions, inversions, duplications, and translocations of nucleotide sequences. Mutations occur in one of two ways. (1) Spontaneous mutations arise occasionally in all cells and in the absence of any added agent. (2) Induced mutations are the result of exposure to a mutagen, which can be either a physical or a chemical agent. Mutations are characterized according to either the kind of genotypic change that has occurred or their phenotypic consequences.

SPONTENEOUS MUTATIONS

 Spontaneous mutations result from errors in DNA replication, spontaneously occurring lesions in DNA, or the action of mobile genetic elements such as transposons. Replication errors can occur when the nitrogenous base of a nucleotide shifts to a different form {isomer) called a tautomeric form. Nitrogenous bases typically exist in the keto form but are in equilibrium with the rarer imino and enol forms . The shift from one form to another changes the hydrogen-bonding characteristics of the bases, allowing purine for purine or pyrimidine for pyrimidine substitutions that can eventually lead to a stable alteration of the nucleotide sequence. Such substitutions are known as transition mutations and are relatively common. On the other hand, transversion mutations-mutations where a purine is substituted for a pyrimidine or a pyrimidine for a purine-are rarer due to the steric problems of pairing purines with purines and pyrimidines with pyrimidines. Replication errors can also result in the insertion and deletion of nucleotides. These mutations generally occur where there is a short stretch of repeated nucleotides. In such a location, the pairing of template and new strand can be displaced by the distance of the repeated sequence, leading to insertions or deletions of bases in the new strand. Spontaneous mutations can originate from lesions in DNA as well as from replication errors. For example, it is possible for purine nucleotides to be depurinated; that is, to lose their base. This results in the formation of an apurinic site, which does notbase pair normally and may cause a mutation after the next round of Wild type replication.

INDUCED MUTATION

 Any agent that damages DNA, alters its chemistry, or in some way interferes with its functioning will probably induce mutations. Mutagens can be conveniently classified according to their mode of action. Three common types of chemical mutagens are base analogues, DNA-modifying agents, and intercalating agents. A number of physical agents (e.g., radiation) are mutagens that damage DNA. Base analogues are structurally similar to normal nitrogenous bases and can be incorporated into the growing polynucleotide chain during replication. Once in place, these compounds typically exhibit base-pairing properties different from the bases they replace and can eventually cause a stable mutation. A widely used base analogue is 5-bromouracil, an analog of thymine. It undergoes a tautomeric shift from the normal keto form to an enol much more frequently than does a normal base. The enol tautomer forms hydrogen bonds like cytosine, pairing with guanine rather than adenine. The mechanism of action of other base analogues is similar to that of 5-bromouracil. There are many DNA-modifying agents-mutagens that change a base's structure and therefore alter its base-pairing specificity. Some of these mutagens are selective; they preferentially react with certain bases and produce a particular kind of DNA damage. For example, methyl-nitrosoguanidine is an alkylating agent that adds methyl groups to guanine, causing it to mispair with thymine. A subsequent round of replication can then result in a GC-AT transition. Hydroxylamine is another example of a DNA-modifying agent. It hydroxylates the nitrogen attached to the number 4 carbon (C-4) of cytosine , causing it to base pair like thymine. Intercalating agents distort DNA to induce single nucleotide pair insertions and deletions. These mutagens are planar and insert themselves (intercalate) between the stacked bases of the helix. This results in a mutation, possibly through the formation of a loop in DNA. Intercalating agents include acridines such as proflavin and acridine orange.

SEXUAL REPRODUCTION AND GENETIC VARIABILITY

 The transfer of genes from parents to progeny is called vertical gene transfer. This type of gene transfer is observed in all organisms. In eukaryotes capable of sexual reproduction, vertical gene transfer is accompanied by genetic recombination, which occurs in two ways. The first is from crossing-over between sister chromosomes during meiosis. This creates new mixtures of alleles on homologous chromosomes. The second type of recombination results from fusion of gametes. Since each gamete contains alleles from the parent, when the gametes fuse, the parental alleles are combined in the zygote. As a result of these two types of recombination events, progeny organisms are not identical to their parents or to each other.

HORIZONTAL GENE TRANSFER: Creating variability the asexual way.

 Bacteria and archaea do not reproduce sexually. This suggests that genetic variation in populations of these microbes should be relatively limited, only occurring with the advent of a new mutation and its transfer to the next generation by vertical gene transfer. However, this is not the case. Bacteria and archaea have evolved three different mechanisms for creating recombinants. These mechanisms are referred to collectively as horizontal (lateral) gene transfer (HGT). HGT is distinctive from vertical gene transfer because genes from one independent, mature organism are transferred to another mature organism, often creating a stable recombinant having characteristics of both the donor and the recipient.

BACTERIAL CONJUGATION

Conjugation, the transfer of DNA by direct cell-to-cell contact, depends on the presence of a conjugative plasmid. plasmids are small, double-stranded DNA molecules that can exist independently of host chromosomes. They have their own replication origins, replicate autonomously, and are stably inherited. Some plasmids are episomes, plasmids that can exist either with or without being integrated into host chromosomes. Perhaps the best-studied conjugative plasmid is F factor. It plays a major role in conjugation in E. coli, and it was the first conjugative plasmid to be described. The F factor is about 100,000 bases long and bears genes responsible for cell attachment and plasmid transfer between specific E. coli cells. Most of the information required for plasmid transfer is located in the tra operon, which contains at least 28 genes. Many of these direct the formation of sex pili that attach the p+ cell (the donor cell containing an F plasmid) to an F- cell. Other gene products aid DNA transfer. In addition, the F factor has several IS elements that assist plasmid integration. into the host cell's chromosome. Thus the F factor is an episome that can exist outside the bacterial chromosome or be integrated into it.

F+ x F- Mating

 In 1952 William Hayes (1913-1994) demonstrated that the gene transfer observed by Lederberg and Tatum was unidirectional. That is, there were definite donor (F+, or fertile) and recipient (F-, or nonfertile) strains, and gene transfer was nonreciprocal. These results are now understood and readily explained in the following way. An p+ strain contains an extrachromosomal F factor carrying the genes for sex pilus formation and plasmid transfer. The sex pilus is used to establish contact between the p+ and F- cells. Once contact is made, the pilus retracts, bringing the cells into close physical contact. The p+ cell prepares for DNA transfer by assembling a type IV secretion apparatus, using many of the same genes used for sex pilus biogenesis; the sex pilus is embedded in the secretion structure. The F factor then replicates by rolling-circle replication. 0 Conjugation: Transfer of the F Plasmid During rolling-circle replication, one strand of the circular DNA is nicked, and the free 3' -hydroxyl end is extended by replication enzymes. The 3' end is lengthened while the growing point rolls around the circular template and the 5' end of the strand is displaced to form an ever-lengthening tail, much like the peel of an apple is displaced by a knife as an apple is pared. The single-stranded tail may be converted to the double-stranded form by complementary strand synthesis. We are concerned here with rolling-circle replication of a plasmid. However, rolling circle replication is also observed during the replication of some viral genomes (e.g., phage lambda). Rolling-Circle Replication During conjugation, rolling-circle replication is initiated by a complex of proteins called the relaxosome, which are encoded by the F factor. The relaxosome nicks one strand of the F factor at a site called oriT (for origin of transfer). Relaxase, an enzyme associated with the relaxosome, remains attached to the 5' end of the nicked strand. As F factor is replicated, the displaced strand and the attached relaxase enzyme move through the type IV secretion system to the recipient cell. During plasmid transfer, the entering strand is copied to produce double-stranded DNA. When this is completed, the Frecipient cell becomes F+.

 Hfr Conjugation

 By definition, an F+ cell has the F factor free from the chromosome, so in an F+ X F- mating, chromosomal DNA is not transferred. However, within this population, a few cells have the F plasmid integrated into their chromosomes. This explains why not long after the discovery ofF+ X F- mating, a second type ofF factor-mediated conjugation was discovered. In this type of conjugation, the donor transfers chromosomal genes with great efficiency but does not change the recipient bacteria into F+ cells. Because of the high frequency of recombinants produced by this mating, it is referred to as Hfr conjugation and the donor is called an Hfr strain. Hfr strains contain the F factor integrated into their chromosome, rather than free in the cytoplasm. When integrated, the F plasmid's tra operon is still functional; the plasmid can direct the synthesis of pili, carry out rolling-circle replication, and transfer genetic material to an F-recipient cell. However, rather than transferring just itself, the F factor also directs the transfer of the host chromosome. DNA transfer begins when the integrated F factor is nicked at oriT. As it is replicated, the F factor begins to move into the recipient. Initially only part of the F factor is transferred, followed by the donor's chromosome. If the cells remain connected, the entire chromosome with the rest of the integrated F factor will be transferred; this takes about 100 minutes to accomplish. However, the connection between the cells usually breaks before this process is finished. Thus a complete F factor is rarely transferred, and the recipient remains F-.

F' Conjugation

 Because the F plasmid is an episome, it can leave the bacterial chromosome and resume status as an autonomous F factor. Sometimes during excision an error occurs and a portion of the chromosome is excised, becoming part of the F plasmid. Because this erroneously excised plasmid is larger and genotypically distinct from the original F factor, it is called an F' plasmid. A cell containing an F' plasmid retains all of its genes, although some of them are on the plasmid. It mates only with an F- recipient, and F' X F- conjugation is similar to an p+ X F- mating. Once again, the plasmid is transferred as it is copied by rolling-circle replication. Bacterial genes on the chromosome are not transferred, but bacterial genes on the F' plasmid are transferred. These genes need not be incorporated into the recipient chromosome to be expressed. The recipient becomes F' and is partially diploid because the same bacterial genes present on the F' plasmid are also found on the recipient's chromosome. In this way, specific bacterial genes may spread rapidly throughout a bacterial population.

Other Examples of Bacterial Conjugation

 Although most research on plasmids and conjugation has been done using E. coli and other Gram-negative bacteria, conjugative plasmids are present in Gram-positive bacteria belonging to genera such as Bacillus, Streptococcus, Enterococcus, Staphylococcus, and Streptomyces. Much less is known about these systems. It appears that fewer transfer genes are involved, possibly because a sex pilus may not be required for plasmid transfer. For example, Enterococcus faecalis recipient cells release short peptide chemical signals that activate transfer genes in donor cells containing the proper plasmid. Donor and recipient cells directly adhere to one another through plasmid-encoded proteins released by the activated donor cell.

BACTERIAL TRANSFORMATION

The second way DNA can move between bacteria is through transformation, discovered by Fred Griffith in 1928. Transformation is the uptake by a cell of DNA, either a plasmid or a fragment of linear DNA, from the surroundings and maintenance of the DNA in the recipient in a heritable form. In natural transformation, the DNA comes from a donor bacterium. The process is random, and any portion of the donor's genome may be transferred. When bacteria lyse, they release considerable amounts of DNA into the surrounding environment. These fragments may be relatively large and contain several genes. If a fragment contacts a competent cell-a cell that is able to take up DNA and be transformed-the DNA can be bound to the cell and taken inside The transformation frequency of very competent cells is around 10-3 for most genera when an excess of DNA is used. That is, about one cell in every thousand will take up and integrate the gene. Competency is a complex phenomenon and depends on several conditions. Bacteria need to be in a certain stage of growth; for example, Streptococcus pneumoniae becomes competent during the exponential phase when the population reaches about 107 to 108 cells per milliliter. When a population becomes competent, bacteria such as S. pneumoniae secrete a small protein called competence factor that stimulates the production of eight to 10 new proteins required for transformation. Natural transformation has been discovered in some archaea and in members of several bacterial phyla (e.g., DeinococcusThermus, Cyanobacteria, Chlorobi, Proteobacteria, and Firmicutes). Natural transformation occurs in soil and aquatic ecosystems and in vivo during infection; it also is an important route of HGT in biofilm and other microbial community. A competent cell binds a double-stranded DNA fragment if the fragment is moderately large; the process is random, and donor fragments compete with each other. The DNA then is cleaved by endonucleases to double-stranded fragments about 5,000 to 15,000 base pairs in size. DNA uptake requires energy expenditure. One strand is hydrolyzed by an envelope-associated exonuclease during uptake; the other strand associates with small proteins and moves through the plasma membrane. The single-stranded fragment can then align with a homologous region of the genome and be integrated into the chromosome. Transformation in Haemophilus influenzae, a Gramnegative bacterium, differs from that in S. pneumoniae in several respects. H. influenzae does not produce a protein factor to stimulate the development of competence, and it takes up DNA from only closely related species (S. pneumoniae and most other naturally competent bacteria are less particular about the source of the DNA). Double-stranded DNA, complexed with proteins, is taken in by membrane vesicles. The specificity of H. injluenzae transformation is due to an 11 base pair sequence that is repeated over 1,400 times in the H. injluenzae genome. DNA must have this sequence to be bound by a competent cell. The protein complexes that take up free DNA must be able to move it through the bacterial cell wall. As expected, the machinery is quite large and complicated. The protein PilQ aids in the movement across the outer membrane, and the pilin complex (PilE) moves the DNA through the peri plasm and peptidoglycan. ComE is a DNA-binding protein; N is the nuclease that degrades one strand before the DNA enters the cytoplasm through the transmembrane channel formed by ComA.. It is localized to the poles of the cell, and many of the components are similar to those of N. gonorrhoeae: the pilin complex (ComGC), DNA-binding protein (CornEA), nuclease (N), and channel protein (ComEC). ComFA is a DNA translocase that moves the DNA into the cytoplasm. A Gram-negative equivalent of ComFA has not been identified yet in N. Gonorrhoeae. Bacterial Transformation Microbial geneticists exploit transformation to move DNA (usually recombinant DNA) into cells. Because many species, including E. coli, are not naturally transformation competent, these bacteria must be made artificially competent by certain treatments. Two common techniques are electrical shock and exposure to calcium chloride. Both approaches render the cell membrane temporarily more permeable to DNA, and both are used to transform E. coli cells that have been made artificially competent. To increase the transformation frequency with E. coli, strains that lack one or more nucleases are used. These strains are especially important when transforming the cells with linear DNA, which is vulnerable to attack by nucleases. It is easier to transform bacteria with plasmid DNA since plasmids can replicate within the host and are not as easily degraded as are linear fragments .

TRANSDUCTION

The third mode of bacterial gene transfer is transduction, which is mediated by viruses. It is a frequent mode of horizontal gene transfer in nature. Indeed evidence suggests that the number of genes moved by marine viruses from one host cell to another is huge (perhaps 1024 per year}. Furthermore, viruses in marine environments and hot springs move genes between organisms in all three domains of life. virus particles are structurally simple, often composed of just a nucleic acid genome protected by a protein coat called the capsid. Viruses are unable to multiply autonomously. Instead, they infect and take control of a host cell, forcing the host to make many copies of the virus. Viruses that infect bacteria are called bacteriophages, or phages for short. Virulent bacteriophages multiply in their bacterial host immediately after entry. After the progeny phage particles reach a certain

number, they cause the host to lyse, so they can be released and infect new host cells. Thus this process is called the lytic cycle. Temperate bacteriophages, on the other hand, do not immediately kill their host. Many temperate phages enter the host bacterium and insert their genomes into the bacterial chromosome. The inserted viral genome is called a prophage. The host bacterium is unharmed by this, and the phage genome is passively replicated as the host cell's genome is replicated. The relationship between these viruses and their host is called lysogeny, and bacteria that have been lysogenized are called lysogens. Temperate phages can remain inactive in their hosts for many generations. However, they can be induced to switch to a lytic cycle under certain conditions, including UV irradiation. When this occurs, the prophage is excised from the bacterial genome and the lytic cycle proceeds. Transduction is the transfer of bacterial or archaeal genes by virus particles. It is important to understand that host genes are packaged in the virus particle because of errors made during the virus's life cycle. The virion containing these genes then transfers them to a recipient cell. Two kinds of bacterial transduction have been described: **generalized and specialized**.

Generalized Transduction

 Generalized transduction occurs during the lytic cycle of virulent and some temperate phages. Any part of the bacterial genome can be transferred. During the assembly stage, when the viral chromosomes are packaged into capsids, random fragments of the partially degraded bacterial chromosome may mistakenly be packaged. Because the capsid can contain only a limited quantity of DNA, the viral DNA is left behind. The quantity of bacterial DNA carried depends primarily on the size of the capsid. The P22 phage of S. Typhimurium can carry about 1% of the bacterial genome; the Pl phage of E. coli and a variety of Gram-negative bacteria can package about 2.0 to 2.5% of the genome. The resulting virion often injects the DNA into another bacterial cell but cannot initiate a lytic cycle. This phage particle is known as a generalized transducing particle and is simply acarrier of genetic information from the original bacterium to another cell. As in transformation, once the DNA fragment has been injected, it must be incorporated into the recipient cell's chromosome to preserve the transferred genes. The DNA remains double stranded during transfer, and both strands are integrated into the recipient's chromosome. About 70 to 90% of the transferred DNA is not integrated but often is able to remain intact temporarily and be expressed. Abortive transductants are bacteria that contain this nonintegrated, transduced DNA and are partial diploids.

Specialized Transduction

 In specialized transduction, only specific portions of the bacterial genome are carried by transducing particles. Specialized transduction is made possible by an error in the lysogenic life cycle of phages that insert their genomes into a specific site in the host chromosome. When a prophage is induced to leave the host chromosome, excision is sometimes carried out improperly. The resulting phage genome contains portions of the bacterial chromosome (about 5 to 10% of the bacterial DNA) next to the integration site, much like the situation with F' plasmids . However, the transducing particle is defective because it lacks some viral genes and cannot reproduce without assistance. In spite of this, it will inject the remaining viral genome and any bacterial genes it carries into another bacterium. The bacterial genes may become stably incorporated under the proper circumstances. The best-studied example of specialized transduction is carried out by the E. coli phage lambda. The lambda genome inserts into the host chromosome at specific locations known as attachment or att sites. The phage att sites and bacterial att sites are similar and can complex with each other. The att site for lambda is next to the gal and bio genes on the E. coli chromosome; consequently when lambda excises incorrectly to generate a specialized transducing particle, these bacterial genes are most often present. The product of cell lysis (lysate) resulting from the induction of lysogenized E. coli contains normal phage and a few defective transducing particles. These particles are called lambda dgal if they carry the galactose utilization genes or lambda dbio if they carry the bio from the other side of the att.

**MICROBIAL RECOMBINATION**

Bacterial recombination is a type of genetic recombination in bacteria characterized by DNA transfer from one organism called donor to another organism as recipient. This process occurs in three main ways;

* Transformation; the uptake of exogenous DNA from the surrounding environment
* Transduction, the virus mediated transfer of DNA between bacteria
* Conjugation, the transfer of DNA from one bacterium to another via cell-cell contact.

The final result of conjugation, transduction and transformation is the production of genetic recombinants, individuals that carry not only the genes they inherited from their parent cells but also the genes introduced to their genomes by transduction, conjugation or transformation.

Recombination in bacteria is ordinarily catalyzed by a RecA type of recombinase. These recombinases promote repair of DNA damages by homologous recombination. The ability to undergo natural transformation is present in at least 67 bacterial species. Natural transformation is common among pathogenic bacterial species. In some cases, the DNA repair capability provided by recombination during transformation facilitates survival of the infecting bacterial pathogen. Bacterial transformation is carried out by numerous interacting bacterial gene products.