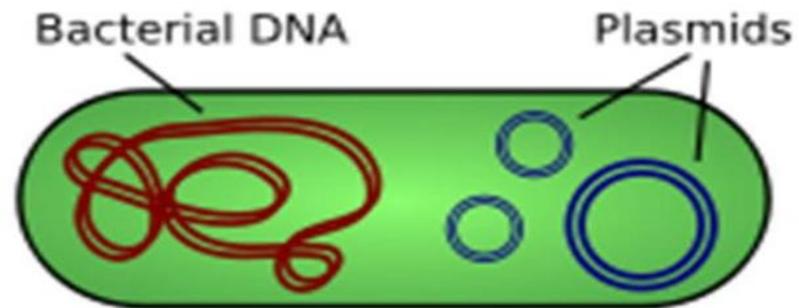


# Microbial Genetics

- Plasmids: Types and their importance
- Mutation: Types and their significance

# Plasmids

- Some bacterial cells contain one or more small circular macromolecules of DNA that store additional specialized information.
- Plasmids contain only 1 – 5% as much DNA as in the bacterial chromosome.
- They confer resistance to antibiotics tolerance to heavy metals.
- Gene products of plasmids may permit the survival of bacteria under conditions that are normally unfavourable for growth



# Types of plasmids

Types of plasmids based on function. There are five main classes:

1. Fertility F-plasmids, which contain tra genes. They are capable of conjugation and result in the expression of sex pilli.
2. Resistance plasmids, which contain genes that provide resistance against antibiotics or poisons. They were historically known as R-factors, before the nature of plasmids was understood.
3. Col plasmids, which contain genes that code for bacteriocins, proteins that can kill other bacteria.
4. plasmids, which enable the digestion of unusual substances, e.g. toluene and salicylic acid.
5. Virulence plasmids, which turn the bacterium into a pathogen.

# Importance of plasmids

- Plasmids used in genetic engineering are called vectors.
- Plasmids serve as important tools in genetics and biotechnology labs, where they are commonly used to multiply (make many copies of) or express particular genes.
- Many plasmids are commercially available for such uses.
- The gene to be replicated is inserted into copies of a plasmid containing genes that make cells resistant to particular antibiotics.
- The gene is also inserted into a multiple cloning site (MCS, or polylinker), which is a short region containing several commonly used restriction sites allowing the easy insertion of DNA fragments

- Next, the plasmids are inserted into bacteria by a process called transformation.
- Then, the bacteria are exposed to the particular antibiotics. Only bacteria that take up copies of the plasmid survive, since the plasmid makes them resistant.
- In particular, the protecting genes are expressed (used to make a protein) and the expressed protein breaks down the antibiotics.
- In this way, the antibiotics act as a filter, selecting only the modified bacteria.
- Finally, these bacteria can be grown in large amounts, harvested, and lysed (often using the alkaline lysis method) to isolate the plasmid of interest.

- Another major use of plasmids is to make large amounts of proteins.
- In this case, researchers grow bacteria containing a plasmid harboring the gene of interest.
- Just as the bacterium produces proteins to confer its antibiotic resistance, it can also be induced to produce large amounts of proteins from the inserted gene.
- This is a cheap and easy way of mass producing a gene or the protein it then codes for; for example, insulin or even antibiotics.

# Mutation

- **Mutation** is an inherited change in the base sequence of the nucleic acid comprising the genome of an organism a mutant.
- A mutant may differ from its parent strain in genotype sometimes in phenotype nutritional mutant that has a requirement for a growth factor an auxotroph wild-type parent a prototroph.
- Mutation can be either **spontaneous or induced**.
- Spontaneous mutation occurs naturally.
- Mutation involving one or a very few base pairs are **point mutations**.
- Mutation involving change in base pairs without causing change in the amino acid **silent mutation**.

Mutation involving change in base pair which codes for a different amino acid is called **missense** mutation. Mutation may result in premature termination of translation resulting in incomplete protein **non-sense** mutation.

Agents that induce mutations are called mutagens chemical or physical agents. Nitrous acid ( $\text{HNO}_3$ ), Hydroxylamine ( $\text{NH}_2\text{OH}$ ) UV and ionizing radiation (x-rays).

# Mutations

	No mutation	Point mutations			
		Silent	Nonsense	Missense	
				conservative	non-conservative
DNA level	TTC	TTT	ATC	TCC	TGC
mRNA level	AAG	AAA	UAG	AGG	ACG
protein level	Lys	Lys	STOP	Arg	Thr

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# Causes of Mutations

- In the living cell, DNA undergoes frequent chemical change, especially when it is being replicated (in S phase of the eukaryotic cell cycle).
- Most of these changes are quickly repaired.
- Those that are not result in a mutation.
- Thus, mutation is a failure of DNA repair

# Significance of mutation

- Gene duplication has occurred repeatedly during the evolution of eukaryotes.
- Genome analysis reveals many genes with similar sequences in a single organism.
- Presumably these paralogous genes have arisen by repeated duplication of an ancestral gene.
- Such gene duplication can be beneficial. Over time, the duplicates can acquire different functions. The proteins they encode can take on different functions; for example, if the original gene product carried out two different functions (see "pleiotropy"), each duplicated gene can now specialize at one function and do a better job at it than the parental gene. But even if they do not, changes in the regulatory sequences of the genes (promoters and enhancers) may cause the same protein to be expressed at different times, at different levels, and/or in different tissues.

- Either situation can provide the basis for adaptive evolution. But even while two paralogous genes are still similar in sequence and function, their existence provides redundancy ("belt and suspenders").
- This may be a major reason why knocking out genes in yeast, "knockout mice", etc. so often has such a mild effect on the phenotype. The function of the knocked out gene can be taken over by a paralog.
- After gene duplication, random loss — or inactivation — of one of these genes at a later time in one group of descendants different from the loss in another group could provide a barrier (a "post-zygotic isolating mechanism") to the two groups interbreeding.
- Such a barrier could cause speciation: the evolution of two different species from a single ancestral species.