BACTERIAL MUTATION; TYPES, MECHANISMS AND MUTANT DETECTION METHODS: A REVIEW

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Abstract

Mutation is a very important concept in biology today that leads to variations in genes. A mutation is a permanent alteration in the sequence of nitrogenous bases of a DNA molecule. The result of a mutation is generally a change in the end-product specified by that gene. In some cases, a mutation can be beneficial if a new metabolic activity arises in a microorganism, or it can be detrimental if a metabolic activity is lost. Mutations can be spontaneous, or induced by a mutagen in the environment. Mispairing is probably mostly due to cellular processes such as Tautomeric shift of bases , oxidative damage to DNA , Depurination and Deamination or caused by "environment", i.e, chemicals, radiation, viruses, diet and lifestyle (Mutagens). Substitution of a nucleotide and Deletion or addition of them is two mechanisms of mutation. Mutation in bacteria has some results such as missense, nonsense, silent, frameshift, lethal, suppressor and conditional lethal mutation. Identifying these mutations requires detection methods. Classic methods i,e, Replica plating, Penicillin enrichment, Ames test, Use of chromogenic substrate and novel tests such as Polymerase chain reaction (PCR) and Gel electrophoresis, Gene probes and Southern blotting, DNA sequencing and DNA microarray are some of these methods that are highlighted in this review.

Keywords: Mutation, Spontaneous and induced mutation, mechanisms of mutation and their results, detection methods

Introduction

Today bacteria are an important tool in the study of genetics and biotechnology, but for 40 years after the rediscovery of Mendel's work and the rebirth of genetics, they were considered too simple to have genes, undergo mutation, or reproduce sexually. This is not surprising - bacteria are so small that it's very difficult to study individuals. Scientists had long observed differences between bacterial colonies, but had never realized that these differences were the results of mutations. Mutations are a very important concept in biology today. Mutations lead to variations in genes. These genes can have a good or bad influence in the characteristics of an organism. Variations are also very important in evolution. Without variations evolution would not be possible, and changes in any part of the environment affecting the organism could make the organism become extinct [1,2,3].

A mutation is any heritable change in DNA sequence. This may, or may not, affect the phenotype of the organism [4,5,6,7,8].   The term "mutation" was coined by Hugo de Vries, which is derived from Latin word meaning "to change"[5].

The process of mutation is called mutagenesis and the agent inducing mutations is called mutagen. (Organisms selected as reference strains are called wild type, and their progeny with mutations are called mutants). Changes in the sequence of template DNA (mutations) can drastically affect the type of protein end product produced [4,7].

Types of mutations

Mutations can be classified by the kinds of alterations in the DNA, or by whether the mutation was spontaneous, or induced by a mutagen in the environment. Mispairing is probably mostly due to cellular processes such as Tautomeric shift of bases , oxidative damage to DNA , Depurination and Deamination or caused by "environment", i.e, chemicals, radiation, viruses, diet and lifestyle (Mutagens)[4,5,6,8,9].

Mechanisms of mutation

a. Substitution of a nucleotide: Base substitution, also called point mutation, involves the changing of single base in the DNA sequence. This mistake is copied during replication to produce a permanent change. If one purine [A or G] or pyrimidine [C or T] is replaced by the other, the substitution is called a transition. If a purine is replaced by a pyrimidine or vice-versa, the substitution is called a transversion. This is the most common mechanism of mutation.

b. Deletion or addition of a nucleotide: deletion or addition of a nucleotide during DNA replication. When a transposon (jumping gene) inserts itself into a gene, it leads to disruption of gene and is called insertional mutation [2, 4,5,6,7].

Mutation Sources

Mutations arise from a variety of sources, including:

Tautomeric shift of bases

Each of the bases in DNA can appear in one of several forms, called tautomers, which are isomers that in the positions of their atoms and in the bonds between the atoms. The keto(C-O) and amino($C-NH_2$) form of each base is normally present in DNA, whereas the imino(C=NH) and enol(C-OH) forms of the bases are rare (Figure 1).Conversion of keto group in thymine & guanine to enol form and changing amino group in adenine & cytosine to imino form, are examples of tautomerization. Naturally, A in amino form pairs with T in keto form, whereas A in its imino form pairs with C and T in enol form match with G [1,6,8] (Figure 2).
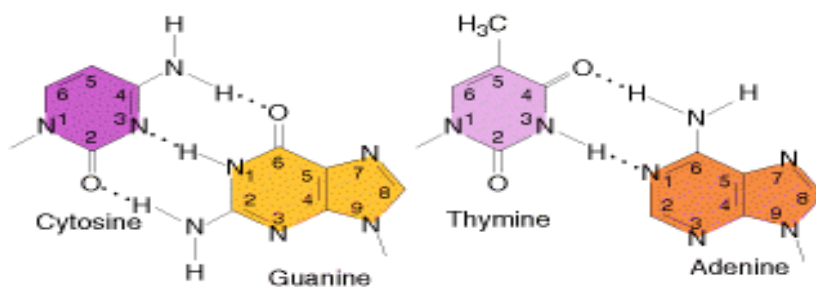

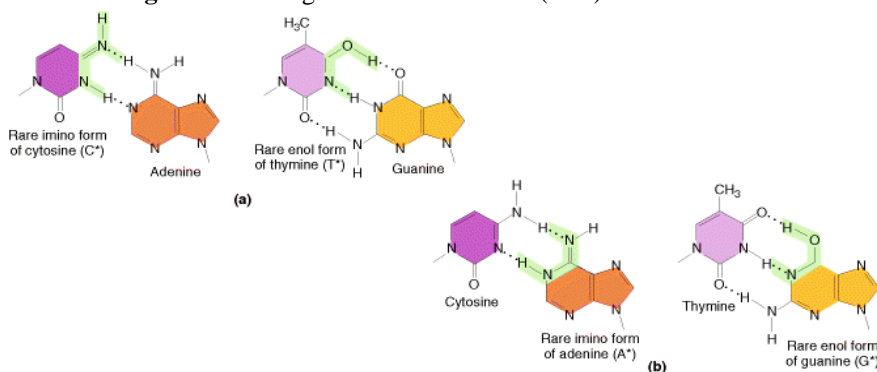**Figure 1 :** Pairing between the normal (keto) forms of the bases.

Figure 2 : Mismatched bases. (a) Mispairs resulting from rare tautomeric forms of the pyrimidines; (b) mispairs resulting from rare tautomeric forms of the purines.

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Depurination

Depurination consists of the interruption of the glycosidic bond between the base and deoxyribose and the subsequent loss of a guanine or an adenine residue from the DNA. in replication, the resulting apurinic sites cannot specify a base complementary to the original purine. So, efficient repair systems remove apurinic sites. Under this certain conditions, a base can be inserted across from an apurinic site; this insertion will frequently result in a mutation [6] (Figure 3) .
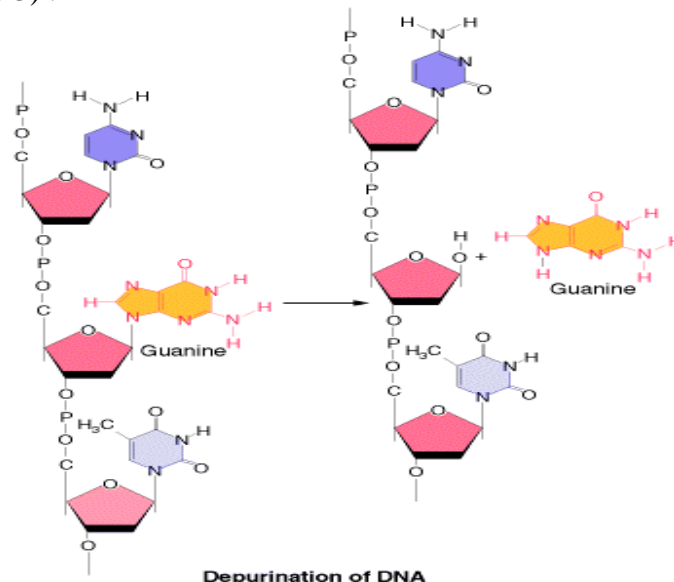

Depurination of DNA

Figure 3 : The loss of a purine residue (guanine) from a single strand of DNA. The sugar-phosphate backbone is left intact.

Deamination

The deamination of cytosine yields uracil. Unrepaired uracil residues will pair with adenine in replication, resulting in the conversion of a G–C pair into an A–T pair (a GC → AT transition). Another example for deamination, is conversion of 5-methylcytosine to thymine [1,2,6] (Figure 4) .
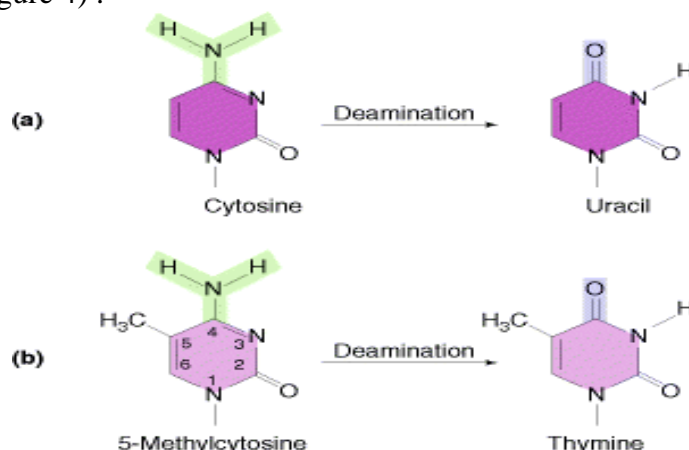

Figure 4 : Deamination of (a) cytosine and (b) 5-methylcytosine.

Oxidatively damaged bases

Active oxygen species, such as superoxide radicals ($O_2\cdot$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH\cdot$), are produced as by-products of normal aerobic metabolism. They can cause oxidative damage to DNA. For example, The 8-oxo-7-hydrodeoxyguanosine (8-oxodG, or GO) product frequently mispairs with A, resulting in a high level of G → T transversions. Thymidine glycol which induced of hydroxyl radical attack to thymine , blocks

DNA replication if unrepaired but has not yet been implicated in mutagenesis [6,10] (Figure 5) .
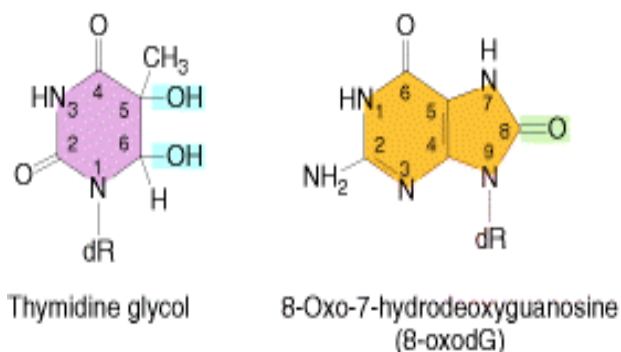

Thymidine glycol   8-Oxo-7-hydrodeoxyguanosine
                          (8-oxodG)

Figure 5 : DNA damage products formed after attack by oxygen radicals.

Ultraviolet irradiation

Ultraviolet light (opt 250 nm) causes bonds to form between adjacent pyrimidine residues (commonly referred to as thymine dimers, although the effect can also occur with cytosine) in the same polynucleotide strand. These are called pyrimidine dimers. UV irradiation can result in the formation of covalent links between pyrimidine dimers (Figure 6) .These bonds distort the DNA conformation and inhibit DNA replication and transcription [1,2,6,7,11].
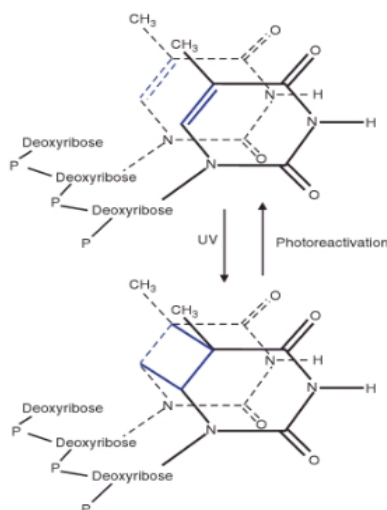

Figure 6 : Structure of thymine dimers.

Chemical mutagens

Variety of chemical mutagens have been identified that are classified into three groups based on their function [1,2,6,8].

First group are those agents that act by chemically modifying a base on the DNA so that it resembles a different base. For example, nitrous acid causes an oxidative deamination in which amino groups are converted to keto groups and thus cytosine residues for example will be converted to uracil. Uracil will be capable of pairing with adenine, thus causing a change from a C–G pair to T–A. Similarly deamination of adenine creates the base hypoxanthines, which will base-pair with cytosine. Some types of chemical agent are not incorporated into the DNA but instead alter a base, causing specific mispairing. They act against the DNA within cells, rather than against isolated DNA. Certain alkylating agents, such as ethylmethanesulfonate (EMS) and nitrosoguanidine (NG), add alkyl groups (an ethyl group in EMS and a methyl group in NG) to many positions on all four bases, mutagenicity is best correlated with an addition to the oxygen at the 6 position of guanine to create an O-6-

alkylguanine. This addition leads to direct mispairing with thymine, and would result in GC → AT transitions (Figure 7) .
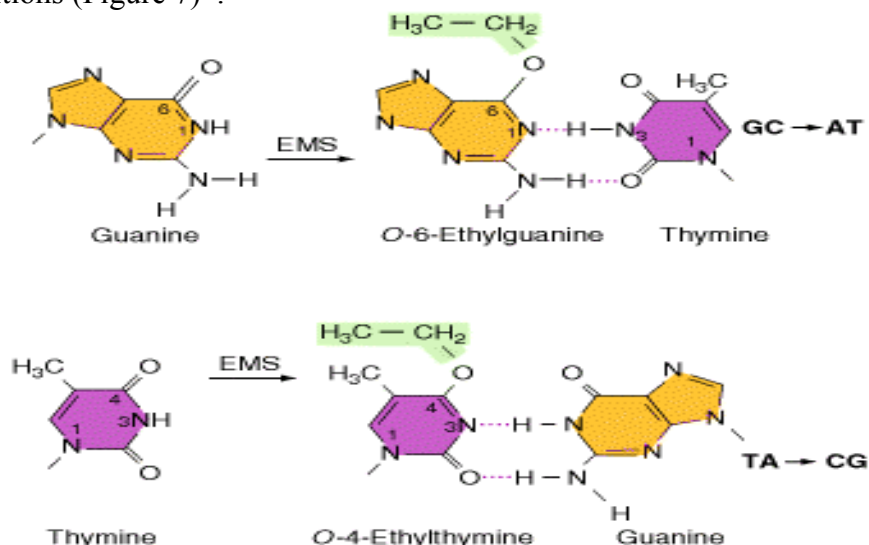

Figure 7 : Alkylation-induced specific mispairing. The alkylation (in this case, EMS-generated ethylation) of the O-6 position of guanine and the O-4 position of thymine can lead to direct mispairing with thymine and guanine, respectively.

Second group are mutagens that induct frameshift formation. The interchalating agents that include proflavin, acridine orange and ethidium bromide are planar molecules, which mimic base pairs and are able to slip themselves in (interchalate) between the stacked nitrogen bases at the core of the DNA double helix. In this intercalated position, the agent can cause single-nucleotide-pair insertions or deletions. Intercalating agents may also stack between bases in single-stranded DNA; in so doing, they may stabilize bases that are looped out during frameshift formation.

Third group are type of agent that acts only against growing cells consists of the base analogues such as 5-bromouracil. Despite its name, this is an analogue of thymine and adenine. 5-BU causes mutations when it is incorporated in one form and then shifts to another form (Figure 8). In its normal keto state, 5-BU pairs like thymine (5-BU$_T$). Thus, 5-BU is incorporated across from adenine and subsequently mispairs with guanine, resulting in AT → GC transitions, but in its enol form, 5-BU pairs like cytosine (5-BU$_C$), so 5-BU is misincorporated across from guanine and subsequently pairs with adenine, resulting in GC → AT transitions (Figure 9).
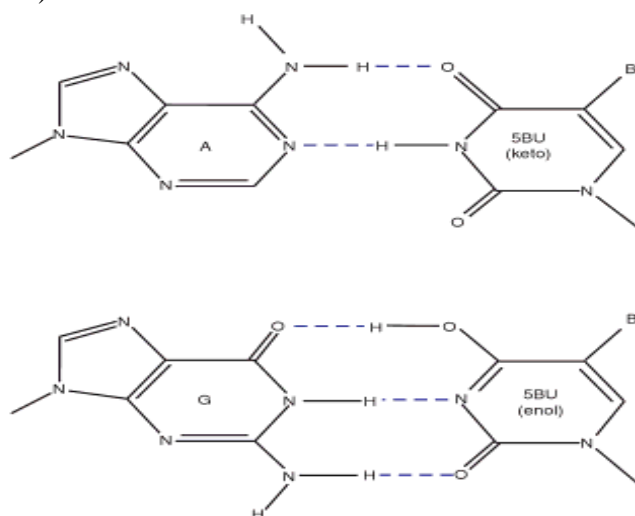

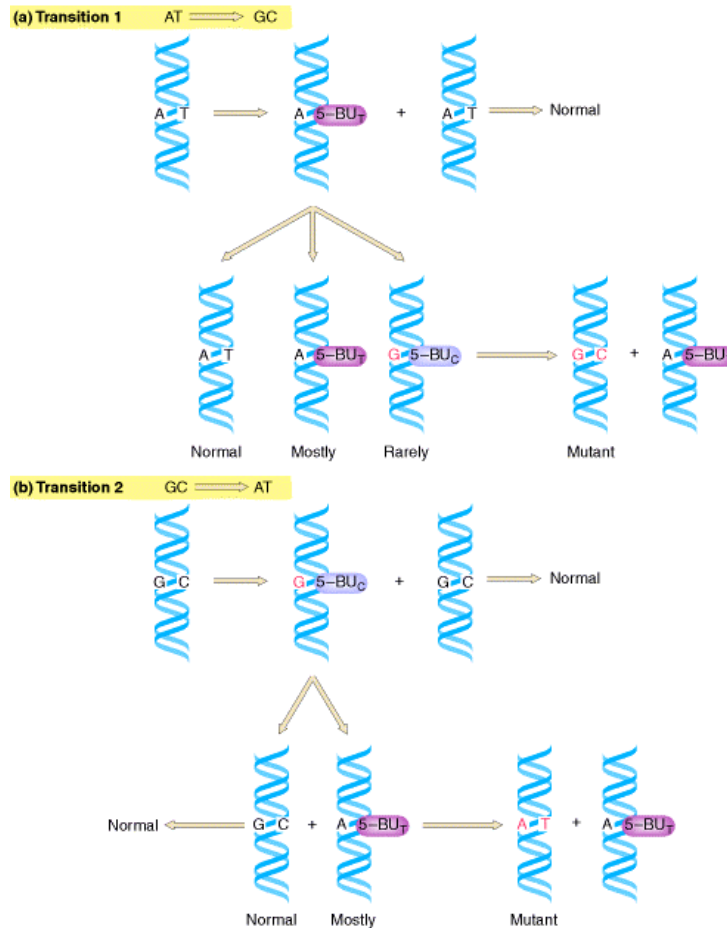**Figure 8 :** Enhanced tautomerism by the base analogue 5-bromouracil.

Figure 9 : The mechanism of 5-BU mutagenesis. (a) In its normal keto state, 5-BU pairs like thymine (5-BU$_T$). Thus, 5-BU is incorporated across from adenine and subsequently mispairs with guanine, resulting in AT → GC transitions. (b) In its ionized form, 5-BU pairs like cytosine (5-BU$_C$). Thus, 5-BU is misincorporated across from guanine and subsequently pairs with adenine, resulting in GC → AT transitions.

Results of mutation

Missense mutation: Missense mutations are DNA mutations which lead to changes in the amino acid sequence (one wrong codon and one wrong amino acid) of the protein product [1,4,5].

Nonsense mutation: A mutation that leads to the formation of a stop codon is called a nonsense mutation. Since these codon cause the termination of protein synthesis, a nonsense mutation leads to incomplete protein products [1,4,5].

Silent mutation: Sometimes a single substitution mutation change in the DNA base sequence results in a new codon still coding for the same amino acid. Since there is no change in the product, such mutations are called silent [1,4,5] (Figur 10).
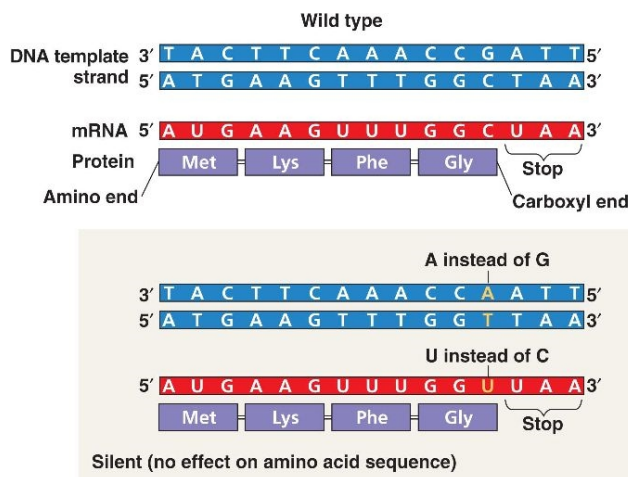
Figure 10 : Mechanism of silent mutation.

Frameshift mutation: Frameshift mutations involve the addition or deletion of base pairs causing a shift in the "reading frame" of the gene. This causes a reading frame shift and all of the codons and all of the amino acids after that mutation are usually wrong. Since the addition of amino acids to the protein chain is determined by the three base codons, when the overall sequence of the gene is altered, the amino acid sequence may be altered as well [1,4,5,6].

Lethal mutation: Sometimes some mutations affect vital functions and the bacterial cell become nonviable. Hence those mutations that can kill the cell are called lethal mutation [5,7].

Suppressor mutation: It is a reversal of a mutant phenotype by another mutation at a position on the DNA distinct from that of original mutation. True reversion or back mutation results in reversion of a mutant to original form, which occurs as a result of mutation occurring at the same spot once again [1,2,4,5,12] (Figur 11).
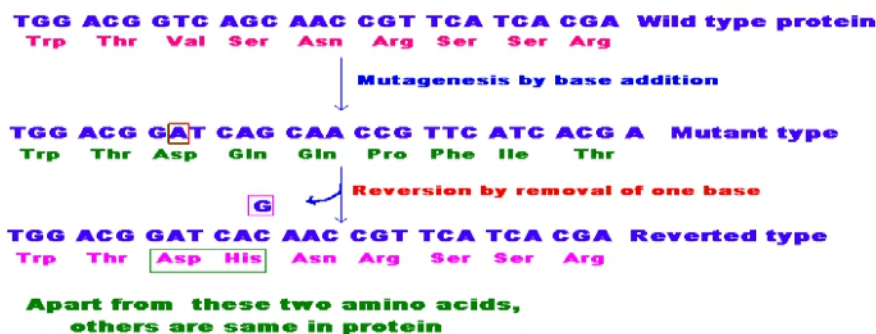

Figure 11 : Mechanism of suppressor mutation.

Conditional lethal mutation: Sometimes a mutation may affect an organism in such a way that the mutant can survive only in certain environmental condition. Example; a temperature sensitive mutant can survive at permissive temperature of 35°C but not at restrictive temperature of 39°C [1,5,7].

Isolation and identification of mutants

In general, changes at the amino acid level in a protein will have a consequential effect on the protein˙s activity, usually detrimental. Thus, an important metabolic pathway

may be blocked by the lack of functional enzyme resulting in identifiable phenotypes [8].This phenotypes can be classified in to three main groups:

1-   mutants that are resistant to antibiotics, or to specific bacteriophages, toxic chemicals or any other agents that are usually lethal or inhibitory to the parent cell;

2-   auxotrophs, i.e. mutants that require some additional growth factor, such as an amino acid;

3-   mutants that are unable to use a particular growth substrate (usually a sugar)[13].

Methods for mutant selection
Replica plating
In this procedure, the mutagenized culture is plated out to obtain single colonies on a nutrient medium on which mutants and parents will grow. After incubation, the colonies are replicated, using a sterile velvet pad, onto a minimal agar plate and then a similar plate to which the appropriate supplement (in this case, histidine, since we are looking specifically for histidine auxotrophs) has been added. Histidine requiring auxotrophs will be unable to grow on the first plate, but will grow on the second one. Thus, mutant colonies can be identified with localization of colonies that have failed to grow in second plate in comparison with first one [1,5,14] (Figur 12).
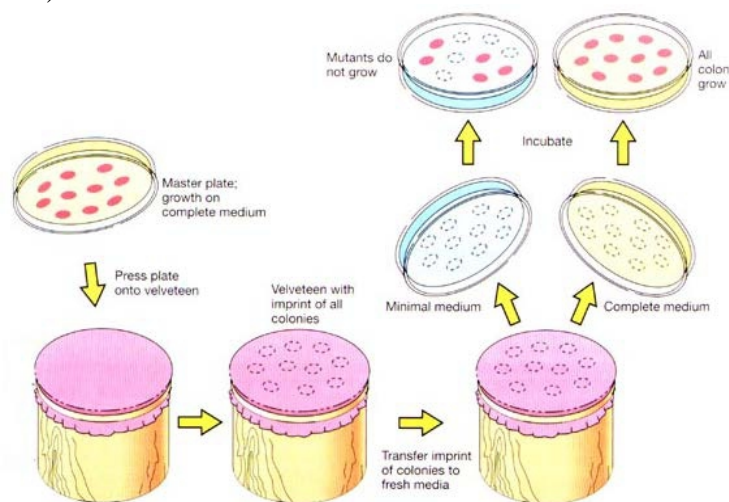

Figure 12 : Replica plating to isolate auxotrophic mutants.

Penicillin enrichment
Some antibiotics (i.e penicillin) acts only against growing bacteria. Cells that are stopped their growth for any reason, relatively not sensitive. Resuspending the bacteria in a minimal medium and adding penicillin will kill the parental cells, which are able to grow, while allowing the non-growing mutants to survive [1,5,14,15,16] (Figur 13).
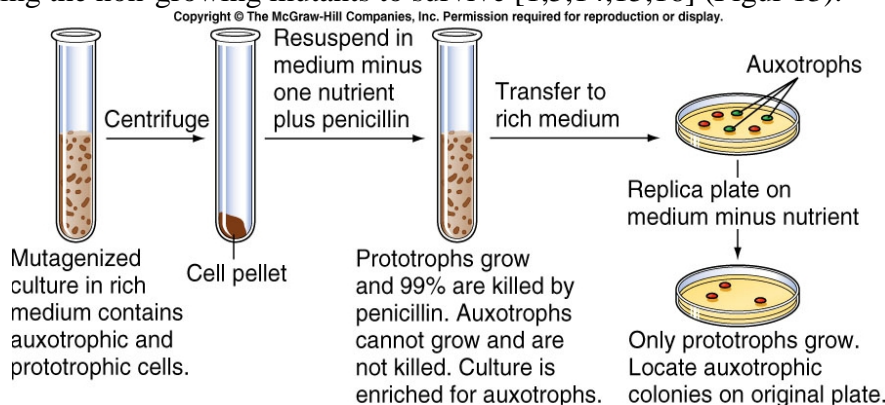

Figure 13 : Mutant screening using penicillin enrichment.

Ames test

The Ames test uses several strains of the bacterium *Salmonella typhimurium* that carry mutations in genes involved in histidine synthesis i.e. it is an auxotrophic mutant, so that they require histidine for growth. The method tests the capability of mutagen in creating mutations that can result in a reversion back to a non-auxotrophic state so that the cells can grow on a histidine-free medium. The bacteria are spread on an agar plate with a small amount of histidine. This small amount of histidine in the growth medium allows the bacteria to grow for an initial time and have the opportunity to mutate. When the histidine is depleted, only bacteria that have mutated to gain the ability to produce their own histidine will survive. The plate is incubated for 48 hours. The mutagenicity of a substance is proportional to the number of colonies observed [7,14 ,17,18] (Figur 14).
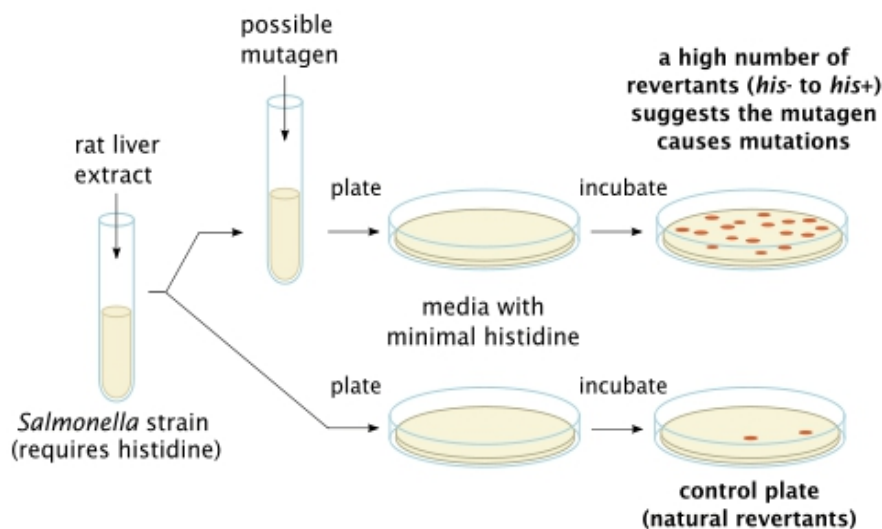

Figure 14 : Ames test procedure.

Use of chromogenic substrate

Mutants that are not able to utilize a particular carbon source (lactose, for example) can be isolated with use a chromogenic substrate that shows an easily detectable color change when acted on by the enzyme concerned. In this case the enzyme is *β*-galactosidase, which catalyses the hydrolysis of lactose into its constituent sugars glucose and galactose. A commonly used chromogenic substrate for *β*-galactosidase is 5-bromo-4-chloro-3-indolyl-*β*-D-galactoside, more popularly known as X-gal. This is a synthetic analogue of the natural substrate, containing a dye linked to galactose. X-gal itself is colorless; the color of the dye is only manifest when it is released by hydrolysis of the linkage by *β*-galactosidase. Lac+ colonies will be blue on a medium containing X-gal and colonies that do not produce *β*-galactosidase will be white[1,19].

Polymerase chain reaction (PCR) and Gel electrophoresis

In this method, target gen (mutant sequence in this case) can be increased exponentially. If the mutation has caused a significant change in the size of a specific gene (such as an insertion or a deletion) it will be detect by a change in the size of the PCR product, using gel electrophoresis [1,20,21,22,23].

Gene probes and Southern blotting

This method involves separating fragments of DNA by electrophoresis in an agarose gel and transferring them to a filter which can then be hybridized with the labeled probe. By using highly specific probes, detection of difference in the sequence is possible [1,14,21,23].

DNA sequencing

DNA sequencing template is amplified using PCR and oligonucleotide primers flanking the region of interest. The amplified fragment is directly cycle sequenced using fluorescent sequencing primers, Sanger dideoxy sequencing chemistry and an enzyme mixture of a mutant Taq DNA polymerase and thermostable pyrophosphatase. The sequence ladders produced are analyzed on a real-time, automated four-color sequencing system. The method produces sequence ladders from unpurified PCR fragments of sufficiently high quality such that heterozygotes can be reproducibly detected and identified by software that recognizes signal-strength patterns indicative of mixed-base positions [1,24,25,26].

DNA microarray

Base-pairing or complementarity is the principle behind this emerging technology. The potential applications of microarray technology are gene expression profiling and identification of gene sequences (including sequences that bear mutations). In this method, a large number of DNA fragments are placed on a glass slide. The fragments are allowed to complement or bind with the labeled DNA (probes), which hybridize with the DNA on the glass slide. The amount of hybridization is then analyzed in each spot on the slide. The genes are given a color where the hybridized ones are colored red and the genes that are hybridized least are colored green. It has been reported that microarray technology could be used in monitoring chromosome gains and losses, tumor classification, drug discovery and development [27,28,29].

Conclusion

Mutations lead to variations in genes that are very important in evolution. A mutation is any heritable change in DNA sequence. Mutation can be beneficial or it can be detrimental. Mutations can be spontaneous due to cellular processes, or induced by a mutagen in the environment. Substitution of a nucleotide and Deletion or addition of them is two mechanisms of mutation. Mutation in bacteria has some results that may, or may not; affect the phenotype of the organism. Several methods have been developed for the mutant detection, of these methods molecular methods Due to the high sensitivity and specificity, and also more time saving than the classical methods is recommended.

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