(A REVIEW)

MUTATION AND ITS ROLE IN BIOTECHNOLOGY

Ismaila Y. Sudi[1] and Umaru Ali-Dunkrah[2*]

1. Department of Biochemistry, Federal University of Technology, Yola. Nigeria
2*. Correspondence author: Chevron Biotechnology Centre, Federal University of Technology, Yola. Nigeria.

Abstract

Mutations is the process by which a gene or chromosome changes; structurally and the end result of that process. All mutations are not harmful' as beneficial mutations occur frequently among various viruses, and bacteria and also in higher organisms. The Biotechnological role of mutations will be reviewed and discussed.

Keywords: Mutations, Harmful, Beneficial, Biotechnology application.

INTRODUCTION

The genetic variability between individuals is what makes us all different from each other. That is, even brothers and sisters with the same parents look similar to each other but there are always significant differences between them (unless they happen to be identical twins). The differences between close relatives are due mostly to a "shuffling of existing genetic material into new combinations. In addition to this, however is a new source of variation that originates from the mutation of existing genes (Allan and Greenwood, 2001). Mutations (which is a term coined by Hugo de Veries in 1900, a rediscover of Mendels principles) is both the process by which a gene or chromosome changes, structurally and the end result of that process (Tamarin, 1999;Weaver, 1999). Small mutations arise at random as the result of physical damage or inherent errors in the replication process. A mutation that increases the chances of survival of the individual increases the likelihood that the mutation will be passed on to the next generation (Voet,

et al, 1999). While most mutations are harmful, there are significant number that are thought to be "Silent" and do not appear to have any effect on the individual. On rare occasions, a mutation may even prove to be beneficial (Allan and Greenwood, 2001; Voet, et al, 1999) and tend to spread rapidly through a population; deleterious changes tend to die along with the organism that harbours them.

In multicelular organisms, mutations are usually notable only when they occur in germ-line cell so that the change is passed on to all the cells of the organism's offspring. Damage to the DNA of a somatic cell, in contrast rarely has and affect beyond that cell, unless the mutation contributes to a malignant transformation (Voet, et al, 1999). The special value of mutations if expressed is that, they form the basis of a comparative study with the normal cells (Wilson and Walker, 1995). Allan and Greenwood (2001) indicated that mutations create new alleles and form an important part of the evolutionary process.

Biotechnology has been defined by different organisation in different ways. But a more unified definition was given by Smith (1996) as "the integrated use of biochemistry, microbiology and engineering sciences in order to achieve biotechnological (industrial) applications capabilities of micro organisms, cultured tissue cells and parts thereof". Shelton (2003) reported that for the past 10,000 years, humans have used selective animals and plants breeding to have desirable characteristics. The result is that the plant we have consumed today would be largely unrecognizable to our ancient ancestors. Scientists consider the techniques of biotechnology to be an aid in the selective breeding to have far more potentials for providing desirable effects.

In essence, this paper intends to dwell on the change(s) in a DNA sequence or cell chromosome and its possible effects/benefits in biotechnological applications.

2.0 MUTATION

A mutation is a change in DNA sequence. It is a misspelling in the code for a protein that can be passed from one generation to the next (Biospace, 2003). It may occur within a gene or in the intergenic regions. If it occurs in the intergenic regions, it will probably be "silent" and hence will have no discernible effect on the cell. However, if it occurs in a gene, it may alter the gene product and generate an observable change in the organism (change in phenotype). An organism displaying the usual phenotype for that species is

called a wild-type" and an organism whose phenotype has been altered by mutation is called a mutant (Brown, 1989). The natural rate at which a gene will undergo a change is normally very low, but this rate can be increased by environmental factors (mutagens), such a ionizing radiations and mutagenic radiations (Brown, 1989; Tamarin, 1999).

2.1 BIOCHEMCAL BASIS OF MUTATION

A mutant is an organism in which either the base sequence of DNA or the phenotype has been changed except for the case of silent mutation, which has no detectable effect on the phenotype of the cell. The chemical and physical properties of each protein are determined by its amino acid sequence and that a single change is capable of inactivating a protein. This was first demonstrated by Vernon Ingram who found that the mutant hemoglobin molecule obtained from patients of sickle cell anemia differed from normal hemoglobin in a single glutamic acid in the normal protein replaced by valine in the mutant. Mutant production can occur as a result of replication errors or can be induced by inserting incorrect base, substitution of tautomerized inserted base having different base-pairing specificity, by deleting one or more bases during replication (Freifelder, 1983., Mays, 1988., Voet, et al, 1999).

2.2 Frequency of Mutations

The natural rate at which a gene will undergo a change is normally very low, but this rate can be increased by environmental factors (mutagens), such as ionising and mutagenic radiations (Brown, 1989; IUPAC 1992; Tamarin, 1999). In humans, it is estimated that an Alu transposition event takes place in every new births today, but may have occurred at a rate of one jump per live birth at one time (Gee, 2001). In general, rates of spontaneous mutation vary between one in 10^4 and one in 10^8 gene per generation. (See table 1)

Table 1: Frequencies of Mutations (Freifelder, 1983).

Genome alteration	Event	Frequency
Very frequent	♦ Lost of various plasmids	10^{-2}-10^{-3}
	♦ Loss of tandem duplication	10^{-1}-10^{-2}
	♦ Occurrence of duplication of a given region	10^{-3}
	♦ Site-specific recombination	10^{-1}-10^{-2}
Frequent	♦ Spontaneous "knock-out" of a gene	10^{-5}
	♦ Transposition of Tn to new site	10^{-4}-10^{-7}
Detectable	♦ Typical spontaneous reversion	Frequent of frame shift
	♦ Missense or nonsense mutation	10^{-4}-10^{-8}
	♦ Precise excision of a transposon	10^{-6}-10^{-9}
	♦ Spontaneous deletion	10^{-6}-10^{-7}
	♦ Transposition of Tn to a particular site	?

Humans inherit 3 x 10^9 base pairs of DNA from each parent. Since no process is 100% accurate, in humans and other mammals, mutations occur at the rate of about 1 in every 50 million (5 x 10^7) nucleotides added to the chain (Allan and Greenwood, 2001; Taylor, et al, 2002). They further said that males contribute more mutations than females and that children of aged fathers suffer more genetic disorders than those of young fathers, but aneuploidy are more apt to arise in eggs than in sperm.

2.3 TYPES OF MUTATIONS

Mutations can be categorised in several ways

2.3.1 Mutation at DNA Sequence

Changes in nucleotide sequence cause alterations within a DNA molecule. These may include;

(i) Point mutation (replacement of one nucleotide by another); which can be transition event, as purine to purine (A-->G) or pyrimidine to pyrimidine (C-->T) or transversion event as punie to prymidine (A or G-->T or C) or vice versa (Brown 1989).

(ii) Insertion/Deletion mutation caused by addition or deletion of DNA base sequence that may result in alteration of the genetic code (frame shift). Insertion and deletions of three nucleotide or its multiples may be less serious because they preserve the reading frame. Insertion mutations destroy the affected region encoded. They typically cause polarity due to their encoding of RNA termination signals and can occasionally provide new promoters reading into the flanking regions (Roberts, 2000). He further stated that a number of inherited human disorders caused by the insertion of triplet nucleotide are Huntington disease and fragile x syndrome. Meissner, et al, (2004) reported that deletion of the intestinal peptide transporter affect insulin and TOR signaling in *C.elagans*.

(iii) Inversion mutation occurs if there is an excision of a portion of the double helix followed by its re-insertion at the same position but in the reverse orientation (Mays, 1988; Brown, 1989).

2.3.2 Mutation at Gene Level

When mutations occurs in an intergenic region whether point, insertion, deletion or inversion may be silent, but if it occurs in the intragenic region, the result is different.

i. Silent mutation within a gene occurs when a point changes takes place at the third nucleotide e.g TTA (leu-->TTG (leu) position of a codon, it changes the codon, but due to degeneracy of the genetic code, the amino acid is not changed. Hence will have no effect on amino acid sequence of the gene

product and will not give rise to mutant phenotype and is not detected without sequencing the gene or its mRNA (Brown, 1989).

ii. **Non wild-type (Missense)** mutations when the base substitution changes cause an amino acid to be inserted and differs from that in a wild-type protein. This can give rise to normally active proteins, inactive proteins, less active proteins, unstable proteins (where the protein is prematurely degraded by proteases within the cell), conditionally active proteins (where the conditional step is either at the level of protein stability or protein synthesis) hyperactive proteins, proteins with new function, charge-altered proteins, proteins that are unprocessed (e.g where processing is required for insertion into or extrusion through the membrane), and finally longer proteins (where the terminal signal is altered). The typical result will be non-functional product (Brown, 1989; Roberts, 2000).

iii. Wild-type (Same sense") mutation:

Where the base substitution mutation causes the same amino acid to be inserted as was found in the wild type. Though such a mutation will probably not affect the phenotype, it just might affect DNA structure, sites for DNA modification, sites involved in mRNA synthesis, sites necessary for mRNA stability or instability because of binding of protective or degradative proteins, changes in mRNA structure and effects on translation through changes in codon choice or context (Roberts, 2000).

iv. Nonsense mutation

With a nonsense mutation, the new nucleotide changes a codon that specified an amino acid to one of the stop codons (TAA, TAG, or TGA). Translation of mRNA from this mutant gene will stop prematurely. The earlier this occurs in gene; the more truncated the protein product (Brown, 1989; Freifelder, 1983). Nonsense mutation yields shortened polypeptide that typically has little or no activity and tends to be highly unstable because of intracellular proteases. Such

mutation often displays some polarity onto transcriptionally downstream genes (Roberts, 2000). An example of nonsense mutation is sickle cell disease.

A-->T transversion at the 17^{th} nucleotide of the gene for the Beta-chain of hemoglobin changes glutamic acid to valine (Mays, 1988).

v. Frame shift mutation

This occurs as a result of insertion event that is not in multiple of three nucleotides. These tend to have a profound effect on a protein product including both loss of function and instability. Since frame shift mutations put ribosome out of the proper reading frame, they often disclose nonsense codons which then results in polarity (Brown, 1989, Roberts, 2000).

2.3.3 Mutation at Organism Level

To provide a mutant phenotype, the nucleotide sequence alteration must give rise to mutated gene products unable to perform its function in the cell. In most cases the cell will not tolerate this loss of functions and will die (lethal mutation). However, there are other mutations that produce phenotype changes, but not lethal.

i Auxothropic mutant lacks a gene product involved in the synthesis of essential metabolite such as amino acids. However, these mutants may be kept alive if supplied with this metabolite as nutrient in culture medium (Brown, 1989). Glimelius et al (1978) also observed that when two parents *N. tabacum* plant that were nitrate reductase deficient mutants and could be grown with nitrate as sole nitrogen source, but their hybrids regenerate shoots in the nitrate medium

ii Conditional lethal mutant can survive if cultured under a particular set of conditions. The most common example is temperature sensitive mutant which are able to survive at one temperature range but die if the temperature is raised above a permissive threshold. The mutated protein is easily denatured and inactivated by heat (Brown, 1989; Bernnet, et al, 1992).

iii Leaky mutant: lethal, auxothropic and conditional lethal mutants can be leaky. The mutated gene product of leaky mutant is that, it produces a different structure but retain the ability to function (Brown, 1989).

2.3.4 **Back Mutations second site reversion and suppression.**

A mutant phenotype can spontaneously or by induction mutate back to the wild-type phenotype although not necessarily to the wild type. This is known as reverse mutation or reversion (Brown, 1989). Reverse mutation has been exploited in development of Ames test (Ames, et al, 1973; McCann, et al, 1975 a,b).

i. Back mutation restores the original nucleotide sequence of DNA molecule. A point mutation can be reversed by second point mutation, an insertion event by subsequent deletion and so on. Back mutations are not very likely unless the site at which the original mutation occurred has some natural predisposition towards mutation.

ii. Second site-reversion restores the original phenotype but does not return the DNA sequence to its precise unmutated wild-type genotype.

For A-->T, changing a leucine codon (TTA) into a codon for Phenylalanine (TTT), changing T-->C (second mutation), becomes CTT (leu) once again. Thus the missense mutation has been corrected and the gene product returned to normal even though the sequence is different from the original (Brown, 1989).

iii. Suppression

Suppression is a second mutation that restores a function lost by the primary mutation. A suppressor mutation that occurs within the same gene is called "intragenic suppressor" and a suppressor mutation that occurs in a different gene is called an "intergenic suppressor" (Hartman and Roth, 1973; Prelich, 1999).

2.3.5. **Recombination**

Recombination and mutation are not related except that both cause alteration to the genetic material and some recombination events leads to phenotype changes that are

grouped as mutations. It results in a re-arrangement in the genetic material of the cell. It happens in several ways that may be quite different. Recombination events occur in Eucaryotes during meiosis and bring about recombination, which scrambles genes of maternal and paternal chromosomes so that non-parental combinations occur in progeny (Voet, et al, 1999; Weaver, 1999).

2.3.6 Transposition

Transposition is the movement of a DNA element from one locus to another, generally requires little, if any, sequence similarity between the recombining DNAs. It is sometimes called "illegitimate recombination"(Weaver, 1999). At times, random movement of DNA causes problems in a genome. Transposable elements often excise and integrate imprecisely, often promoting the joining together of unrelated DNA segement and other DNA rearrangement (Cullum 1985). They have been implicated in various human diseases such as hemophilia A and B, SCID, porphyria, APC mutations leading to Colon Polyps breast cancer and Duchenne muscular dystopy. They can also cause rearrangement of exons, increasing genome diversity by creating new genes (Gee, 2001) Transposable elements are important

in bacteria since they carry genes for antibiotic resistance and may mediate spread of resistance in natural populations.

In higher organisms, the retroviruses, which include some viruses implicated in cancer as well as the AIDS viruses, resemble transposable elements. In the laboratory, they are used as mutagens because they can inactivate genes they transpose onto (polar mutations) or possibly modulate the expression of a gene downstream from an insertion site; this causes a phenotype change indistinguishable from a mutation (Allan and Greenwood, 2001; Cotton, 2001).

2.4 MECHANISM OF MUTATION AND SPONTANEOUS MUTAGENESIS

The production of a mutant requires that a change occur in the base sequence. This can occur by induction with mutagen or spontaneously replication errors (see table 2) or by positive selection for forward mutants using analogues substances.

TABLE 2: Types of Mutagens, Freifelder (1983).

Mutagens	Mode of action	Example	Consequences
Base analogue	Substitutes for a standard base and causes a new base pair to appear in daughter cells in a later generation	5-Bromouracil	A.T→ G.C and G.C → A.T
Chemical	Chemically alters a base so that a new base pair appears in daughter cells in later generation	Nitrous acid Hydroxylamine Ethyl methane sulponate (EMS) NNG Ultraviolet light	G.C → A.T and A.T → G.C G.C→ A.T G.C → A.T, G.C → C.G and G.C →T.A Same as EMS All single changes are possible
Inter-calating agents	Addition or deletion of one or more base pairs	Acridines	Frames shifts
Mutator genes	Excessive insertion of incorrect bases or lack of repair of incorrectly inserted bases	-	All single base changes are possible
None	Spontaneous deamination of 5-methycytosine (MeC)	-	G. MeC → A.T

The mutagens interact directly with DNA molecule by a variety of means, stimulating the introduction of errors.

(1) **Base–analogue mutagens substitutes during replication.**

Examples of base analogue are 5-bromouracil (5-bu) and 2 amino purine (AP). 5-bromouracil is an analogue of T and is expected to base pair with A, it can also undergo a slight change in its chemical structure (tautomeric shift) after it base pairs with G. If this happens during DNA replication, then one of the daughter molecules will have 5-bu G base pair instead of A:T. Another round gives G:C pair in one double helix and 5-bu:G or 5-bu:A in the other daughter helix. This gives rise to point mutation. Another useful base analogue is AP which substitutes for A. AP does not readily tautomerises but forms base pairs with both T and C. Pairing with C is weak but strong enough to allow occasional incorporation of a C during subsequent replication. This gives A:C-->G:C pair transition after two rounds of replication (Freifelder, 1983; Brown, 1989).

(2) **Chemical mutagens**

By a chemical mutagen is meant a substance that can alter a base that is already incorporated in DNA and thereby change its hydrogen-bonding specificity. Four powerful chemical mutagens are nitrous acid (NA), Hydroxylamine (HA) ethyl methane sulfonate (EMS) and N– methyl–N– nitro–N-nitrosoguanidine-NNG (Friefelder, 1983; Mays, 1988; Brown, 1989; Voet, et al, 1999).

The premutational lesion caused by NA is deamination of nucleotides. The amino group removed by NA is replaced with keto group. The deamination of A gives the base hypoxanthine (HX), which has a tendancy to pair with C rather than with T. This change results in

A:T-->HX-->G-->G:C. Thus NA is like the base analogs and can cause transition in either direction.

Hydroxylamine reacts specifically with C and converts it to a modified base that pairs only with A, so that G:C pair ultimately becomes A:T pair. EMS and related MMS; EES, and NNG are alkylating agents that reacts with bases. Other alkylating mutagens include carcinogenic epoxides and mustard gas. The bulky alkylating group evidently cause the DNA to be distorted, thus they are excised and replaced by the DNA repair systems, some of which are error prone.

Alkylation of G or T can actually result in mispairing directly rather than excision and misrepair. All of the alkylating mutagens produce a wide variety of mutations, including transitions and transversion (Mays, 1988; Voet, et al, 1999). Same authors indicated that several types of radiations (X-rays, uv-light, gamma ray) are mutagenic, for instance, uv – radiation of about 260nm wavelength is absorbed by purine and pyrimidines and can cause chemical changes in their structure, by the formation of dimers between adjacent pyrimidine rings in a polypeptide, most often between Ts. Dimerization causes the bases to stack closer together and can give rise to deletions during DNA replication.

(3) Intercalating agents

Acridine orange, proflavine, acriflavine and ethiduim bromide are planar, three – ringed molecules whose dimensions are roughly the same as those of a purine – pyrimidine pair. These substances in aqueous solution form stacked arrays and are also able to stack with a base pair (insertion between two base pairs) called intercalation. This cause frame shift mutation, if it happens within the gene. Since frame shift mutations gives rise to phenotypic changes, intercalating agents are popular mutagens for generating mutants to be used in research (Mays, 1988; Voet, et al, 1999).

(4) Mutagenesis by transposable element.

E. coli and many other organisms contain long DNA segments (hundreds to thousands bp long) that are mobile (transposable). Transposable elements replicate; one replica remains at the original insertion site and the other replica inserted in another region of the chromosome. This process is called transposition. When it occurs, the sequence frequently inserts itself into a bacterial gene, thereby mutating that gene (Voet, et al 1999).

(5) Mutator genes

These are genes when in mutant state cause mutations in other genes. It is believed that polymerase makes occasional errors and corrects these errors by

means of $3'$ or $5'$ exonuclease editing functions. An alteration in the region of the polymerase molecule that reduces or eliminates the editing function constitutes a mutator. Mutations occur when random errors are not corrected.

About 5% of the C in a typical DNA is in the methylated form, 5–methylcytosine (Mec). This is subject to alteration by spontaneous deamination to give 5-methyluracil (another name for T); therefore G:Mec-->G:T, which in subsequent replication yields an A:T pair. Spontaneous deamination can also occur in a non-replicating DNA molecule and the dam – gene product will equally methylate both strands. Thus, the mismatch repair system receives no signal indicating that the G:C pair is the correct one and could just convert the G:T-->A:T pair. Mec has been found to account for most hot spots for spontaneous mutagenesis (Brown 1989; Freifelder, 1983). Although different mutations do not have a selective affinity for certain genes, on a finer scale different mutagens do exhibit characteristically different attack sites and give typical resultant mutations (Mays, 1988). McClintock earlier reported in 1984 that genomic stress also release dominant transposable elements resulting in a mutator activity.

(6) Positive selection for forward mutations

The method was first used in *E. coli* by Alper MD et al (1975) to generate galactose resistance mutants when galactose analogue, 2'deoxygalactose was used as sole carbon source. Many of the resistant mutants were due to insertion sequence element insertion into the *gal* operon (Cullum 1985).

Similar reports were obtained in some gram +ve bacteria eg *Streptomyces* where 2'deoxygalactose resistance mutants were isolated (Kendall 1984, Kendall et al 1987, Umaru Ali-Dunkrah 1988), 2'deoxyglucose resistant mutants (Fisher et al

1987) and selenate mutants (Lydiate et al 1987) were also isolated respectively. Resistant mutants isolated were not IS element associated, but other forms of DNA rearrangement, e.g. deletions (U Ali-Dunkrah 1988 and U Ali-Dunkrah et al 1990) was implicated.

3.0 EFFECT OF MUTATIONS

It is not correct to assume that all mutations are harmful. There are numerous documented cases where a beneficial mutation with a survival advantage has arisen in a population. Such beneficial mutations occur frequently among viruses and bacteria, but also occur in higher organisms as well (Brown, 1989).

3.1 HARMFUL MUTATIONS

There are many documented mutations that cause harmful effects. In humans, there are more than 5,000 physiological diseases in single genes and over one hundred syndromes known to be caused by chromosomal abnormality which arise due to defect in a chromosome or in arrangement of the genetic material on chromosome. The number of genetic disorders that are identified increases every year. Few examples include;

(A) Sickle cell disease/sickle cell anemia, is an inherited disorder that affects hemoglobin and causes the RBC to sickle or become crescent – shaped. This is as a result of autosomal recessive mutation (involves substitution of one base for another in the HBB gene on chromosome 11) that codes for the (beta – chain of hemoglobin) causing a single amino acid to be altered, valine. At least 476-beta globin gene variants exists and several results in life threatening illness (Allan and Greenwood, 2001). Aluoch, (1997) reported that individuals of African, Mediterranean, Caribbean, South and Central American, Arab, and East Indian descent exhibit the highest frequency of risk genotypes. It was also reported by the same author that this high frequency of sickle cell trait (Hb AS) in individuals with African and Mediterranean ancestry have been maintained due to the reduced mortality from

malaria infections when compared with individuals who do not carry the haemoglobin variant (Hb AA).

(B) Thalassaemia is an inherited condition where the genes controlling hemoglobin production are affected (Bornik and Dowlatabadi, 2004).

Beta – thalassaemia is the most common type of thalassaemia in Asia, Middle East and Mediterranean affecting 1% of the population. It is an autosomal recessive mutation of the HBB gene (located on chromosome 11), coding for hemoglobin beta–chains. This may arise through a gene deletion or a nucleotide deletion or insertion. It causes severe anaemia during the first few years of life, patient require frequent blood transfusion to cause iron build up in the heart, and other organs (Allan and Greenwood, 2001). However, co-inheritance of the alpha globin gene variants – located on chromosome 16 (alpha - thalassaemia) in individuals with sickle cell disease appear to be protective against some sickle cell complications such as acute chest syndrome, anaemia and cardiovascular accidents (Gill et, al, 1995; Powars and Hiti, 1993).

(C) Cystic fibrosis or mucoviscidosis is an antosomal recessive gene mutation (Located on chromosome number 7). Over 500 different mutations (deletion, missense, nonsense, terminator codon) of the CFTR gene have been identified. Its outlook could be boosted by gene transfer therapy, inserting normal CFTR gene using adenovirus vectors and liposomes (Allan and Greenwood, 2001).

(D) Huntington disease is a genetic disorder of the central nervous system with symptoms usually appearing in adults within the third and fourth decade of life, although symptoms may occur in individuals younger or older than this. Symptoms include involuntary movements, loss of motor control, dementia or loss of memory and decreased mental capacity (Collins, 1999). The disease is an autosomal dominant mutation of the HD gene (located on chromosome 4) caused by an increase in the length (36–125 against 11–30) of a CAG repeat region. Research is underway to develop drugs that interfere with the Huningin protein. Genetic Counseling and genetic screening of embryos may also be developed into the future (Allan and Greenwood, 2001).

(E) Aneuploidy is a condition of non – disjunction of homologous chromosomes during the host first cycle – reduction division. One or more chromosomes are missing from or added to the normal somatic chromosomes number. Polyploid cells resulting from abnormal mitotic division of primary ocytes and spermatocytes were revealed to affect both somatic and sperm cells (Fedorova, 1997). Faulty gamete production results into a number of medical conditions, which include:

(i) Jacob syndrome is normally seen in males (XYY, and XYYY) apparently normal, tall and aggressive.

(ii) Klinfelter syndrome observed in males (XXY, XXXY, XXXXY) usually sterile, because penis and testis are underdeveloped, resulting in low levels of testosterone and mental retardation increases with the increase in chromosome number.

(iii) Turner syndrome observed in females (XO). If one of the chromosomes is only present once, it is called monosomy. A monosomy in all body cells is mostly not viable except for monosomy of the X – chromosome (Ravenswaaji, 1998).

(iv) Super females, XXX, XXXX, XXXXX,. The significance of this is that it has one active X – chromosome in the cell and the other one (s) form barr bodies (Allan and Greenwood, 2001).

(v) Down Syndrome is a trisomy of the sex chromosome (XXX, XXY, or XYY). It is a pattern of mental retardation and physical abnormalities often including heart attack. Incidence rate is 1 in 800 birth for 30 – 35 years women. The chances of having child with a chromosome inheritance error become greater as women grow older (March and Dimes, 2004; Taylor, et al, 2002).

- Patau syndrome is a trisomy with incidence rate of 1 in 3,000 live births (with maternal age effect). Its feature includes retarded mental and physical development, eye defect, defects of internal organs, polydactyl, malformed ears, low set, cleft lip (CL) and deft palate (CP).
- Edward syndrome is another trisomy with incidence rate of 1 in 5.000 live births (with maternal age effect). Its feature include severe mental

retardation, malformed ears, congenital heart defects small mouth and rocker – bottom feet (Allan and Greenwood, 2001).

3.2 BENEFICIAL MUTATIONS

3.3

Scientists have shown that beneficial mutations do occur to produce brand new alleles (variant of genes) that improve an organism's chance of survival in a particular environment. Examples of beneficial mutations are: -

(a). **Cholesterol Tolerance**

It has been found that about 40 Limone villagers in Italy, showed cholesterol tolerance. They are found to posses a genetic mutation, which produces an altered protein with just one amino acid different from the rest of us (Allan and Greenwood, 2001). It was further indicated by same authors that such individuals has the advantage to tolerate high cholesterol level in their body without developing coronary artery disease.

(b). **Reduced Susceptibility To Disease**

It is believed that the unusual high frequency of sickle cell trait (Hb AS) in individuals with African and Mediterranean ancestry has been maintained due to the reduced mortality from malaria infections when compared with individuals who do not carry the hemoglobin variant (Hb AA). Co-inheritance of the (HB S) variant and alpha globin gene variant (alpha thalassaemia) protect individuals against some sickle cell complications such as acute chest syndrome, anemia, and cardiovascular accidents (Gill, et al, 1995; Powars and Hiti, 1993; Krusz, 1995).

(c). **Blood Clotting**

Congenital factor XIII (FXIII) deficiency is potentially a severe bleeding disorder, but in some cases, the symptoms may be mild. It was reported by Mikkola, et al. (1997) that thymine (T)→cytosine (C) transition at position +6 of intron C in heterozygous for the Arg 661→stop mutation affected splicing of FXIII mRNA resulting in low steady state levels of several variant in RNA transcripts and permit correct splicing of FXIII mRNA. This is a rare example of an inherited

human disorder in which a mutation affecting the splicing still permits some correct splicing to occur and this has a beneficial effect to the phenotype of the patients.

(d). **Treatment of Heart Disease**

(i.) Plasminogen Activator Inhibitor-1 (PAI–1) Plasma levels have been consistently related to a polymorphism of PAI–1 gene and is used as a thrombolytic agent for the treatment of myocardial infarction and studying structure – function relationship (Margaglione, et al, 1998; Wurm, 1991). An insertion/deletion polymophism of angiostensin – converting enzyme (ACE) gene has been related to plasma cellular ACE levels. Gene variants of PAI–1 and ACE account for a significant portion between individual variability of circulating PAI–1 antigen concentrations in a general population without clinical evidence of arteriosclerosis (Margaglione, et al, 1998). Virchow, et al, (1998) when studying C825T polymorphism in the gene encoding for the G beta –3– sub-unit of hetrotrimeric G proteins indicated that the 825T allele is associated with a novel splice variant (G beta–3-s), enhanced signal transduction and enhanced immune cell function in humans. It was also reported by Iacoviello, et al (1998) that the polymorphism involving R 353 Q and hyper-variable region 4 of the factor VII gene showed patients with QQ or H7H7 genotype had decreased risk of myocardial infarction

(ii) Lipoprotein lipase (LPase) is the rate-limiting enzyme in the lipolysis of triglyceride rich lipoproteins and the gene coding for LP is therefore a candidate gene in the artherogenesis. Substitution mutations (Asn 291--> Ser; Asp 9-->Asn and Ser 499-->stop) affect LPase activity, HDL cholesterol and triglycerides levels. This confers a protective effect against the development of *artherosderosis* and coronary artery disease (Galton, et al, 1996).

(e) **Evolution of Unicellular Organism Into A Multicellular Species.**

Mutation accumulates changes and leads to biodiversity. Selective pressure has been observed to convert single cellular forms into multicellular forms. This type

of beneficial mutation was reported by Boraas (1983) to occur as a result of induction by predation of a multicellularity in a strain of *Chlorella pyrenoidosa* (reclassified as *C. vulgaris*). Papadopoulos, et al, (1999) indicated that each population of bacterial strain had different genetic fingerprints from its ancestor over time and tremendous diversity accumulated after 10,000 generations.

(f) **Improvement of Yeast Cells.**

Growth of yeast cell in a chemostat with limited phosphate (due to high pH) allows evolution of a single clonal line of beer yearst with mutations in the permease and phosphatase enzyme. This results in cell clump, which increased population density to improve cell growth (Francis and Hansche, 1973, 1973; Hansche, 1975)

(g) **New Cultural Condition**

Bernnet, et al, (1992) reported that a single clone of *E. coli* cultured at 32^{0C} and another at 37^{0C} for about 2000 generations showed that they are more fit than the ancestor populations to low and high temperatures respectively. This agrees with Krusz (1995) that evolution is due to beneficial mutations that are passed on to offspring, which convey some procreative advantage to the organism.

(h) **Euploidy**

Failure of chromosomes to separate during the process of meiosis or failure of the cell to divide after the chromatids have separated creates double number of chromosomes and may give rise to sterile or fertile offspring (Allan & Greenwood, 2001; King, 1999). An example of such beneficial allolopolyploids is Triticale (McClean, 1997).

(i) **Evolution of New Metabolic Pathway.**

Organisms are capable of evolving whole new metabolic pathways when exposed to new environment rather than just improving the existing pathways.

- **Modifying the fucose pathway to metabolize propanediol.**

  In normal anaerobic *E. coli* metabolism L–fucose is converted to dihydroxyacetone phosphate and lactaldehyde (waste). The lactaldehyde is then converted to propanediol, which is excreted from the cell. When *E. coli* lines are exposed to an aerobic environment rich in propanediol, some are able to utilize the waste as a source of energy. This is made possible by a change to the enzyme that formally converted propanediol to lactaldehyde. The lactaldehyde can then be processed by the previously aerobic pathways that use lactaldehyde as a carbon and energy source (Lin and Wu, 1984)

- **Metabolism of Exotic Five – Carbon Sugars**

  Some five – Carbon Sugars are very rare in nature, so very few organisms have the ability to use these exotic compounds in their metabolism. Hartley (1984) reported that *Klesiella aerogenes* was not able to metabolize D–arabinose and xylitol, but able to evolve such a capability. *K. aerogenes* has no isomerase that converts arabinose to D–rinbulose. However, the isomerase for L–fucose has a low activity for D–arabinose. Mutations occurred in few *K. aerogenes* that allowed the fucose isomerase to be produced at all times – not just when L–fucose is present. In this situation the mutation is a good thing and allows the cell to survive in arabinose environment. Generally spontaneous mutation (with U.V. or chemical) in some bacterial strains use ribitol dehydrogenase in the cell to convert xylitol to D–ribulose for which pathway already exist. This is an example of complete new metabolic pathway being developed through duplication and modification of an existing pathway. This was also confirmed by Brown, et al (1998) that when microbes evolve in a continuos nutrient limited environment, natural selection favours genetic changes that give cells greater access to limiting substrate.

- **Metabolism of Lactose**

  Deletion of an important gene in an *E. coli* allows it to use lactose as a food and energy source (Kenneth, 1999). The evolved beta – galactosidase enzyme of *E.*

coli can hydrolyse lactose and fall into; class I mutants use only lactose and class II mutants use lactulose as well as lactose. Neither class uses galactosylarabinose effectively. Galactosylarabinose utilization can evolve as a consequence of sequential spontaneous mutations and via intragenic recombination in crosses between class I and class II e.g. A^+ mutant strains (Hall and Zuzel, 1980).

- **Nylon Degradation.**

  Frame shift mutations in *Flavobacterium nucleatum* allow it to metabolize nylon waste. Nylon wasn't invented until 1937 and neither did nylon eating bacterium. Examination of DNA sequences of the original bacterium and of the Nylon – eating version (Nylon bug) show identical gene for a key metabolic enzyme, with only one difference in over 400 nucleotides. This single micro evolutionary addition of a single thymine (T) nucleotide caused the bacterium's enzyme to be composed of a completely novel sequence of amino acids via frame shift mutation. Most frame shift of a key enzyme, destroy the enzyme, resulting in immediate death of the organism. This drastic mutation gave an ordinary sugar–eating bacterium, the unusual ability to digest nylon (Susumu, 1984; Thwaites 1985; Sieji, et al, 1992).

4.0 ROLE OF MUTATION IN BIOTECHNOLOGY

Some mutation events are used either directly as novel protein products or adapted in the synthesis of biologically active compounds. Plants can be transformed by infecting them with laboratory-developed mutants whose genes have been replaced with specifically chosen DNA to be transformed into production facilities for "designer foods" pesticides, antimicrobial agents, herbicides, chemical feedstocks, human genes and pharmaceuticals (Lesney, 1999). Valuable approaches of potential mutational applications include:

4.1.0 **Medicine and Pharmaceuticals.**

Over the last decade, scientists registered a considerable progress in identifying antigen therapeutic proteins and their genes for conferring immunity and treatment of disease

4.1.1 a protein produced from deletion of an allele gene of chemokinin receptor–5 protein of the T–lymphocytes for certain strain of human immuno-deficiency Virus type-1 (HIV–1) confers immunity to individuals exposed to HIV virus and slow disease progression in an infected individuals (Dean, et al, 1996).

4.1.2 Alpha polypeptide hemoglobin produced by variant Hb As due to mutation on the alpha polypeptide of hemoglobin molecule in individual with African and Mediterranean ancestry appears to be resistant to malaria infection (Aluoch, 1997).

4.1.3 Mutation in infectious organisms is used to reduce their infective ability. For example, *Helicobactor pylori*, which require flagella for, stomach infection, mutants become non-motile and hence reduced infection (Ottemann and Lowenthal, 2002).

4.1.4 Activase Recombinant Tissue Plasminogen activator (rtPA) developed by systematic clustered amino acid exchange mutation of the entire length of the polypeptide is used as a thrombolytic agent for the treatment of myocardial infarction (Wurm, 1991).

4.2 Agriculture

A wide range of biotechnologies has been developed over the years. Mutational events are now used in crucial areas of crop and animal selection, breeding and reproduction to have desirable characteristics such as improved taste, enhanced yield and pest resistance.

4.2.1 Mutation breeding in local germ plasm of crop plant cells or seed are used for crop improvement/ breeding through easy selection of the desired traits and improve roots nodulation (Nichterleim, 1998; Bhatia et al., 2001 and Jain, 2002).

4.2.2 Euploidy/Allopolyphoids mutations are applied in plant breeding. This leads to the formation of new traits that are not seen in other species. It can also give rise to sterile or fertile offspring. (McClean, 2002).

4.2.3 Spontaneuos mutants derived from their wild type have been used to control diseases. For example, bacterial blight which is caused by *Xanthomonas compestris pv perlorgonii* (the most serious disease of geranium) is controlled by h-(host-range) mutants bacteriophages which attack an extended range of host (Harbaugh, et al, 1996)

4.3 Fermentation Industries.

Induced mutations in the permease and phosphates enzymes of yeast cells for beer fermentation leads to increase cell growth rates (increase fermentation process) and cell clumping, that make cells not easily washed out of chemostat. (Francis and Hansche, 1972, 1973; Hansche, 1975).

4.4 Environmental Sustainability and Management.

For any technology to be considered sustainable, it must not degrade the environment through either the overuse of resources or the creation of unbearable ecological burden (Smith, 1996).

4.4.1 Allopolyploid mutation of *spartina alterniflora*, evolved a new grass S. townsendii. Such grass is widely used in Holland and other places to stabilise dikes (McClean, 2002). This prevents erosion.

4.4.2 Frame shift mutation in bacterium *Flavobacterium nucleatum* makes it able to metabolize nylon waste (an environmental pollutant that is not easily degraded) which constitute a menace to the environment and agricultural lands (Susumu, 1984,Thwaites, 1985, and Seiji *et al.*, 1992).

4.5 Research.

We need to know and understand the structure–function of genes in an organism before attempting to manipulate it for human welfare.

4.5.1 Direct Assay of Potential Carcinogen was difficult and expensive until

after Ames and co developed an indirect method based on reversion of mutation in the histidine operon in the bacterium *Salmonella typlimuruim* (Ames, et al, 1973; McCann, et al, 1975a,b). Ames test, though with many modifications, is the best-known test for mutagens (Hassall, 1990).

4.5.2 Transposable elements have contributed to unlock the potential in the human genome. Now fragmentation is used to build alternative version of protein from the same information by shuffling exons (Gee, 2001).

4.5.3 Manipulation of structure and regulatory elements as well as gene products themselves encompasses recombinant DNA technology, which allows precise changes in manageable segments of the genome with relatively little effort. For example, site directed mutagenesis is a widely used method (Cosby and Lesley, 1997) and homogenotisation mechanism could be used to transfer alleles in both directions between high copy number plasmid and chromosomes (U Ali-Dunkrah 1988).

CONCLUSION/ SUGGESTIONS

The implications of mutations in biotechnology are enormous and yet to be skillfully exploited fully. This could possibly be attributed to the initial held notion that mutation is totally deleterious! However, the vast potential of mutation, using biotechnological approach can be tapped to improve human well fare. To realize this, I strongly suggest the following: -

i. Biotechnologists should research more into beneficial mutations, for example the isolation of biodegrading microbes to help combat environmental pollution and its sustainability.

ii. Government and companies should invest more in biotechnological endeavors.

iii. Government should introduce and fully fund biotechnology courses in all its universities leading to the production of graduates specialized in various areas of biotechnology.

iv. Government should make a legislation that will encourage biotechnology research.

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