STRAIN IMPROVEMENT FOR AMYLASE PRODUCTION BY INDUCED MUTATION

Sangavai C[1] , Roja B[2] , Haseena M[3] , Muthukrishnan K[4]

1Assistant Professor and Head,Department of Biotechnology,Dhanalakshmi Srinivasan College of Arts & Science for Women, (Autonomous),Perambalur.

***corresponding author: sangavai12@gmail.com**

Abstract

Mutations are changes in the nucleotide sequence of an organism's genetic material. Mutations can be caused by copying errors in the genetic material during cell division, exposure to ultraviolet or ionizing radiation, chemical mutations, or viruses, or by cellular processes, such as hyper mutation. In multicellular organisms with dedicated reproductive cells, mutations can be divided into germ line mutations, which can be passed on to offspring via reproductive cells, and somatic mutations, which involve cells outside a specific reproductive group and are not usually transmitted to offspring. In this work the organism producing amylase was isolated from the soil and identified based on morphological and biochemical experiments.

Temperature of various carbon and nitrogen sources, pH changes in amylase production. By using UV, edium bromide and nitrosoquanidine the organism becomes mutated and the amylase production is checked, the genetic DNA mutation is checked and the amylase-producing genes are amplified by PCR.

Key word: Mutation PCR Amylase Biochemical test

Introduction

In biology, mutations are changes in the nucleotide sequence of an organism's gene. Mutations can be caused by copying errors in the genetic material during cell division, exposure to ultraviolet or ionizing radiation, chemical mutations, or viruses, or by cellular processes, such as hyper mutation. Mutations create variation within the gene pool. Natural selection can reduce the frequency of positive (or detrimental) mutations in the gene pool, while more positive (beneficial or positive) mutations can accumulate and lead to adaptive evolutionary changes. For example, a butterfly may produce offspring with new mutations. Most of these mutations have no effect; But one can change the color of one of the butterfly's offspring, This makes it harder (or easier) to spot predators. If this color change is positive, the chances of this butterfly surviving and producing its own offspring are slightly better, and over time the number of butterflies with this mutation may generate a higher percentage of the population.

Neutral mutations are defined as mutations that do not affect a person's fitness. These accumulate over time due to genetic slip. It is believed that the majority of mutations do not have a significant impact on the fitness of an organism. Furthermore, DNA repair mechanisms are able to repair most changes before they become permanent mutations, and many organisms have mechanisms to remove permanently transplanted somatic cells.

Mutation may occurs in various methods like ,By effect on function,By inheritance, *By pattern of inheritance,*By impact on protein sequence,

Frameshift mutation is a mutation caused by the insertion or removal of many nucleotides that are not evenly divided into three parts from the DNA sequence. Due to the triple nature of gene expression by codons, insertion or deletion can disrupt the reading frame or set of codons, resulting in a completely different translation from the original.

Missense mutations or unnamed mutations are types of point mutations where one nucleotide is converted to another amino acid. The resulting protein does not work. Such mutations cause diseases such as epidermolysis pullosa, sickle-cell disease, and SOD1-mediated ALS *(Boillée 2006, p. 39)*

Condition mutation is a mutation that has a wild-type (or less severe) phenotype under certain "permissible" environmental conditions and a mutated phenotype under certain "restricted" conditions.

β Amylase

Amylase is an enzyme that breaks starch down into sugar. Amylase is present in human saliva, where it begins the chemical process of digestion. Foods that contain much starch but little sugar, such as rice and potato, taste slightly sweet as they are chewed because amylase turns some of their starch into sugar in the mouth. The pancreas also makes amylase (alpha amylase) to break down dietary starch into di- and trisaccharides which are converted by other enzymes to glucose to supply the body with energy. Plants and some bacteria also produce amylase. As *diastase*, amylase was the first enzyme to be discovered and isolated (Anselme Payen in 1833). Specific amylase proteins are designated by different Greek letters. All amylases are glycoside hydrolases and act on α-1,4-glycosidic bonds. β-amylase is also synthesized by bacteria, fungi, and plants. Working from the non-reducing end, β-amylase catalyzes the hydrolysis of the second α-1,4 glycosidic bond, cleaving off two glucose units (maltose) at a time.

α-Amylase

The α-amylases are calcium metalloenzymes, completely unable to function in the absence of calcium. By acting at random locations along the starch chain, α-amylase breaks down long-chain carbohydrates, ultimately yielding maltotriose and maltose from amylose, or maltose, glucose and "limit dextrin" from amylopectin. Because it can act anywhere on the substrate, α-amylase tends to be faster-acting than β-amylase. In animals, it is a major digestive enzyme and its optimum pH is 6.7-7.0.

γ-Amylase

(Alternative names: Glucan 1,4-α-glucosidase; amyloglucosidase; Exo-1,4-α-glucosidase; glucoamylase; lysosomal α-glucosidase; 1,4-α-D-glucan glucohydrolase) In addition to cleaving the last α(1-4)glycosidic linkages at the nonreducing end of amylose and amylopectin, yielding glucose, γ-amylase will cleave α(1-6) glycosidic linkages. Unlike the other forms of amylase, γ-amylase is most efficient in acidic environments and has an optimum pH of 3.

Uses

Amylase enzymes are used extensively in bread making to break down complex sugars such as starch (found in flour) into simple sugars. Yeast then feeds on these simple sugars and converts it into the waste products of alcohol and CO_2. This is the reason for long fermented dough's such as sour dough. Modern bread making techniques have included amylase enzymes (often in the form of malted barley) into bread improver thereby making the bread making process faster and more practical for commercial use. Bacillary amylase is also used in detergents to dissolve starches from fabrics.

Materials and methods

All general chemicals used were analytical grade and were obtained from Qualigens (India). Peptone, Beef Extract and other dehydrated media were obtained from Himedia (India). The reconstituted media were sterilized by autoclaving at 121^{o}C for 15min.

Characterization of the pure culture isolates

Staining was done by Gram's staining and Negative staining biochemical tests was done for fermentation of carbohydrates, Catalase activity,Hydrogen sulphide production test, Indole production test, Methyl-red and voges-proskauer tests, Citrate utilization test, Urease test and Growth characteristics

Estimation of protein by biuret method

Dispense the standard BSA solution aliquots 0.2 to 1ml in clean labeled tubes.Makeup the volume to 2ml with distilled water.To each tube,add 3ml of biuret reagent and mix well.A blank was taken without BSA but only biuret reagent and 2ml water. Heat the tubes in boiling water bath for 10 min and cool.Read the OD in a colorimeter at 520nm against a blank. Then take the 0.5ml of supernatant of mutant UV,Ethidium bromide and Nitroso guanidine and makeup the volume to 2ml.Then add 3ml of biuret reagent and read as above.

Quantititive estimation of glucose (Reducing Sugar) by DNS method)

S.D. Glucose solution aliquots 0.1 to 1.0 ml in clean labeled tubes. Make a volume of 2 ml in distilled water. For each tube, add 2 ml of DNS reagent and mix well. A blank is taken without glucose, but only with DNS regeneration and 2 ml of water. Heat the tubes in boiling water for 10 ml and let cool.

Amylase production

Flask fermentation: 30 ml of starch broth was converted into 100 ml cone flask and sterilized. In each of these, individual isolates were inoculated in an orbital shaker at 200 RPM injection at 37oC for 24 h. (Unless otherwise stated and all enclosures were made under the conditions mentioned above.)

Amylase enzyme preparation: After incubation, 2ml of the broth was taken into an appendorff tube and centrifuged at 8000rpm for 15minutes. The supernatant thus obtained was used as a crude enzyme.

Study of amylase activity:

Add 1 ml of 1% starch solution to 1 ml of the enzyme sample and soak in water at 37 C for 30 min. A blank should be maintained with 1 ml of 1% starch solution and 1 ml of distilled water. Add 1 ml 2 n NaOH to stop the enzyme reaction immediately after maturation. Add 1 ml of DNS regenerator to each tube and place in a boiling water bath for 10 minutes and cool in tap water.

NOTE: 1 unit of amaylase activity is expressed as the amount of enzyme which produced 1Mm/minute of reducing sugar with glucose and standard.

Evaluation of reducing sugars

The 1 ml sugar sample was transferred to test tubes containing 1 ml DNS regenerated and placed in a water bath at 100oC for 10 min. After incubation, the volume in each tube was prepared in distilled water up to 5 ml and allowed to cool to room temperature and the absorption was read at 540nm.

Effect of substrate concentration

To find the optimal concentration of the substrate for the production of amylase, 30 ml of starch broth containing soluble starch of varying concentration (1–5% w / v) was prepared and sterilized, and this 1% active inoculum was added and incubated. The optimum concentration of the substrate that can be used for amylase production was then determined by making an amylase estimate of the fermented broth.

Effect of temperature

Optimum temperature required for production of amylase was determined as follows. 30ml of starch broth was prepared in flasks and sterilized. The flasks were incubated at different temperatures with 1% inoculum.

Effect of pH

Optimum pH required for production of amylases was determined as follows. 30ml of the starch broth was prepared and sterilized. The pH of the media was adjusted to 3-8 using 1N NaOH and 0.5N H_2SO_4. To these, 1% active inoculum was added aseptically and incubated. Amylase assay of fermented broth was then performed.

Conformation test for amylase production:

Starch agar test:

Prepare the starch agar medium after autoclave, inoculate the organism by streak plate technique, and incubate at 37°C in incubator. After incubation then add few drops of iodine and observed the color change.

Effect of various carbon sources:

The effect of various carbon sources on amylase production was observed by incubating the culture in 50 ml production medium-1 containing various carbon sources like maltose, Glucose, Galactose, dextrose, Lactose, Xylose etc. in 250 ml conical flasks, incubated with active culture of *Bacillus cereus* strain. The incubation was carried out for 48 hour and the amylase production was estimated at an interval of 24^{th} and 48^{th} hour using the procedure mentioned earlier.

Effect of Nitrogen sources:

The influence of various nitrogen sources on amylase production was investigated by using different nitrogen sources at a concentration of 2gm /l in the medium. The various nitrogen sources tested are Casein, Peptone, Yeast extract, and Urea. The production medium with these nitrogen sources was inoculated with active culture of *Bacillus cereus* and incubated at 37^0 C for 24 hrs on shaker at 250 rpm / min.

Comparative studies of mutant bacillus cereus

Production of enzyme amylase by mutant bacillus cereus like u.v mutant and different concentration of ethidium bromide and mutagenic compound Nitroso guanidine.

Estimation of protien by biuret method:

Maintain different concentration of protein as a standard values. Maintain the over night L.B broth with different mutagenic compounds and inoculated the organism ***Bacillus cereus***. Centrifuged at 10,000 rpm 10min. take the supernant and make up to 2ml with distilled water. 3ml of biuret reagent added all the tubes. Incubated at room temperature at 10 min. Finally the absorbents read at 520nm**.**

Genomic DNA isolation:

Grow the bacterial culture overnight in nutrient broth. Transferred 2 ml to a 2-ml micro centrifuge tube and spin 2 min. Decanted the supernatant. . Resuspended the pellet in 500 µl TE buffer by repeated pipetting. Added 30 µl of 10% SDS and 3 µl of 20 mg/ml proteinase K, Lysozyme, mix, and incubated 1 hr at 37 ° C. Added an equal volume of phenol/chloroform and

mix well but very gently to avoided shearing the DNA by inverting the tube until the phases are completely mixed. Carefully transferred the DNA/phenol mixture into a Phase Lock Gel^{TM} tube (green) and spin at 12,000 RPM for 10 min. The upper aqueous phases were transered to a new tube and add an equal volume of phenol/chloroform. Again mixed well and transfered to a new eppendorf tube and spin 10 min. Transfered the upper aqueous phase to a new tube. Added 1/10 volume of sodium acetate and 10 micro liters of RNAase and Mix well. Added 0.6 volumes of isopropanol and mix gently until the DNA precipitates. DNA was washed by dipping end of rod into 1 ml of 70% ethanol for 30 sec. Resuspend DNA in at least 200 µl TE buffer. After DNA has dissolved, determine the concentration by measuring the absorbance at 260nm and 280nm.

Polymerase chain reaction:

Forward primer

5'-ATCTGAATTCATCGGTACAA-3'

Reverse primer

5'-GTACAAAAAGAAGTAGCC-3'

PCR Mixture

The PCR mixtures were prepared with H_2O (Mili-Q grade), 2 µl of 20 pmol of both forward and reverse primers, 1µl of 10 mM dNTP, 5 µl of 1U Taq DNA polymerase, 5 µl of 10X PCR buffer, 4.0 µl of 25 mM $MgCl_2$, 1µl DNA Sample. Water was added to adjust the final reaction volume to 50µl.

PCR Conditions

Amplification with both the primers was optimized and is as follows:

95^0 C for 5 min	(Initial denaturation step)
94^0 C for 2 min	
56^0 C for 2 min	35 cycles
72^0 C for 1 min	
72^0 C for 7 min	(Final extension step)

PCR Products were analyzed with 2% Agarose gel electrophoresis.

Result

Colonies of amylolytic bacterial isolates grown in starch agar medium by streak blade method. (Plate 1) .One gram of stained violet stems were found. From the above observation it is said to be one gram positive bacteria (plate 2). Negative staining spherical cells occurring in clusters appear transparent (colorless) against a red posterior ground. From this observation it is said to be a capsulated organism (plate 3).

Fermentation of Carbohydrates

After 48 hours of incubation, it was possible to see that the sugars that are glucose are used by the organism and the acids are produced. No acid formation was found in sucrose. The organism produced the acid using all the glucose and sugar, so it was positive. This is negative for sucrose (plate 4).

Catalase activity

After 24 hours of incubation when four drops of hydrogen peroxide was added to the slants slow appearance of gas bubbles was observed. After the addition of hydrogen peroxide gas bubbles

were observed which is the indication of positive test **(Plate 5).**

Hydrogen sulphide production test

After 24 hours of incubation, no black spot was seen on the puncture line. No black color was found on the punch line. Therefore The organism may be H2S negative (plate 6).

Indole production test

After 24 hours of incubation development of cherry (deep) red color in the top layer of the tube is not observed. As development of cherry red color is not observed in the top layer of the tube It was negative **(Plate 7).**

Methyl-Red and Voges-Proskauer tests

After the incubation of tubes for 3 days the tube in which methyl red was added pink color was observed and after 2 days of incubationin the V-P test tubes in which V-P reagents I & II were added no red color was observed.

MR test result:

As in the methyl red test pink color is observed hence, it is positive test **(Plate 8 a).**

VP test results:

In the VP test no red color was observed hence, it is Negative test **(Plate 8 b).**

Citrate utilization test

After 24 hours of incubation it was observed that there is no change in the medium color. From this observation it is concluded that it was negative to this test **(Plate 9).**

Urease test

After 24 hours of incubation few drops of phenol red indicator was added and it was observed that there is formation of pink color. From the above observation it was concluded that it is positive test **(Plate 10).**

Biuret method

From the protein estimation it was observed that mutant of Ethidium bromide gave the better result i.e 0.921,then result for mutant of UV was0.820 and for Nitroso guanidine was 0.520 **(Table 1,Graph 1).**

Dns method

Amylolytic bacteria obtained were further tested for quantitative amylase activity using soluble starch as a substrate in flask level fermentation. The extra cellular enzyme was collected by centrifugation; amylase activity was performed after 24hrs.

Optimization of different fermentation parameters like temperature, pH of the media is necessary for efficient production of amylases. Hence a study was made to know the effect of above parameters on amylase production using the *Bacillus cereus* with best substrate, soluble starch **(Plate 11).**

EFFECT OF SUBSTRATE (SOLUBLE STARCH) CONCENTRATION ON AMYLASE Production

Starch (soluble) as substrate taken and concentration (%w/v) of substrate plays an important role in the amylase production. The production of amylase by the selected isolates RF4 in the presence of various concentrations of starch was estimated. The production of enzyme was maximum with 3%(w/v) soluble starch, enzyme activity was 90 U/ml **(Table 2, Graph 2).**

Effect of temperature on amylase production

Some amylases are thermo-stable in nature. The enzyme production by related bacteria was highest at 40^{o}C which produced 39U/ml.(4.13). When tested with variation in temperature

effect the enzyme production. The optimum temperature range is 30-40 ^{0}C for highest enzyme production **(Table 3, Graph 3).**

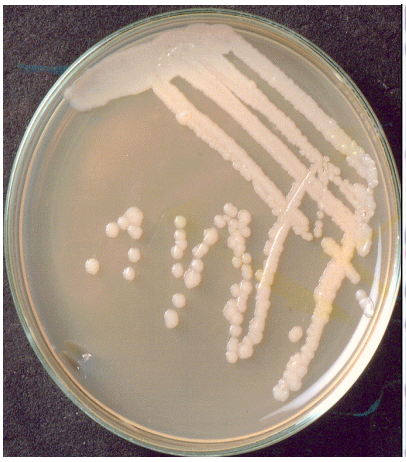
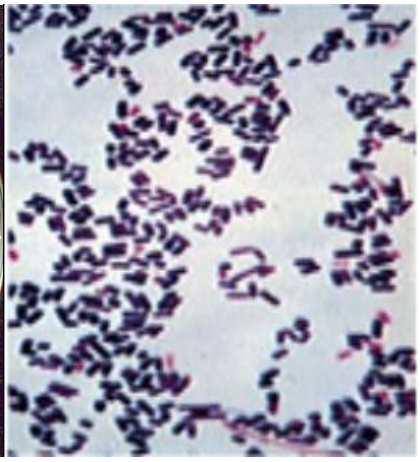
Effect of pH on amylase production

pH of the growth medium plays an important role by inducing the morphological changes in the organism and in enzyme secretion. The production of amylase is very sensitive to pH of fermentation medium. The enzyme production was highest at pH 7.0, which yielded 78 U/ml (Fig 15). When tested with variation in pH results from substrate consumption (e.g. protein hydrolysis) and/or metabolite production (e.g. organic acids). Results indicate that enzyme production was generally stable at pH range from 6-8 which indicates good buffering property of the substrates used for fermentation **(Table 4, Graph 4).**

Starch agar test:

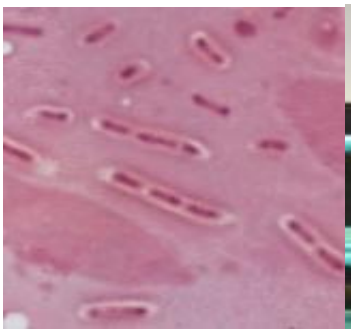
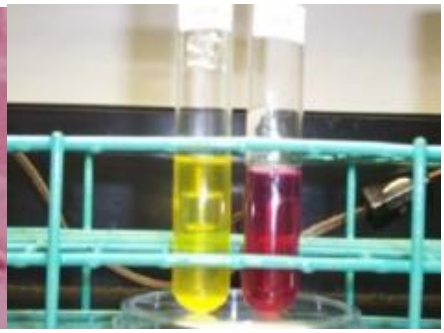
Amylolytic bacterial colony in Starch agar medium stained with iodine solution. Zone of starch hydrolysis can be seen around the colony with halo **(Plate 12).**

PLATE-1;STREAK PLATE TECHNIQUE **PLATE-2: GRAM STAINING**

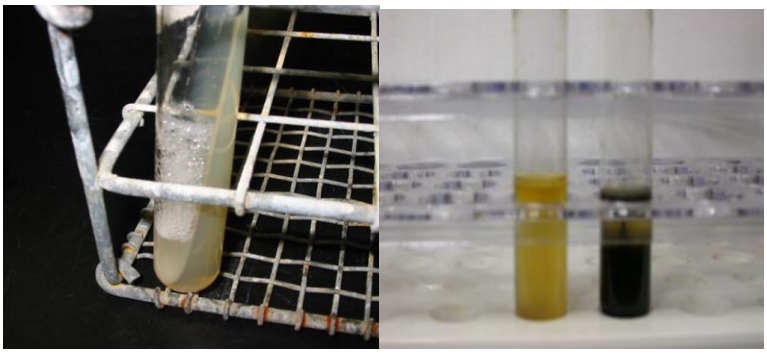
Violet colour rods-Gram positive bacteria

PLATE-3: NEGATIVE STAINING, PLATE-4: FERMENTATION OF CARBOHYDRATES

Capsulated organism **A** B **(**A: negative; B: Positive**)**

PLATE 5: CATALASE ACTIVITY **PLATE 6: HYDROGEN SULPHIDE PRODUCTION TEST**

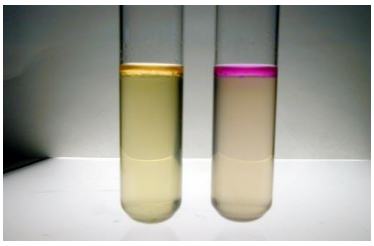


Bubbles were observed (Positive). A B

(A. Left side negative test; B. Right side positive test)

No black colour (Negative).

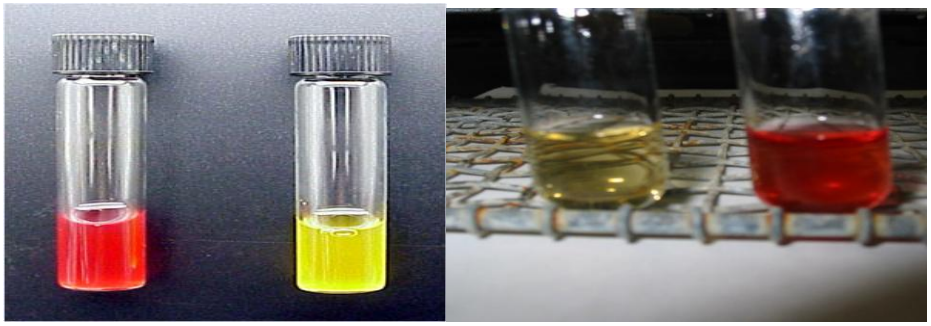
PLATE 7: INDOLE PRODUCTION TEST



A B **(**A: negative. B: Positive**)**

No cherry red colour on top layer (Negative).

PLATE 8a: METHYL RED TEST **PLATE 8b: VOGES-PROSKAUER TEST**



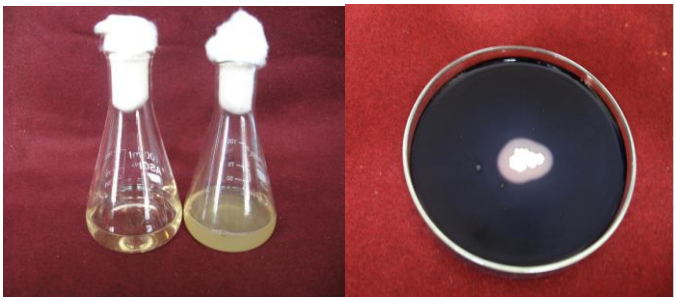
A. **B** A B (A- Negative B- positive)

Pink color was observed (Positive).

PLATE 9: CITRATE UTILIZATION TEST **PLATE 10: UREASE TEST**

A B A B (A – Positive.B – negative)

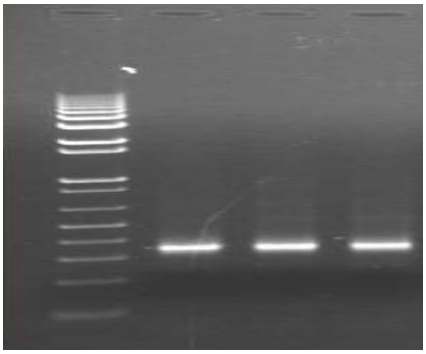
PLATE 11: DNS METHOD PLATE 12: STARCH AGAR TEST



A B A B

Fermentation broth containing 1% soluble starch used for amylase production in flask fermentation .A-Sterile broth broth before inoculation
B-Fermented broth with bacterial (*Bacillus cereus*) growth after incubation. Amylolytic bacterial colony in Starch agar medium stained with iodine solution. Zone of starch hydrolysis can be seen around the colony with halo.

FIGURE 1:GENOMIC DNA ISOLATION



M(10kb) DNA (5KB)

FIGURE 2:PCR M(5kb) DNA(2kb)

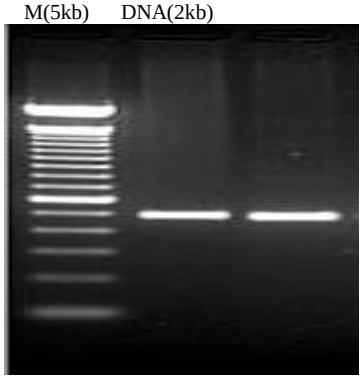
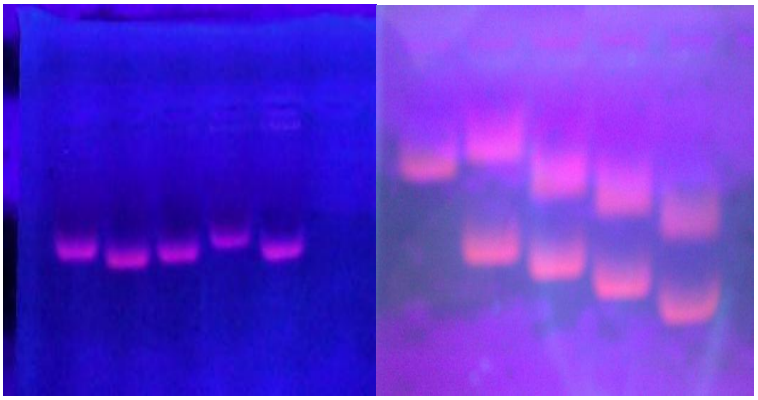


FIGURE 3: UV MUTANT *Bacillus cereus* DNA **IGURE 4: ETHIDIUM BROMIDE MUTANT *Bacillus cereus* DNA**

1 2 3 4 5 1 2 3 4 5



Here
1: normal *Bacillus cereus*
2: 0.2% Ethidium bromide mutant *Bacillus cereus*
3: 0.4% Ethidium bromide mutant *Bacillus cereus*
4: 0.6% Ethidium bromide mutant *Bacillus cereus*
5: 0.8% Ethidium bromide mutant *Bacillus cereus*

1: normal *Bacillus cereus*
2: 5min U.V mutant *Bacillus cereus*
3: 10 min U.V mutant *Bacillus cereus*
4: 15min U.V mutant *Bacillus cereus*
5: 20min U.V mutant *Bacillus cereus*

NITROSO GUANIDINE MUTANT *Bacillus cereus* DNA

1 2 3 4 5

1: normal *Bacillus cereus*
2: 1% Nitroso guanidine mutant *Bacillus cereus*
3: 2% Nitroso guanidine mutant *Bacillus cereus*
4: 3% Nitroso guanidine mutant *Bacillus cereus*
5: 4% Nitroso guanidine mutant *Bacillus cereus*

TABLE 2: ESTIMATION OF GLUCOSE STANDARDS BY DNS METHOD

Glucose concentration	O.D. at 520nm
100µg	0.042
200 µg	0.140
400 µg	0.240
600 µg	0.330
800 µg	0.420
1000 µg	0.520

GRAPH 2:Glucose (mg/ml) standard curve by DNS method (standard concentration taken was 1mg/ml).

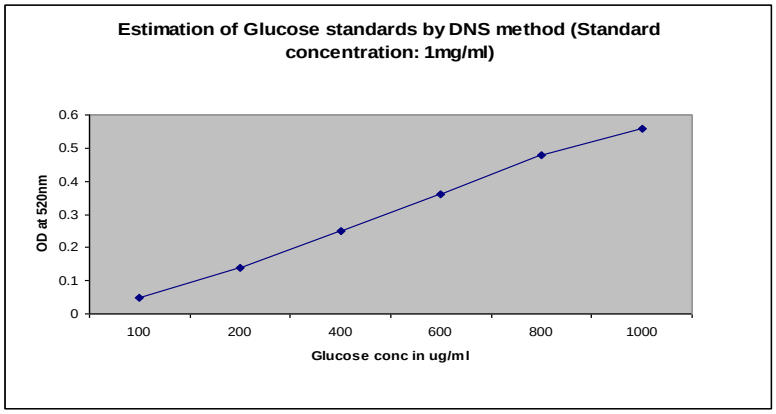


TABLE 3: EFFECT OF SUBSTRATE (SOLUBLE STARCH) CONCENTRATION ON AMYLASE PRODUCTION

Concentration of substrate	Enzyme activity U/ml
1%	70
2%	75
3%	94
4%	80
5%	78

GRAPH 3:Effect of substrate (soluble starch) concentration on amylase production by *Bacillus cereus.*

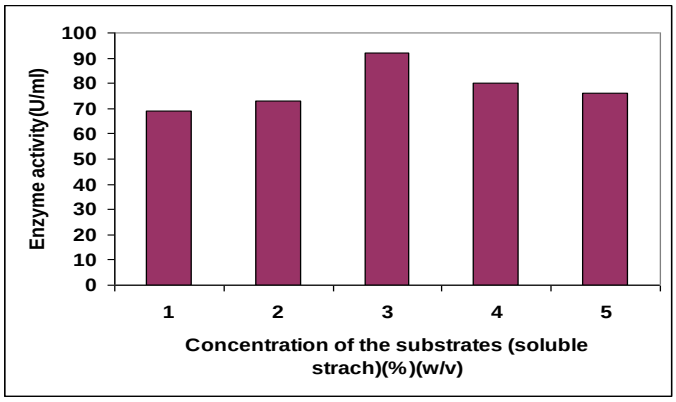
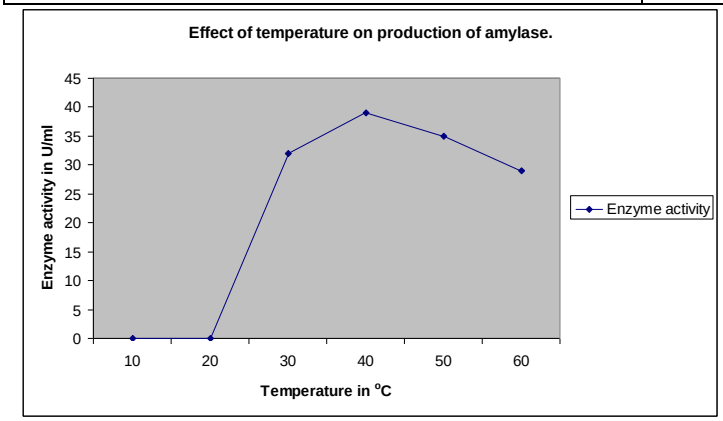


TABLE 4: EFFECT OF TEMPERATURE ON AMYLASE PRODUCTION

Different temperatures	Enzyme activity (U/ml)
20°c	04
30°c	30
40°c	39
50°c	37
60°c	27

GRAPH 4: Effect of temperature on amylase production

Different pH	Enzyme activity(U/ml)
4.0	25
5.0	30
6.0	35
7.0	40
8.0	45



Optimum temperature: 40 °C; Maximum enzyme activity: 39U/ml

TABLE 5: EFFECT OF pH ON AMYLASE PRODUCTION

GRAPH 5: Effect of pH medium on amylase production by *Bacillus*

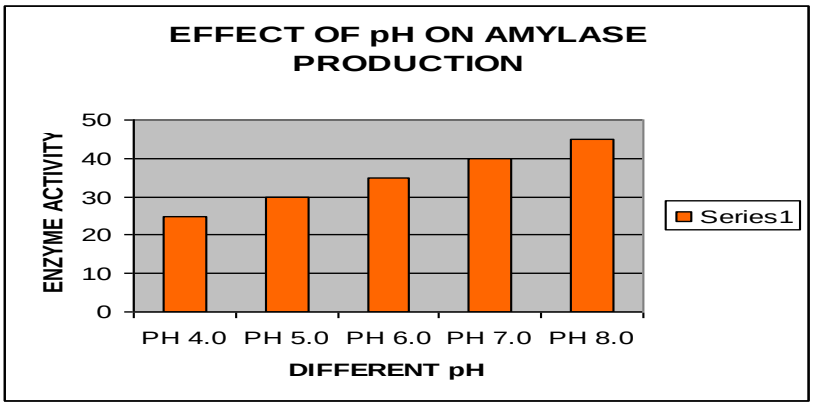


TABLE 6:EFFECT OF DIFFERENT CARBON SOURCE ON PRODUCTION OF ENZYME AMYLASE

Different carbon source	O.D at 600nm
Glucose	0.621
Galactose	0.059
Maltose	0.425
Dextrose	0.528

GRAPH 6: Effect of carbon source on amylase production

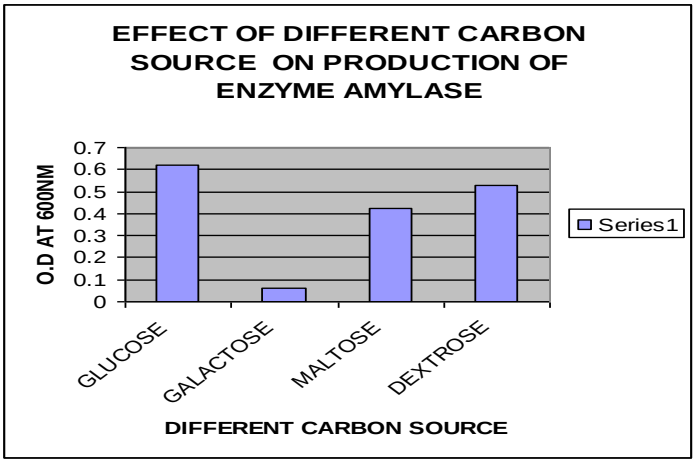
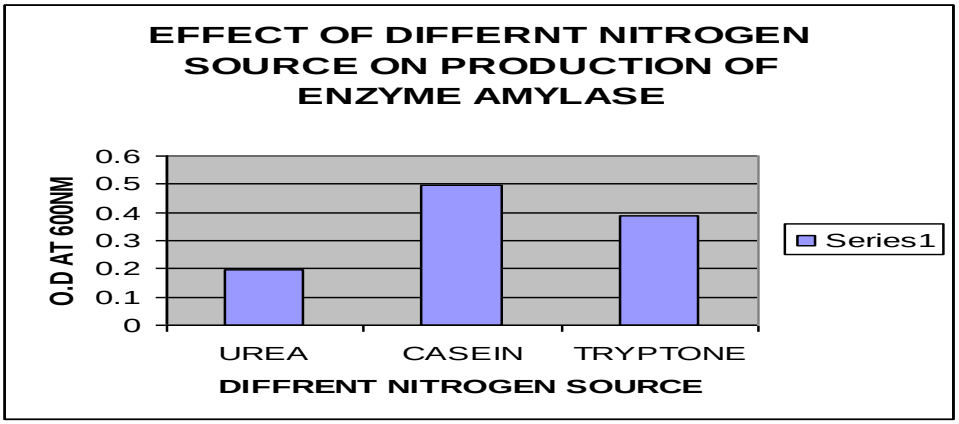


TABLE 7: EFFECT OF DIFFERENT NITROGEN SOURCE ON PRODUCTION OF ENZYME AMYLASE

Different Nitrogen Source	O.D at 600nm
Urea	0.198
Casien	0.497
Tryptone	0.385

GRAPH 7:Effect of nitrogen source on amylase production



COMPARATIVE STUDIES ON MUTANT *Bacillus cereus*
TABLE 8: PRODUCTION OF ENZYME AMYLASE BY U.V MUTANT *Bacillus cereus*

Type Of *Bacillus cereus*	O.D at 600nm
Normal culture	0.607
Mutant 5min	0.642
Mutant 10min	0.623
Mutant 15min	0.608
Mutant 20min	0.614

GRAPH 8: Production of amylase by uv mutant *Bacillus cereus.*

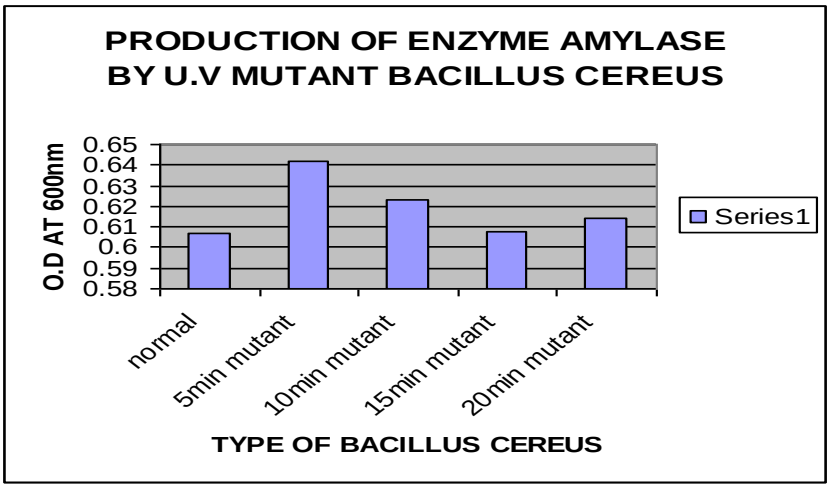


TABLE 9: PRODUCTION OF ENZYME AMYLASE BY ETHIDIUM BROMIDE MUTANT *Bacillus cereus*

Type Of *Bacillus cereus*	**O.D at 600nm**
Normal culture	0.607
0.2 concentrate	0.808
0.4 concentrate	0.817
0.6 concentrate	0.799
0.8 concentrate	0.808

GRAPH 9: Production of amylase by ethidium bromide mutant *Bacillus cereus.*

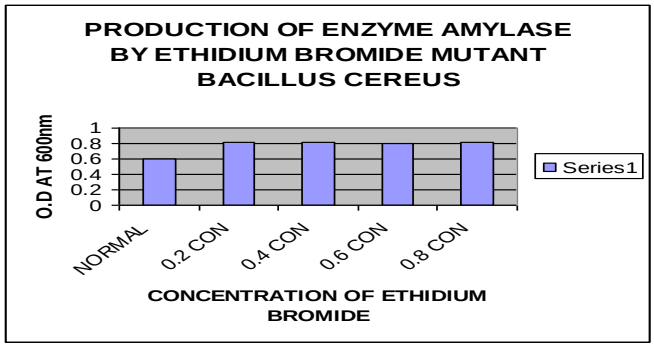


TABLE 10: PRODUCTION OF ENZYME AMYLASE BY NITROSO GUANIDINE MUTANT *Bacillus cereus.*

Type of *Bacillus cereus*	**O.D at 600nm**
Normal	0.607
0.1% concentration	0.563
0.2% concentration	0.520
0.3% concentration	0.350
0.4% concentration	0.280

GRAPH 10: Production of amylase by nitrosso guanidine mutant *Bacillus cereus.*

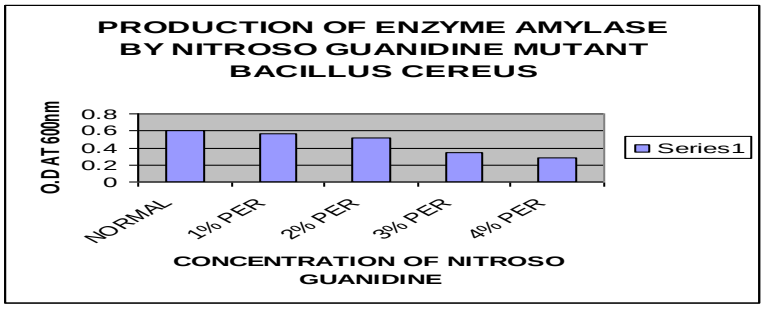
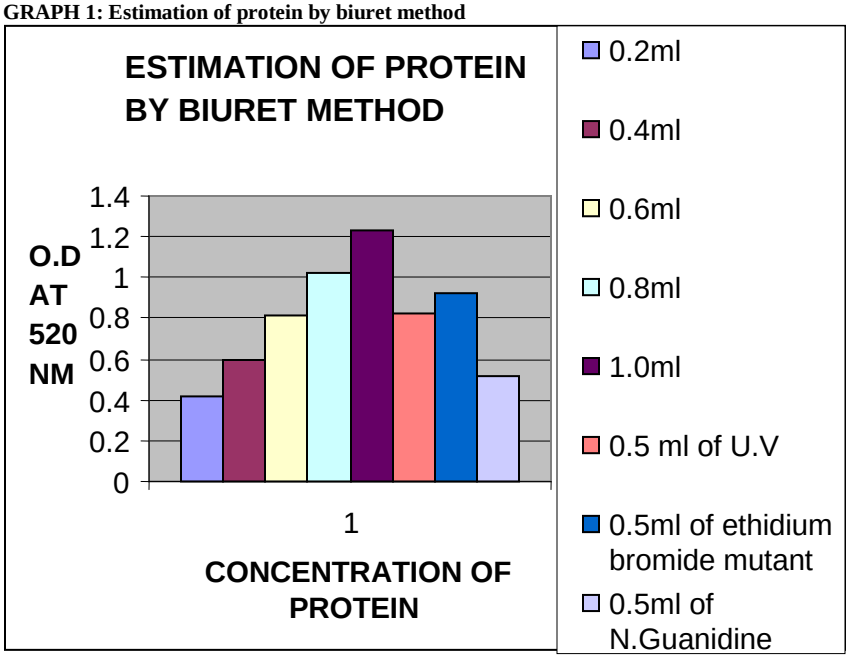


TABLE 1: ESTIMATION OF PROTEIN BY BIURET METHOD

s.no	Vol of standard	Concentration of standard	Distilled water	Biuret reagent	O.D at 520nm
1	0.2ml	200µg	1.8ml	3ml	0.42
2	0.4ml	400 µg	1.6ml	3ml	0.60
3	0.6ml	600 µg	1.4ml	3ml	0.81
4	0.8ml	800 µg	1.2ml	3ml	1.02
5	1.0ml	1000 µg	1.0ml	3ml	1.23
6	0.5 ml of U.V mutant		1.5ml	3ml	0.820
7	0.5ml of ethidium bromide mutant		1.5ml	3ml	0.921
8	0.5ml of Nitroso Guanidine		1.5ml	3ml	0.520

GRAPH 1: Estimation of protein by biuret method



Result and discussion

In general, the appearance of a new mutation is a rare event. Most mutations that were originally studied occurred spontaneously. This class of mutation is termed spontaneous mutations. Historically, geneticists recognized these in nature. The mutations were collected, and the inheritance of these mutations was analyzed. But these mutations clearly represent only a small number of all possible mutations. To genetically dissect a biological system further, new

mutations were created by scientists by treating an organism with a mutagenizing agent. These mutations are called induced mutations.

The spontaneous mutation rate varies. Large gene provides a large target and tends to mutate more frequently. A study of the five coat color loci in mice showed that the rate of mutation ranged from 2×10^{-6} to 40×10^{-6} mutations per gamete per gene. Data from several studies on eukaryotic organisms shows that in general the spontaneous mutation rate is $2\text{-}12 \times 10^{-6}$ mutations per gamete per gene. Given that the human genome contains 100,000 genes, we can conclude that we would predict that 1-5 human gametes would contain a mutation in some gene.

Mutations can be induced by several methods. The three general approaches used to generate mutations are radiation, chemical and transposon insertion. The first induced mutations were created by treating Drosophila with X-rays. Using this pproach Mueller to induce lethal mutations. In addition to X-rays, other types of radiation treatments that have proven useful include gamma rays and fast neutron bombardment. These treatments can induce point mutations (changes in a single nucleotide) or deletions (loss of a chromosomal segment).

Chemical mutagens work mostly by inducing point mutations. Point mutations occur when a single base pair of a gene is changed. These changes are classified as transitions or transversions. Transitions occur when a purine is converting Ed to a purine (A to G or G to A) or a pyrimide is converted to a pyrimidine (T to C or C to T). A transversion results when a purine is converted to a pyrimidine or a pyrimidine is converted to a purine. A transversion example is adenine being converte d to a cytosine. You can determine other examples.

Two major classes of chemical mutagens are routinely used. These are alkylating agents and base analogs. Each has a specific effect on DNA. Alkylating agents [such as ethyl methane sulphonate (EMS), ethyl ethane sulphonate (EES) and musta rd gas] can mutate both replicating and non-replicating DNA. By contrast, a base analog (5-bromouracil and 2-aminopurine) only mutate DNA when the analog is incorporated into replicating DNA. Each class of chemical mutagen has specific effects that can lead to transitions, transversions or deletions.

Scientists are now using the power of transposable elements to create new mutations. Transposable elements are mobile pieces of DNA that can move from one location in a geneome to another. Often when they move to a new location, the result is a new mutan t. The mutant arises because the presence of a piece of DNA in a wild type gene disrupts the normal function of that gene. As more and more is being learned about genes and genomes, it is becoming apparent that transposable elements are a power source f or creating insertional mutants.

The detailed knowledge of the structure and funciton of transposable elements is now being applied in the pursuit of new mutations. Stocks are created in which a specific type of element is present. This stock is then crossed to a genetic stock that doe s not contain the element. Once that element enters the virgin stock, it can begin to move around that genome. By carefully observing the offspring, new mutants can be discovered. This approach to developing mutants is termed insertional mutagenesis.

Mutations create variation within the gene pool. Less favorable (or *deleterious*) mutations can be reduced in frequency in the gene pool by natural selection, while more favorable (*beneficial* or *advantageous*) mutations may accumulate and result in adaptive evolutionary changes. For example, a butterfly may produce offspring with new mutations. The majority of these mutations will have no effect; but one might change the color of one of the butterfly's offspring, making it harder (or easier) for predators to see. If this color change is advantageous,

the chance of this butterfly surviving and producing its own offspring are a little better, and over time the number of butterflies with this mutation may form a larger percentage of the population.

Neutral mutations are defined as mutations whose effects do not influence the fitness of an individual. These can accumulate over time due to genetic drift. It is believed that the overwhelming majority of mutations have no significant effect on an organism's fitness. Also, DNA repair mechanisms are able to mend most changes before they become permanent mutations, and many organisms have mechanisms for eliminating otherwise permanently mutated somatic cells.

Mutation is accepted by the scientific community as the mechanism upon which natural selection acts, providing the advantageous new traits that survive and multiply in offspring or disadvantageous traits that die out with weaker organisms.

The thermostable a-amylases from Bacillusspp. Play important roles in textile and paper industries, starch liquefaction, food, adhesive, and sugar production, and various other industries and therefore are of considerable commercial interest (Babu K.R,1995).It was reported earlier from this laboratory that the a-amylase of Bacillus licheniformisCUMC305 shows great promise because of its excellent activity at high temperatures (optimum, 91°C [retaining high activity even at110°C]) and over a wide pH range (pH 5 to 10.0)(Nigam .P,1995,M.V.Ramesh,1987).

Narrow pH range for other a-amylases (Tanyildizi .M.S,2005,G.Feller,1998,S.Mishra,2005).

In our previous studies of o-amylase production by this strain as well as studies of a-amylase production by other organisms, peptone-beef extract media were primarily used. Several studies have been under taken to define ideal culturing and nutritional conditions for obtaining higher yields of the enzyme (Francis.F,2003,L.Bunni,1989). Various carbohydrate sources have been tested as enzyme production increasing agents for different strains (Vishwanathan.P,2001).

Complex fermentation media which give optimum production of a-amylase have been reported(Gomes.I,2003) but no successful attempt to develop cheap substrates for optimum production of the enzyme by *B. licheniformis* has been reported. The regular use of peptone-beef extract-based fermentation media is not commercially viable for industries. For efficient commercial production, a continuous effort is being made to find cheaper substrate sources. Available carbon and nitrogen sources are the decisive factors in the optimum production of enzymes, and these differ very much from substrate to substrate. Defattedor whole vegetable meals like sorghum, wheat bran, cottonseed meal, soybean meal, and alfalfa meal are the most commonly used fermentation additives if not the principal substrates (Hayashida.S,1986).

Several oilseed cakes, because of their abundant availability and low price, are used as cattle feed(Moller.K,2004) fertilizer (Sodhi.H.K,2005), and, in rare cases after proper processing, food for humans (Mielenz.J.R,1983).

The use of complex starchy substances to achieve higher yields of a-amylase has been reported(Jensen.B,1992,B.K.Lonsane,1985). Baked-bean waste (Boyer R.1999) has also been used for accelerating a-amylase production by fungi. The possibility that cheap raw materials like the various oilseed cakes increase the yield of thermo stable amylases has not been much demonstrated. The present study was planned to determine the effect of oilseed cakes on enzyme production by the thoroughly studied organism *B. licheniformis* CUMC305 (TanyildiziM.S,2005). In this paper, were port the enhancement of a-amylase production by most of the oilseed cakes tested. We also attempted to grow *B. licheniformis* CUMC305 in a medium containing some essential minerals and a few oilseed cakes as the sole carbon and nitrogen sources, excluding expensive bacteriological peptone and beef extract from the growth medium

(Ramachandran.S,2004). The purpose of the experiment is to determine whether green tea extract increases survival rates in *E. coli* bacteria exposed to UV light. UV rays cause the formation of cross-linking of adjacent pyrimidine bases thereby forming pyrimadine dimers (Ahmad.A,2000).

These pyrimadine dimers are detrimental to the cell because they halt transcription that will inevitably lead to cell death (Shimoi K). Some strains of *E. coli* have DNA repair enzymes which function in the reversal of dimer formations (Babu.K.R,1995) experiment (B/r WP2 trp-) is one such strain. The DNA of this bacteria strain contains a DNA excise repair mechanism, similar to that of human DNA, only involving fewer genes (Kuroda Y,1988).

It has been suggested that it is this repair mechanism in *E. coli* that works in association with tannic acid and offers an enhanced protection against the mutagenic properties of UV rays (Katiyar S,2001). Green tea, along with other herbal products contains polyphenols, which impart antioxidant and anitmutagenic benefits in animals, including humans (Shizuoka). Should green tea prove to offer some resistance to UV rays in *E. coli*, it may offer some protection in humans as well. The author speculates that daily ingestion of green tea may possibly act in a preventative manner against certain skin cancers caused by sun exposure.

Tannins are found within many species of plants such as oak gulls, hemlock, chestnut and mangroves. Tannic acid, a form of tannin, has a wide variety of medicinal uses that can be traced back to the Middle Ages (Katiyar S,2001). Not only is Tannic acid particularly effective in treating burns; it is also used to treat hemorrhoids, dysentery, conjunctivitis, and various skin diseases (Delpech R,2001). Evidence has shown that tannic acid has antimutagenic and anti-oxidant properties (Freifelder D,1998). Due to its structure, tannic acid is said to have the ability to mediate DNA cleavage (Case C,1998). Tannic acid stimulates the excision repair process of *E. coli* thereby removing pyrimidine dimers and allowing for synthesis of a new DNA strand (Hall BG,1980). Researchers Inoue and Kuroda (Delpech R,2001) have stated that substances showing antimutagenic properties in bacteria also show antimutagenicity in mammalian cells. This study is to see if the tannic acid contained in green tea may enhance inhibition of UV induced fatality in *E. coli*.(Hall BG,1980).

By this work we can say to the society that mutant *Bacillus cereus* of UV(5 min) and mutant of Ethedium bromide(0.4 concentration) will produce high amount of Amylase than normal strain.In other case mutant of Ntrodso Guanidine will yield smll amount of amylase than normal.

Reference

Ahmad A, Hadi S, Khan N. (2000). Anti-oxidant, pro-oxidant properties of tannic acid and its binding to DNA. *Chemico-Biological Interactions* **125**: 177-189.

Aris S. Sideropoulos[1], Patricia Kacsuta[1] and Mary Kay Betz[1] Department of Biological Sciences, Duquesne University, 15219 Pittsburgh, Pennsylvania, USA

Babu K.R, Satyanarayana T; (1995), α-Amylase production by thermophilic *Bacillus coagulans* in solid-state fermentation, *Process Biochem*. **30** 305–309.

Bunni.L, Mc Hale.L, (1989). Production, isolation and partial characterization of an amylase system produced by *Talaromyces emersonii* CBS 814.70, *Enzyme Microb. Technol*. **11** 370–375.

Boyer R. (1999). Concepts in Biochemistry. Brooks/ Cole Publishing Company. 330 p. Vasilieva. 2001. *Para*-aminobenzoic acid inhibits a set of SOS functions in *Escherichia coli* K12. *Mutation Research* **496**: 89-95.

Case C, Funke B, Tortora G. (1998). *Microbiology An Introduction*. 6th ed. The Benjamin/Cummings Publishing Company, Inc. 172 p.

Delpech R. (2001). Using Vibria natriegens for studying bacterial population growth, artificial selection, and the effecs of UV radiation and photo-reactiviation. *Journal of Biological Education.* **35**: 93-97.

Feller.G, Le Bussy.O, Gerday.C (1998.) Expression of psychrophilic genes in mesophilic hosts: Assessment of the folding state of a recombinant a-amylase, *Appl. Environ. Microbiol*. **64** 1163–1165.

Francis.F,. Sabu.A, NampoothiriK.M, Ramachandran.S, Ghosh.S, Szakacs.G, Pandey.A (2003). Use of response surface methodology for optimizing process parameters for the production of α-amylase by *Aspergillus oryzae, Biochem. Eng. J*. **15** 107–115

Freifelder D, Malacinski G. (1998). *Essentials of Molecular Biology*. 3rd ed. Boston: Jones and Bartlett Publishers. P 237-239.

Gomes.I, Gomes.J, Steiner.W(2003). Highly thermostable amylase and pullulanase of the extreme thermophilic eubacterium *Rhodothermus marinus*: Production and partial characterization, *Bioresour. Technol*. ***90*** 207–214

HallBG,ZuzeT. Evolution of a new enzymatic function by recombination within a gene. Proc Natl Acad Sci U S A 1980 Jun 77:6 3529-33.

Hall BG Biochemistry. Changes in the substrate specificities of an enzyme during directed evolution of new functions 1981 Jul 7 20:14 4042-9.

Hayashida.S, Teramoto.Y (1986). Production and characteristics of raw-starch-digesting a-amylase from a protease negative *Aspergillus ficuum* mutant, *Appl. Environ. Microbiol*. **52** 1068–1073.

Jensen.B, Olsen.J (1992). Physicochemical properties of a purified alpha amylase from the thermophilic fungus *Thermomyces lanuginosus*, *Enzyme Microb. Technol*. **14** 112 – 116.

Kang.J,Blaser.M.J(2006) UVr D helicase suppresses recognition and DNA damage-induced delitions.

Katiyar S, Bergamo B, Vyalil P, Elmets C. (2001). Green tea polyphenols: DNA photodamage and photoimmunology. *Journal of Photochemistry and Photobiology B: Biology. 2001*; **65** (109-114).

Kuroda Y, Inoue T. (1988). Antimutagenesis by factors affecting DNA repair in bacteria. *Mutation Research* **202**: 387-391

LonsaneB.K, GhildyalN.P, Budiatman.S, Ramakrishna .S.V(1985). Engineering aspects of solid-state fermentation, *Enzyme Microb*. *Technol*. ***7*** 258-265.

MielenzJ.R (1983). *Bacillus stearothermophilus* contains a plasmid- -borne gene for a-amylase, *Proc*. *Natl*. *Acad*. *Sci*. **80** 5975–5979.

Mishra.S, NoronhaS.B, SuraishkumarG.K (2005). Increase in enzyme productivity by induced oxidative stress in *Bacillus subtilis* cultures and analysis of its mechanism using microarray data, *Process Biochem*. **40** 1863–1870

Molle.K, M.Z. SharifM.Z, L. Olsson.L (2004). Production of fungal a-amylase by *Saccharomyces kluyveri* in glucose-limited cultivations, *J*. *Biotechnol*. **111** 311–318.

Moore M. The American Materia Medica, Therapeutics and Pharmacognosy.(2001). Home page.

Nigam P, Singh D, (1995). Enzyme and microbial system involved in starch processing, *Enzyme Microb*. *Technol*. **17** 770–778.

RameshM.V, B.K. Lonsane B.K, (1987). A novel bacterial thermostable alpha amylase system produced under solid-state fermentation**,** *Biotechnol*. *Lett*. **9** 501–504.

Ramachandran.S, PatelA.K, NampoothiriK.M, Francis.F, Nagy.V, Szakacs.G, Pandey.A (2004). Coconut oil cake – A potential raw material for the production of a-amylase, *Bioresour*. *Technol*. **93** 169–174.

SodhiH.K, Sharma.K, GuptJ.Ka, Soni S.K(2005). Production of a thermostable a-amylase from *Bacillus* sp. PS-7 by solid- state fermentation and its synergistic use in the hydrolysis of malt starch for alcohol production, *Process Biochem*.**40** 525–534.

Shimoi K, Kada T, Nakamura Y, Tomita L. Bio-antimutagenic effects of tannic acid on UV and chemically induced mutagenesis in Escherichia coli B/r.

Shizuoka 422, Laboratory of Health Science, Shizuoka College of Pharmaceutical Sciences, japan.

TanyildiziM.S, Ozer.D, Elibol.M(2005). Optimization of α-amylase production by *Bacillus* sp. using response surface methodology, *Process Biochem*. **40** 2291–2296

Vishwanathan.P, SurlikarN.R,(2001) Production of a-amylase with *Aspergillus flavus* on *Amaranthus* grains by solid-state fermentation, *J*. *Basic Microbiol*. **41** 57–64.