2014

ISSN 2278-5655

Aarhat Multidisciplinary International Education Research Journal (AMIERJ)

(Bi-Monthly)

Peer-Reviewed Journal

Impact factor:0.948

Aug-Sep Issues

Chief-Editor

Ubale Amol Baban

PEER-REVIEWED INTERNATIONAL JOURNAL

[Editorial/Head Office: 108, Gokuldham Society, Dr.Ambedkar chowk, Near TV Towar,Badlapur, MSief

Aarhat Multidisciplinary International Education Research Journal (AMIERJ) (Bi-Monthly)
Peer-Reviewed Journal Vol No III Issues IV
AUG -SEP 2014 ISSN 2278-5655

INDUCTION OF AUXOTROPHIC MUTANTS IN E. COLI K12

Botany

Dr. Geetha G Nair

Professor in Botany

RIE,Mysore-6

Abstract

Mutations are sudden heritable changes seen in organisms. Evolution is brought about through mutations. In the present experiment induction of auxptrophic mutants in E.coli have been brought about using two mutagens, viz., N-methyl –N-nitro-N-nitrosoguanidine and 5-Bromouracil.The number of colonies obtained using Nitrosoguanine (NG) were 35(average) and Bromouracil(BU) were 30(average). The results validate the methodology adopted and prove that the mutagens were effective.

Introduction

Mutations are sudden heritable changes in the structure of genetic material. They constitute the principal raw material with which nature works to bring about evolution.

The frequency of spontaneous mutation in a bacterial population is usually less than 1 in 105. Screening of the desired mutant becomes possible by increasing the occurrence of mutation by the use of mutagens (physical or chemical mutagens which induce changes in the genetic

material leading to altered information). The present experiment deals with the induction of auxotrophic mutants in *E.Coli.*

There are two types of bacteria- the prototrophs and the auxotrophs. Prototrophs are wild type of bacteria which can grow on a minimal medium. Auxotrophs are those bacteria which require supplemented growth factors

In this experiment, the mutagens used are N-methyl –N-nitro-N-nitrosoguanidine and 5-Bromouracil.

Action of the mutagens-

a) 5-Bromouracil

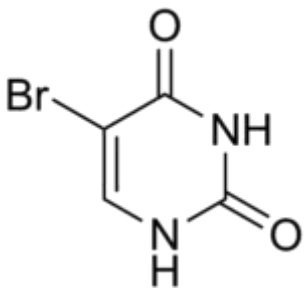

Figure 1(IUPAC name) 5-Bromo-1H-pyrimidine-2,4-dione

5-Bromouracil (or **5-bromo-2,4(1H,3H)-pyrimidinedione** or **5-BrU** or **5-BU**) is a brominated derivative of uracil that acts as an antimetabolite or base analog, substituting for thymine in DNA, and can induce DNA mutation in the same way as 2-aminopurine. It is used mainly as an experimental mutagen, but its deoxyriboside derivative (5-bromo-2-deoxy-uridine) is used to treat neoplasms.

5-BrU exists in three tautomeric forms that have different base pairing properties. The keto form (shown in the infobox) is complementary to adenine, so it can be incorporated into DNA by aligning opposite adenine residues during DNA replication (see below left). Alternatively, the enol (below right) and ion forms are complementary to guanine. This means that 5-BrU can be present in DNA either opposite adenine or guanine.

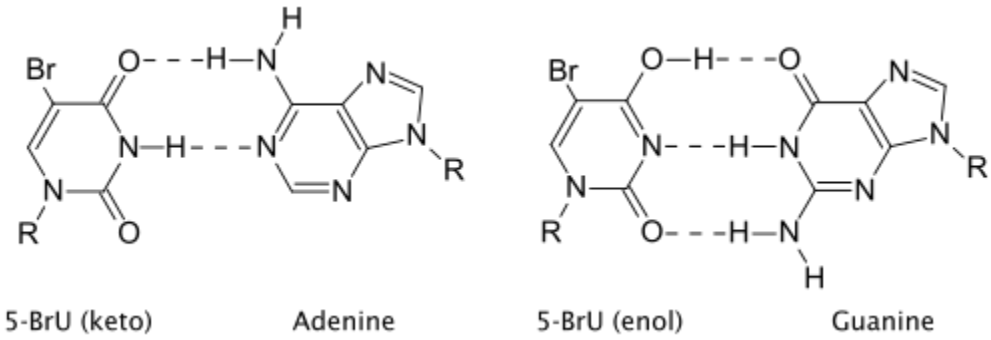

Figure 2 Tautomeric forms of 5-Bromouracil

The three forms frequently interchange so base-pairing properties can become altered at any time. The result of this is that during a subsequent round of replication a different base is aligned opposite the 5-BrU residue. Further rounds of replication 'fix' the change by incorporating a normal nitrogen base into the complementary strand.

Thus 5-BrU induces a point mutation via base substitution. This base pair will change from an A-T to a G-C or from a G-C to an A-T after a number of replication cycles, depending on whether 5-BrU is within the DNA molecule or is an incoming base when it is enolized or ionized.

Bromouracil is an analogue of the uracil and acts in the following manner. At first, it incorporates itself in place of thymine, resulting in pairing with adenine. In the next replication due to presence of the bromine,the bromo-uracil exhibits tautomerism and gets converted to the

enol form. Thus, during the replication the adenine gets replaced by guanine. In the replication which follows, the bromo-uracil is replaced by cytosine. As a result in this generation A-T is replaced by G-C. The whole sequence may be shown as follows

A=T---->A==BU------->GΞBU*------->GΞC

This is known as a transition mutation.

b) Nitrosoguanidine- the actual name is N-methyl-N-nitro N-nitrosoguanidine

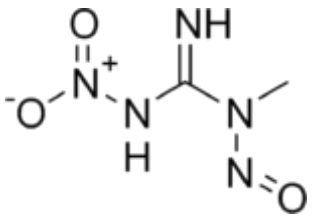

Figure 3 IUPAC name 1-methyl-2-nitro-1-nitrosoguanidine

N-Methyl-*N*-nitroso-*N′*-nitroguanidine

N-Methlyl-*N′*-nitro-*N*-nitrosoguanidine

Methylnitronitrosoguanidine (MNNG or MNG) is a biochemical tool used experimentally as a carcinogen and mutagen. It acts by adding alkyl groups to the O^6 of guanine and O^4 of thymine, which can lead to transition mutations between GC and AT. These changes do not cause a heavy distortion in the double helix of DNA and thus are hard to detect by the DNA mismatch repair system.

In organic chemistry, MNNG is used as a source of diazomethane when reacted with aqueous potassium hydroxide. MNNG is a probable human carcinogen listed as an IARC Group 2A carcinogen.

It works only in the replicating phase. It is an alkylating agent. Therefore , it alkylates guanine base of DNA strand forming an unstable compound and which can be excised from the DNA chain by any other base, either purine or pyrimidine. This reaction occurs at the N7 position of guanine (It is a transversion or transition type of mutation).(www. wikipedia.com)

Wild type of *E.Coli* strain K12 are treated with mutagens and the mutagenised cells are enriched by growth in a nutrient medium. These cells are exposed to antibiotic penicillin which kills growing bacteria by interfering with the cell wall synthesis. Since in minimal medium only the prototrophs will grow, penicillin kills them selectively. Thus an enrichment of auxotrophs occurs.

Methodology

Nutrient broth was prepared by adding 3 gm of beef extract and 5 gms of peptone to 100 ml water and adjusting the pH to 7.4 to 7.6. 15 gms of nutrient agar was added to the above mentioned ingredients The broth was autoclaved and *E.Coli* were transferred from the log culture to the broth in an inoculation chamber. This was kept on a shaker for 498 hours. And thus was obtained the *E.Coli* K12 cultures.

5 ml of the above culture was centrifuged and the broth drained off. The pellet so obtained was suspended in 5 ml of fresh broth(2 sets of test tubes were prepared one for each mutagen) and added .05 ml of NG solution and .05 ml of BU solution separately . Now, these were incubated for 20 minutes at 37 degrees with shaking,centrifuged and washed with saline. Resuspended the pellets in 5 ml of saline(5 gms 100 ml). 0.1 ml of the suspension was transferred to conical flasks containing equal volume of sterilized nutrient broth medium. The rest of the suspension was stored in a refrigerator. The above conical flasks were incubated overnight with shaking to enrich the mutant population.

Next day the mutagenised culture was diluted to 10^{-1} in saline(by adding 1 ml of culture to 10 ml of saline) and added 0.1 ml of this to 10 ml of minimal medium containing penicillin.

The culture was incubated for 6 hours at 37 degree Celsius with shaking during which the phototrophs were killed. Now serial dilution was done upto 10^{-8} with saline. The rest of the culture was stored in the refrigerator

Nutrient agar plates were prepared and the dilutions were plated on them by rotating the plates on a swivel table and holding an L-shaped rod on the agar surface. These plates were then incubated overnight at 37 degree Celsius.

Results and discussions

Mutant colonies were obtained on the agar plates. The number of colonies obtained using Nitrosoguanine (NG) were 35(average) and Bromouracil(BU) were 30(average). The results validate the methodology adopted and prove that the mutagens were effective

References

1.en.wikipedia.org.

2. Ames, B. N. (1979). "Identifying environmental chemicals causing mutations and cancer". *Science* **204** (4393): 587–593. D.S

3. Hayatsu, Hiroka (1991), *Mutagens in Food: Detection and Prevention*, CRC Press, pp. 286 pages,

4.Sanders, E. R. Aseptic Laboratory Techniques: Plating Methods. *J. Vis. Exp.* (63), e3064, doi:10.3791/3064 (2012).

5 Raetz,R.H.C .1975.Isolation of *Escherichia coli* Mutants Defective in Enzymes of Membrane Lipid Synthesis. *Proc. Nat. Acad. Sci*, USA
Vol. 72, No. 6, pp. 2274-2278.

6.www.ncbi.nlm.nih.gov