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**Annals of Biological Research, 2019, 10 (2): 7-13**

*(http://www.scholarsresearchlibrary.com)*

ISSN 0976-1233
CODEN (USA): ABRNBW

**ISSN:0976-1233**

# Assessing of Mutagenicity of Monosodium Glutamate by Using HGPRT Gene Mutation Assay of *Coprinopsis cinerea*

**Nagat S. Elhaddad*, Eman M. Belkasem, Hoda A. Khatab**

*Department of Botany, University of Omer Almukhtar, Al-Baida, Libya*

***Corresponding Author:** Nagat S. Elhaddad, Department of Botany, University of Omer Almukhtar, Al-Baida, Libya, E-mail: enjesaad@yahoo.com*

## ABSTRACT

*The HPRT gene mutation assay is a remarkable tool for testing genotoxic chemicals, allows for the isolation and screening mutation in different living cells. Here, we report the applicability of HGPRT gene to test the mutagenic activity of different concentrations of monosodium glutamate, MSG, (1, 3, 5 and 7 g/l). The mutagenic effect of monosodium glutamate was assessed by decreasing the viability and increasing the HGPRT gene mutation rate of Coprinopsis cinerea. The alterations were proportional to the concentration of MSG up to reach the optimum concentration (7 g/l) when the maximum rate of mutation was 1.7 and the viability was about 27%. The highest viability was 37.4% when oidia treated with 2 g/l of MSG. To determine the optimal mutagenic time of the optimal mutagenic concentration, the period of incubation was ranging from 1 to 4 hours of the cells treated with 7 g/l MSG. Two hours was the optimal mutagenic time that reaches 1.02 mutagenic rate of HGPRT gene mutation and 37.7% of the viability. The highest viability was gained is 43.6% when cells treated with 7 g/l MSG and incubated for one hour. In vitro results indicates that the MSG is mutagenic and subsequently may cause DNA damage. These data do provide an indication of potential genotoxic of MSG to human health. Thus its use as a food additive should be completely avoided and look for a safer alternative.*

**Keywords:** HGPRT gene, Mutagenic, *Coprinopsis cinerea*, Monosodium glutamate.

## INTRODUCTION

Food additives are the substances that are added to food in order to prolong the shelf-life of the food's factories by inhibiting the development of microorganisms such as bacteria and fungi. Some other purposes including coloring, flavoring, sweetening, and thickening are also targeted of the food additives [1]. About 5000 trade name products on a worldwide-basis for about 2500 chemicals that function as food additives [2]. Monosodium Glutamate (MSG) is one of the chemicals that extensively used as a food additive. Plenty of countries, mainly in South East Asia are using MSG as a flavor enhancer in food products. MSG was added to meats, poultry, seafood, snacks, soups and stews and another kind of food, subsequently, humans are daily exposed to these chemical substances [3]. Monosodium glutamate is originally extracted from molasses by microbial fermentation of beet sugar, sugar cane, starch and corn sugar [4]. Burde et al. [5] reveal that damage in nerve cells of both immature rats and mice was induced after applying of MSG by oral administration and subcutaneous injection. The effects of oral dosage of monosodium glutamate applied for short- and long-terms were induced severe damage on the histology and ultrastructure of testes of the adult rats [6]. According to Rundlett and Armstrong [7] many of cytotoxic and genotoxic complications in the immune system, reproductive organs, liver, kidney, hematological parameters, pituitary functions, and oxidative stress were caused by the accumulation of the MSG. Yang et al. [8] determined the optimal concentration of MSG to be between 0.2 and 0.8% for humans is about 60 mg/kg body weight. However, the long term exposure of MSG on humans leads to many complications as Glutamate is an excitatory neurotransmitter in the central nervous system of

mammals [9,10]. In this project we used Hypoxanthine Guanine Phosphoribosyl Transferase (HGPRT) assay for gene mutation at the HGPRT locus, using *Coprinopsis cinerea* to investigate the genotoxic effects of MSG food additive. *Coprinopsis cinerea* is an excellent model that has attracted the attention of geneticists and cytologists in basidiomycetes because of its short life cycle, capability to grow and fruit on artificial media under laboratory conditions [11,12]. This basidiomycete has a small nuclear genome size of 38,000 Kilobase pairs (kb), which facilitates molecular analyses. In addition, genetic studies and experimental manipulation of all phases of its life cycle are straightforward and simple [13]. *Coprinopsis cinerea* is a heterothallic basidiomycete, has two types of mycelia in its life cycle, monokaryon, and dikaryon. The monokaryon is sterile primary mycelium, has simple septa and forms asexual spores that called oidia constitutively in abundant numbers on the oidiophores [14,15]. Whereas, the dikaryon is generated by mating of two compatible monokaryons [16]. *C. cinerea* can complete its life cycle in two weeks in the laboratory. Haploid spores germinate and grow as filaments (hyphae). If filaments of compatible mating type fuse, a new growth pattern is established, the 'dikaryon', in which a single haploid nucleus from each parent is maintained in every cell. The dikaryon can respond to light/dark regime to form fruit bodies [17].

The fact that HGPRT is a non-essential enzyme for *in vitro* cell growth because purine nucleotides can be formed by de novo synthesis is the base of the HGPRT gene mutation assay. The HPRT gene has been cloned and characterized. Thus it has been the most actively studied locus in investigations of mutational agents. The theory is, HGPRT locus mutants are produced by the action of the examined mutagen, lose the function to metabolize purine analogs such as 6-thioguanine (TG) or 8-azaguanine which is thus lethal only to wild-type cells, the mutant cells being easily identified using an agar medium containing TG [18,19]. Purine analogue resistance in mammalian cells results from an inability to convert these analogues to toxic nucleotides, an activity-dependent upon functional HPRT. Thus quantitation of 6-thioguanine or 8-azaguaniner resistant cells provides a simple measure of mutation frequency [20].

## MATERIALS AND METHODS

### *Strain and culture conditions*

*Coprinopsis cinerea* strain AmutBmut is a self-compatible homokaryon that, due to mutations in the A and B mating-type genes, produces fruiting bodies without mating to another compatible monokaryon [21] Boulianne et al. [22] were isolated the hypha from horse dung and cultivated on YMG agar plates (4 g yeast extract, 10 g malt extract, 4 g glucose and 10 g agar per l) for 5 days at 28°C until the mycelium fully covered the substrate. About 50 Petri dishes were cultured, the hypha were harvested and then oidial suspension were prepared [18,23].

### *Hypoxanthine-guanine phosphoribosyl transferase locus (HGPRT) assay*

The HGPRT assay was performed according to Li et al. [18] Experiment was divided to five groups, the first group is the negative control in which the cells were incubated with Distilled Water (DW). Cells in the next four groups were treated with selected concentrations of MSG (1, 3, 5 and 7 g/l) for one hour at 37°C. Three replicate tubes were used for each group. After the incubation, cells were washed twice with DW and centrifuging at 1500 rpm. Treated oidia were then divided and cultured on two different culture medium. Non-selective medium (YMG agar plates) to detect the wild-type phenotype, whereas, the selective medium (YMG agar plates containing 6-TG) to detect the mutant phenotype cells. Plates were incubated at 28°C for 48 hrs. Colonies on both non-selective and selective medium were counted. Three separate experiments (n=3) were carried out for each treatment. Viability was expressed as the number of colonies on the non-selective medium. Mutation frequency was determined phenotypically as the number of mutant colonies in selective medium and the number of colonies in non-selective medium.

### *Determination of optimal mutagenic concentration of MSG*

In this experiment, three tubes were dedicated for control and treated samples, DW and graduate concentration of monosodium glutamate (1, 3, 5 and 7 g/l) were added respectively. Tubes were incubated at 37°C for one hour, oidia then washed twice and cultured on both selective and non-selective medium. Plates were incubated at 28°C for 48 hr, colonies were counted.

### *Determination of optimal mutagenic period of the optimal concentration*

Next to the determination of the optimal mutagenic concentration of MSG (3 g/l), five groups of tubes were prepared. One group was for the control and the other groups were treated with 3 g/l of MSG and incubated for 1, 2, 3 and 4hrs

at 37℃ to determine the optimal mutagenic period. Cells were washed twice and cultured on both selective and non-selective medium. Plates were incubated at 28℃ for 48 hrs, colonies were counted.

***Statistical analysis***

Un-paired t-tests were used to compare the viability and rate of mutation values of both controls and treated samples. For each treatment, three tubes were included and then four different plates were cultured from each tube (4 replicates). Data were performed using SPSS computing software, results with $p<0.05$ were considered to be statistically significant.

## RESULTS

The evaluation of the cytotoxicity of selected doses of monosodium glutamate on *Coprinopsis cinerea* revealed a fluctuation of the viability percentage (Figure 1) when the concentration of MSG increased the averages of the viabilities were 15.6, 37.4, 17.7 and 27% when oidia treated with 1, 3, 5 and 7 g/l of MSG respectively. There was a significant difference between the control and each treatment and also between treatments at (T-test, $p<0.0001$). The highest viability (37.4) was obtained when the cells were treated with 3 g/l of MSG whereas when treated with 1 g/l gives the lowest viability was 15.6%. Comparing between the last two treatments 5 and 7 g/l, viabilities were 17.7 and 27% respectively. This demonstrates a less significant difference (t-test, $p<0.05$) in the number of wild-type colonies than the other tested doses.

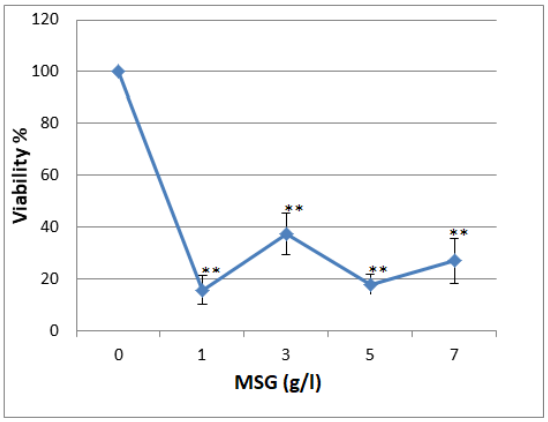


**Figure 1.** Determination of the highest viability using different concentrations of MSG. Values plotted are means of 3 readings from 9 plates. Error bars represent the standard deviation. Values were statistically tested using unpaired t-tests

The mutagenicity test results for the HGPRT assay suggests that all of the selected doses may be mutagenic, as indicated by the fact that the mean number of colonies formed was significantly lower than the control (Figure 2).

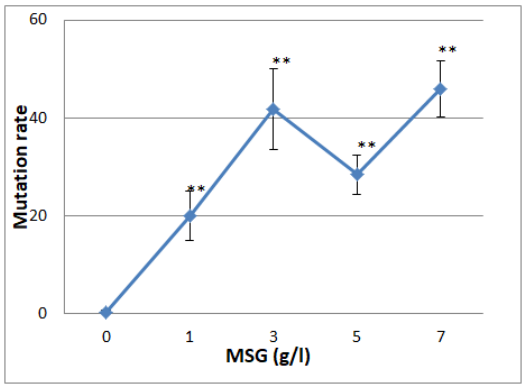


**Figure 2.** Determination of the optimal mutation rate using different concentrations of MSG. Values plotted are means of 3 readings from 9 plates. Error bars represent the standard deviation. Values were statistically tested using unpaired t-tests

In the mutagenicity test, HGPRT assay, the mean number of colonies for the control was, as expected, significantly higher (t-test, $p<0.0001$) than that treated oidia. All the treatments with 1, 3, 5 g/l show a mean number 1.2, 1.1, 1.5

which means that the optimal mutation rate is 1.7 for the 7 g/l and it is the optimal mutagenic concentration. There were significant differences between the first and the last treated cells at $p<0.01$. Regarding the determination of the optimal mutagenic period of the optimal mutagenic concentration for both the viability and mutation rate, cells were treated with MSG (7 g/l) and then incubated for 1, 2, 3 and 4 hrs. Results show that the optimal mutagenic concentration reduces the viability of normal cells through declining of the mean number of treated colonies in comparison to the control (Figure 3). Readings obtained from the wild-type cells were 43.6, 37.7, 35.5 and 35%. The highest significant difference was achieved when control and the first treatment were evaluated (T-test, $p<0.001$).

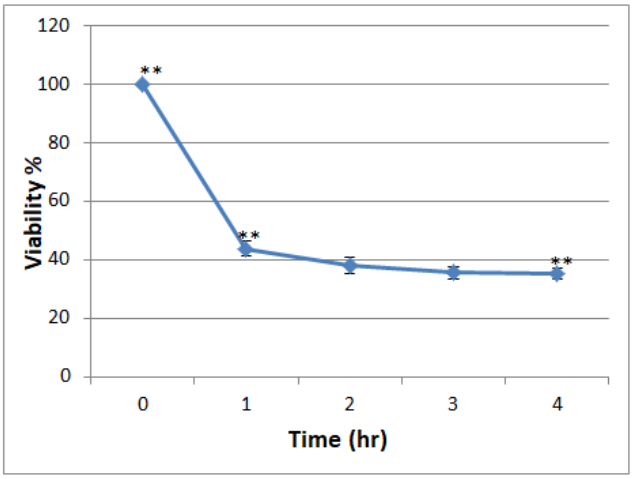


**Figure 3.** Determination of the highest viability using the optimal mutagenic concentrations of MSG (7 g/l). Values plotted are means of 3 readings from 9 plates. Error bars represent the standard deviation. Values were statistically tested using unpaired t-tests

Fewer differences at $p<0.01$ were attained between the remain of verified cells. However, in the mutant frequency experiment to determine the optimal mutagenic time, the results were 0.7, 1.02, 0.9 and 1.0 at 1, 2, 3 and 4 hrs correspondingly (Figure 4). The numbers of counted cells indicate that the 2 hr is the optimal mutagenic period of the optimal mutagenic concentration since the highest mutagenic rate was 1.02. The static analysis revealed that all tested cells were significantly higher than the control cells at $p<0.001$. However, there was a significant difference ($p<0.05$) between oidia that incubated at one hour, two and four hours compared with three hours.

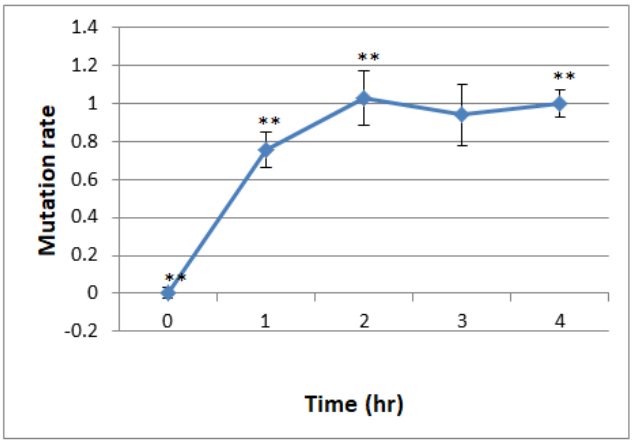


**Figure 4.** Determination of the optimal mutagenic time (hours) using the optimal mutagenic concentrations of MSG (7 g/l). Values plotted are means of 3 readings from 9 plates. Error bars represent the standard deviation. Values were statistically tested using unpaired t-tests

## DISCUSSION

The assessment of DNA-damaging on the Hypoxanthine-Guanine Phosphoribosyl Transferase (HGPRT) locus is expected to be an effective tool for various genetics, medical and agricultural applications. We attempted to compare

mutagenesis and survival cells in *Coprinopsis cinerea* after subjecting the cells to four selected doses of monosodium glutamate.

MSG is the sodium salt of glutamic acid. Nowadays, it is considered a silent killer. It is a common flavor enhancer in nutritional industries. It does not catabolize like other amino acids [24]. It has enormous harmful effects on many organs such as the liver, kidney, immune system, central nervous system, and reproductive organs. It can trigger cognitive functions, inducing cytotoxic and genotoxic effects [25].

The results of the present study revealed that incubation of the asexual cell with MSG induced a highly significant elevation in the mean values of both survival percentage and mutagenic rate comparing to the control group. Our results indicate that the optimal mutagenic concentration of monosodium glutamate was 7 g/l and the optimal mutagenic period was two hours. The reduction in the ability to form wild type colonies on the non-selective plates in Figure 1 might be a result of the cytotoxicity of mono-sodium glutamate that leading to exterminate of the oidia and subsequently less viability. As well as, increasing the incubation time may offers along period to the MSG to reduce the viability with an exemption to the average of the 1 g/l treatment.

In the mutagenicity assay (HGPRT), in the presence of 6-thioguanine, a positive relationship between the rate of mutation and the monosodium glutamate concentrations were remarked, suggesting mutagenesis at the HGPRT locus.

The previous suggestion was agreed with Whong [26] when tested the mutagenic effect of Icr-170 on the conidia of *Neurospora crassa*. Moreover, a haloethanes were reported as cytogenic and also mutagenic of HGPRT gene in Chinese Hamster Ovaries [27]. In addition, the HGPRT gene mutation assay was used to determine the cytotoxicity and genotoxicity of a basidiomycete *Agaricus blazei* [28,29]. The procedure that was followed in this study was previously used by Bennett and Smiters [30]. They reported that including a toxic base analogous in the media would stimulate the non-mutant cells to motivate only through the complementary salvage pathway instead of de nova biosynthetic pathway in DNA replication which subsequent to the cells death.

Generally, HPRT helps purine by recycling the degraded bases via the complementary salvage pathway OD the DNA replication that accounts for most of the cellular requirements. HPRT is an $Mg^{2+-}$ dependent enzyme and recycles hypoxanthine and guanine via transferring phosphoribosyl group from Phosphoribosylpyroph-Osphate (PRPP) to generate Inosine Monophosphate (IMP) and Guanine Monophosphate (GMP), respectively. However, the alteration of the toxic base analogous to the nucleotide via complementary salvage pathway will inhibit the formation of non-mutant nucleotide in the biosynthetic de nova pathway as a result of the feedback in presence of a toxic 6-thioguanine [18,19,31].

Whereas in the selective media that includes 6-TG, in the absence of HGPRTase as a result of the mutation of the HGPRT gene, cells will select biosynthesis purine de nova pathway parting the toxic purine analogous [18,19,31].

## CONCLUSION

Summarizing, our results suggest that the monosodium glutamate concentrations tested all are potentially mutagenic as determined by the HGPRT assay. Furthermore, it is evident that more tests are necessary for the investigation of the cytological effects of MSG applying different assays. These findings indicate mono-sodium glutamate should not be used due to their genotoxicity and care should be taken in the case of use as a food additive. It is recommended to increase health education programs about the health impact of food additives especially monosodium glutamate and trial to substitute it by other safer food additives.

## ACKNOWLEDGMENT

The authors thank all of Dr. Nagah S.A. ABubaker and Dr. Ibrahim S. Eldurssi in the Faculty of Science for their contribution to this research.

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