Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2019 16(1):677-682. **OPEN ACCESS**

Using of *Salmonella typhimurium* as a test for the detection of carcinogens in foods in Iraq

Maitham Ghaly Yousif and Abbas Mayar Hezam

Biology Department College of Science University of Al-Qadisiyah, **Iraq**

***Correspondence:** *matham.yousif@qu.edu.iq abbas.hezam@qu.edu.iq **Accepted:** 31Jan.2019 **Published online:** 12Mar. 2019

Some of the chemicals used by humans, such as food additives are a potential mutagens and carcinogens. The mutagenic activity of food additives were investigated by using histidine auxotrophs *Salmonella typhimirium* in the presence or absence of metabolic activation system (S9). In the current study, the evaluation of potential mutagenic and genotoxicity of some food by using the bacterial reverse mutation test. The results showed there are four foods(Potato chips lays, Custerd, Indian red meat, Milk) are positive for the bacterial reverse mutation test, food that can induce reverse mutation in auxotrophs*S.typhimurium* in the presence or absence of metabolic activation system (S9). Polymerase chain reaction technique (PCR)also used to investigate gene *hisG*in histidine auxotrophs *Salmonella typhimirium* that are grown on Glucose -minimal salt agar without the need to histidine from an external source after treatment with different concentrations of foods under study, the results showed that the presence of *hisG* in all isolates of bacteria treatment with Potato chips, Custerd, Indian red meat in presence or absence of metabolic activation system(S9) and in the Milk in presence of metabolic activation system, while *hisG* not appear in bacteria treatment with milk in absence of metabolic activation system(S9).The difference in the PCR technique results compared with the Ames test may be due to the loss of the bacterial DNA during the extraction, or lack of prefixes association with the target gene appropriately or may be due to the use of non-standard strains and thus the likely occur mutation in histidine operon during replication .

Keywords: food additives, Salmonella typhimurium, hisG, PCR,

INTRODUCTION

Carcinogenesis is a multistep process that is affected by a number of lifestyle factors (Dybing, et al.,2008), and due to the development of modern techniques which aim to the preservation, increase the production and packaging and improve properties, such as color and taste of foods, some substances in foods can induce mutations in the genetic material and thus may induce of tumors (Herceg, (2007).Diet plays an important role in the occurrence of cancer in humans, because food contains many carcinogens and mutagens as my cotoxins , preservatives, flavorings, nitrosamines and polycyclic aromatic hydrocarbons, which are responsible for cancer (Abnet, , (2007)., Benigni, and Bossa, (2008) and Yousif, et al.,2016). Food additives and environmental chemicals are considered mutagens that affect DNA reproductive cells and this may due to the accumulation of mutant genes in Population, leading to damage to the reproductive cells and cause problems in the process of fertilization and the formation of mutations in future generations (genetic disease) or may affect DNA somatic cells, leading to the emergence of cancer (Richardson et al.,2007).Many studies have also shown that there is a relationship between Mutagenesis process and the emergence of cancer, as the substances that cause genetic

mutations may be carcinogenic because the cancer is often associated with mutation (McCann et al.,1983)), and there are evidence enough to indicate that the point mutation in oncogenes and Suppressive tumor in somatic cells involved in the formation of tumors in humans and laboratory animals Erickson, (2010).Because the DNA is similar chemically in all living organisms and thus the chemicals that cause mutations in bacteria may be carcinogenic to humans Mortelmans and Zeiger, E. (2000).To evaluate the mutagenic effect of environmental factors and food additives, many techniques are used ,the primary technique is the Ames test and the basis of this test is convert auxotrophic mutation of histidin (his-) in *S.typhimurium* to the wild-type (his +) in presence of mutagenes (Ajay et al.,2013). Auxotrophs*S.typhimurium* is characterized by membrane cell more permeable large chemicals and the presence of defects in DNA repair system, in addition to contain a mutation in a gene *hisG* responsible for the synthesis of histidine and therefore the bacteria cannot synthesized and the need from an external source Goodson-Gregg, . and De Stasio, (2009), so using these bacteria on a large scale as bacterial system common to test chemicals, food and drugs that are likely to be mutagenic and that can stimulate Second mutation in the original mutation site to come back with bacteria to wild-type prototrophic Gatehouse, (2012).The aim of the present study was to investigate and determine mutagenic and carcinogenic effects of four different food using the bacterial reverse mutation assay (Ames test).

MATERIALS AND METHODS

Preparation of the concentrations of food that used in Ames test

Random samples (30 samples) of food in local markets were tested. Solid samples includes: Potato chips (5 samples), Indomie (1samples), Custard (1 samples) and biscuits (2 samples) has been crushed and prepared a set of concentrations (50, 100, 150, 200, 250)

Bacterial reverse mutation assay

Top agar, glucose- minimal salts agar medium, histidine - biotin solution and S9- mix were prepared according to Ames test Maron. and Ames,(1983)and Mortelmans and Zeiger, (2000)genetic analysis of*S. tyiphimrium* (his-) strains (histidine requirement) were confirmed according to the method (Maron. and Ames,(1983) .Sodium azide was used as positive control and sterile distilled water was used as negative control. The test was conducted in presence or absence of metabolic activation system (S9- mix)according to (Maron. and Ames,(1983) and, Maron. and Ames,(1983)

Culturing of *S. tyiphimrium* (his-) in Nutrient broth at 37 C with shaking for 18-24 hours,then 100 µL of suspension of *S. tyiphimrium*, 500 µL of phosphate buffer (or 500 µL of S9- mix) and 100 µL of the test solution for each concentration added to2 mL of top agar contains His/Bio solution (kept in a water bath at 43-48°C) and the mixture vortexed and pour on GM agar and left to harden at room temperature and then inverted the plate and incubated darkness at 37 C for 48 hours and then the revertant bacterial colonies on each plate were counted manually or by colony count. Samples were tested on triplicate plates. The reading results based on the principle that the average number of colonies of bacteria at the GM agar grown at twice or more than the number of colonies of bacteria for negative control (Kutlu, **et al., 2004)**

Genomic DNA Extraction

Genomic DNA was extracted from bacteria according to company instructions by using Genomic DNA Mini Kit (Geneaid. USA). Then the extracted DNA was checked by Nanodrop spectrophotometer, and stored at -20C at refrigerator until used in PCR amplification.

Polymerase chain reaction (PCR)

Polymerase chain reaction was conducted to investigate of the gene *hisG* responsible for the synthesis of histidine in *S. typhimurium*, the assay was carried out by using specific primer which was designed by using *hisG* genetic sequences found in GenBank at NCBI and using design prefixes Primer3 plus program, to amplify a 305bp fragment of highly conserved regions of *hisG* gene in *S. typhimurium*. *hisG* forward primer (CGACCAGAAAGGCGTCTCTT) and *his G*Reverse primer (CCTCTTCCAGGCGTTCACTT) were provided by Bioneer Company (Korea). Then PCR master mix was prepared by using (AccuPower® PCR PreMix kit. Bioneer. Korea. The PCR premix tube contains freeze-dried pellet of (Taq DNA polymerase 1U, dNTPs 250µM, Tris-HCl (pH 9.0) 10mM, KCl 30mM, MgCl2 1.5mM, stabilizer, and tracking dye) and the PCR master mix reaction was prepared according to kit instructions in 20µl total volume by added 5µl of purified genomic DNA and 1.5µl of 10pmole of forward primer and 1.5µl of 10pmole of reverse

primer, then complete the PCR premix tube by deionizer PCR water into 20µl. The reaction was performed in a thermocycler by set up the following thermocycler conditions; initial denaturation temperature of 95 °C for 5 min; followed by 30 cycles at denaturation 95 °C for 30 s, annealing 59.3°C for 30 s, and extension 72 °C for 1min and then final extension at 72 °C for 5min. The PCR products were examined by electrophoresis in a 1.5% agarose gel, stained with ethidium bromide, and visualized under UV illumination.

Statistical Analysis

The statistical analysis of the data performed by two way ANOVA, followed by LSD (least significant difference). The difference was considered significant when the probability P ≤ 0.05(20)

RESULTS

Phenotypic detectionof carcinogens in food bybacterial reverse mutation assay

The results of bacterial reverse mutation assay are summarized in Table 1, which showed that out of 30foods there are 4foods (Potato chips, Custard, Indian red meat and Milk)were positive results for bacterial reverse mutation test when compared with the negative control in the presence or absence of metabolism activation system (S9) through the growth of auxotrophs *S. Typhimurium* on the glucose- minimal salts agar without the need for a histidine - biotin solution.

Genotypic detection of carcinogens in food by using PCR

Mutagens and carcinogens in foods includes (Potato chips, Custard, indian red meat and Milk) were detected using PCR technology through the investigation of gene *hisG* responsible for the synthesis of histidine in auxotrophs*S. Typhimurium* that has grown at GM agar without the need for histidine from an external source after treatment at different concentrations of substances under the study in addition to the positive and negative control, the results showed that the presence of gene*hisG* in all isolates of auxotrophs*S. Typhimurium* after treatment with Potato chips (Figure1), Custard (Figure 2), Indian red meat (Figure 3) in the presence or absence of metabolism activate system and with Milk (Figure 4) in the presence of metabolism activate system.

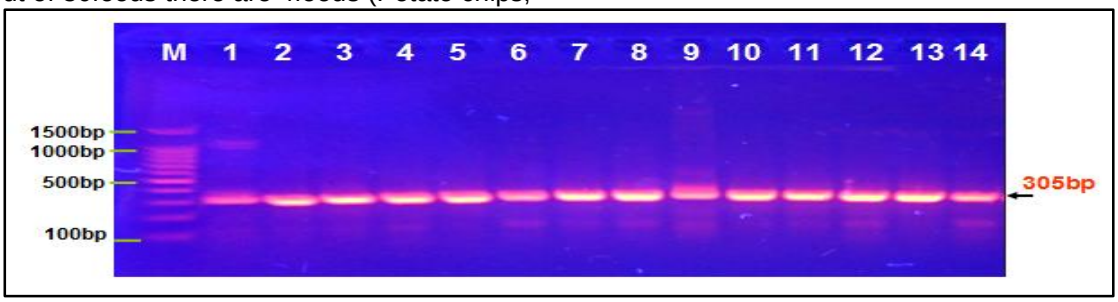

Figure (1): Agarose gel electrophosis image, which show the PCR product results for auxotrophic*S. Typhimurium* of *hisG* gene after treatment withPotatochips at 305bp PCR product size, where M: Marker100bp, Lane (1-14) are positive samples

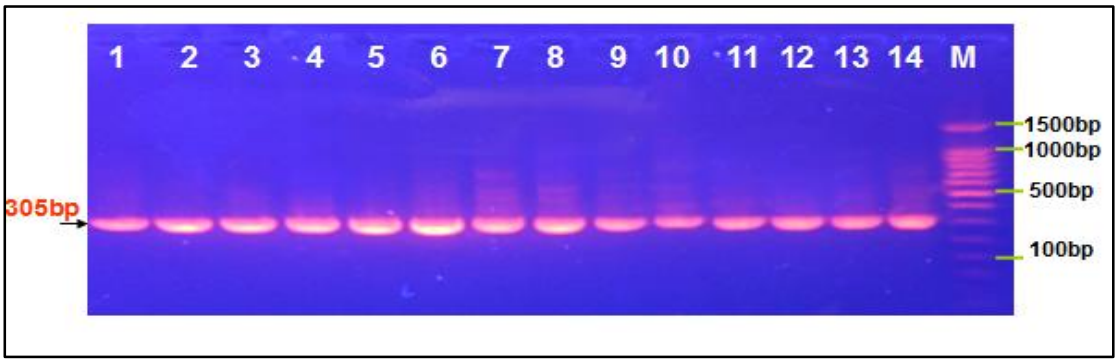

Figure (2) :Agarose gel electrophosis image, which show the PCR product results for auxotrophic*S. Typhimurium* of *hisG* gene after treatment withcustard at 305bp PCR product size,

where M: Marker100bp, Lane (1-14) are positive samples

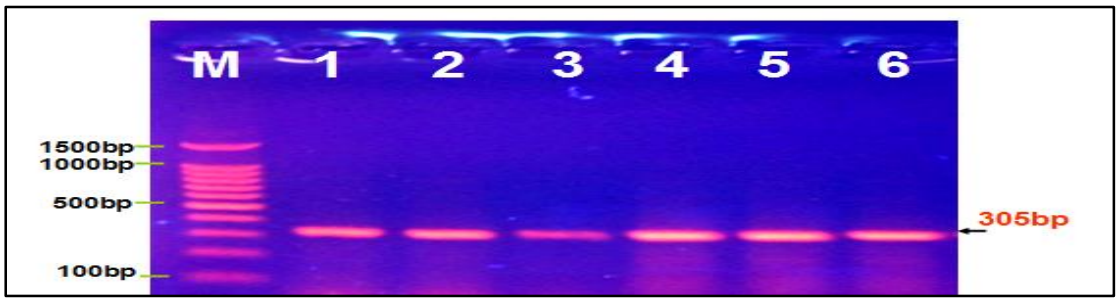

Figure (3): Agarose gel electrophosis image, which show the PCR product results for auxotrophic*S. Typhimurium* of *hisG* gene after treatment withindian red meat at305bp PCR product size, where M: Marker100bp, Lane (1-6) are positive samples

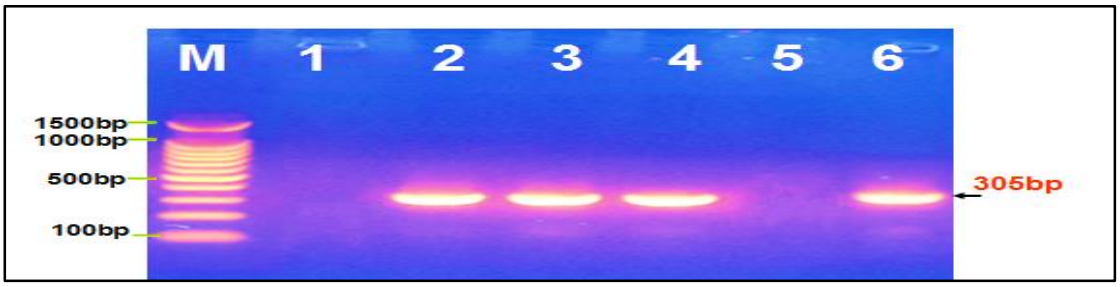

Figure (4): Agarose gel electrophosis image, which show the PCR product results for auxotrophic*S. Typhimurium* of *hisG* gene after treatment with milk at 305bp PCR product size, where M: Marker100bp, Lane (2,3,4,6) are positive samples .

DISCUSSION

Bacterial reverse mutation assay is simple, rapid and inexpensive assay for the detection of genetic damage achieved by different substances, damage of DNA by mutagens may be the main cause of death, accumulating this damage during organism's life, initiates most genetic defects and cancer Goodson-Gregg, . and De Stasio, (2009).and, Mortelmans and Zeiger, E. (2000), the test was conducted in presence or absence of metabolism activation system (S9) because many of the carcinogens is not effective until it is converted enzymatically to form active capable of covalently link with DNA and thus lead to mutation (Hakura et al.,2005) .In the current study. Bacterial reverse mutation assaywas used for detect of the mutagenic and genotoxic potential of (Potato chips, Custard, Indian red meat and Milk) using auxotrophs*S. typhimurium* to detect point mutationsin his-operon,point mutations make bacteria unable to synthesize of the histidine, The his-operon mutation can belong mutated when cultured along with any substance with a mutagenic ability and make the bacteria to again synthesize histidine resulting in revertant colonies formation as revealed in the Ames assay. On the other hand ,the point mutations are the cause of many human genetic diseases and their occurrence in oncogenes and tumor suppressor genes of somatic cells may cause tumor in humans (Malev, O. (2012).The mutagenicity and genotoxicity obtained in present study were believed due to food additives ,in biological systems, the process of oxidation of the chemical compounds and food additives lead to the synthesis of free radicals that interact with DNA and thus lead to genetic mutations (Fischer et al.,2005), also due toadd sodium nitrate for meat as a preservative against microbial contamination, nitrates decomposes to N2 and NH3, the ammonia produced from nitrates are carcinogenic, nitrates also act on Nitrosation of organic compounds and synthesis of nitric oxide NO which has a toxic effect on the genes (Andreassi et al.,2001) ,nitric oxide output of nitrate is believed working to deamination of cytosine in a single or double strand of DNA, as the deamination of cytosine lead to base substitutions

mutations in histidine operon and result in the synthesis of histidine in auxotrophs*S. typhimurium* and thus return the wild type. The classic Ames test was developed to include a molecular genetics investigation, as the PCR used to the investigation of gene *hisG* responsible for the synthesis of histidine in *S. Typhimurium*that has grown on GM agar without the need for histidine from an external source after treatment with different concentrations of the test under study , since base substitutions mutations in the mutant gene site or near the mutant gene can return to the wild-type and therefore the appearance of gene *his G*. There are no adequate studies using PCR technique in the investigation of mutagens and carcinogens in foods and other substances, exception of some the studies conducted for the purpose of investigating the chemical mutagens, as most of the research used phenotypic methods and studies of cancer in laboratory animals. The results of the current study agree with previous studies, the study Goodson-Gregg, . and De Stasio, (2009).that confirmed the presence of gene hisG and gene *hisD* in *S. Typhimurium* after treatment of various chemicals, the study (Abu-Shakra, et al.,2000) that used the PCR technique screening for mutations in conjunction with the traditional Ames test. The difference in the PCR technique results compared with the Ames test may be due to the loss of the bacterial DNA during the extraction, or lack of prefixes association with the target gene appropriately or may be due to the use of non-standard strains and thus the likely occur mutation in histidine operon during replication.

CONCLUSION

Potato chips, Custard, Indian red meat and Milk have a mutagenic effect on auxotrophs *S. Typhimurium* in all treatments studied in the presence or absence of S9 metabolism and may therefore have a role in cancer.

ACKNOWLEGEMENT

Creators earnestly wish to recognize the individuals from the Laboratory of the College of Science, University of AL -Qadisiyah for supporting.

REFERENCES

Abnet, C.C. (2007). Carcinogenic food contaminants. Cancer Invest, 25: 189-96.

Abu-Shakra, A .; McQueen, E. T. and Cunningham, M. L. (2000) . Rapid analysis of base-pair substitutions induced by mutagenic drugs through their oxygen radical or epoxide derivatives. Mutat. Res, 470: 11-18.

Ajay, K ;Kavita , S ; Monika,T; Vinay, M and Sudhir, K. K . (2013). Determination of Mutagenic Potential of Imidacloprid in Salmonella Typhimurium–TA 98 and TA 100 Following Bacterial Reverse Mutation Assay. International Journal of Biotechnology and Bioengineering Research, 4: 703-710.

Andreassi, M.G.; Picano, E.; Del Ry, S.; Botto, N.;Colombo, M.G.; Giannessi, D. ;Lubrano, V.; Vassalle, C. and Biagini, A. (2001). Chronic long-term nitrate therapy: possible cytogenetic effect in humans. Mutagenesis, 10: 512-521.

Benigni, R and Bossa, C. (2008). Structure alerts for carcinogenicity, and the Salmonella assay system: A novel insight through the chemical relational databases technology. Mutation Research/Reviews in Mutation Research, 659(3): 248-261.

Dybing, E; O'Brien, J; Renwick, AG and SannerT .(2008). Risk assessment of dietary exposures to compounds that are genotoxic and carcinogenic--an overview.ToxicolLett, 180: 110-117.

Erickson, R .P. (2010). Somatic gene mutation and human disease other than cancer: An update. Mutation Research/Reviews in Mutation Research, 705: 96-106.

Fischer, J.M.; Robbins, S.B.; Al-Zoughool, M.; Kannamkumarath, S.S. ; Stringer, S.L.; Larson, J.S.; Caruso, J.A.; Talaska, G.; Stambrook, P.J. and Stringer, J.R. (2005). Co-mutagenic activity of arsenic and benzo(a)pyrene in mouse skin. Mutation Research, 588: 35-46.

Gatehouse, D. (2012). Bacterial Mutagenicity Assays: Test Methods. Genetic Toxicology Methods in Molecular Biology. Methods Mol. Biol, 817: 21-34.

Goodson-Gregg, N. and De Stasio, E.A . (2009). Reinventing the Ames test as a Quantitative Lab that Connects Classical and Molecular Genetics. Genetics, 181: 21-30.

Hakura, A., Shimada, H., Nakajima, M., Sui, H., Kitamoto, S., Suzuki, S. and Satoh, T. (2005). Salmonella/human S9 mutagenicity test: a collaborative study with 58 compounds. Mutagenesis. 20(3): 217-228.

Herceg, Z. (2007). Epigenetics and cancer: towards an evaluation of the impact of environmental and dietary factors. Mutagenesis, 22: 91-103.

Kutlu, M.; Aydogan ,G.; Susuz ,F. and Özata, A. (2004). The Salmonella mutagenicity of water and sediments from the Porsuk River in Turkey. Environ. Tox.andPharma, 17: 111-116.

Malev, O. (2012).a dissertation on toxic effects of selected neonicotinoidsthrough different organisational levels: in vitro and in vivo studies.Universityof nova gorica graduate school.

Maron, D.M. and Ames, B.N. (1983). Revised Methods for the Salmonella Mutagenicity Test. Mutation Res, 113: 173-215.

McCann, J.; Spingarn, N.E. ; Kobori, J. and Ames, B.N. (2000). Detection of carcinogens as mutagens: Bacterial Tester Strains with R factor plasmids. Proceedings of the National Academy of Sciences, 72(3): 979-983.

Meeyutem, P. (2010) . Effects of selected thai ancient remedies extracts on mutagenicity and antimutagenicity using Ames test . M.Sc. Thesis. College ofpubic Health Sciences , University of Chulalongkorn.[18] Mortelmans, K. and Zeiger, E. (2000). The Ames Salmonella/microsome mutagenicity assay . Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 455(1): 29-60.

Richardson, S. D.; Plewa, M. J. ; Wagner, E. D. ; Schoeny, R. and DeMarini, D. M. (2007). Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in drinking water: A review and roadmap for research. Mutation Research/Reviews in Mutation Research, 636(1-3): 178-242.

Schielfer,W.C.(1980).Statistics for the biological sciences.2nd ed.Addison .Wesley publComp,California,London.

Yousif, Maitham G. And Al-Shabbani, Hiyam A. (2016) Phylogenetic Analysis of *Bacteroidesfragilis* based on Neuraminidase gene isolated from Chronic Tonsillitis. RJPBCS 7(3) Page No. 1524.