

Short Communication

The mutagenic potentials of potassium bromate and some commonly used food additives

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Accepted 26 January, 2007

Food additives are substances added to preserve flavour or improve the taste and appearance of food. The continuous consumption of these food additives could be hazardous to human health. Food additives including sodium bicarbonate, sodium benzoate, ammonium bicarbonate and potassium bromate were subjected to the Ames Spot Forward Mutation Assay using *Escherichia coli*. The mutant strains of the organism were examined in the presence or absence of rat liver metabolizing enzymes. The result showed that sodium bicarbonate, sodium benzoate and ammonium bicarbonate produced no mutant strain of the organism and no alteration in the phenotypic characteristics of the organism as compared with potassium bromate and the standard mutagen – ethidium bromide, which produced mutant strains of the organism and altered the phenotypic characteristics of the organism. This revealed that potassium bromate is mutagenic in bacteria and could be said to possess carcinogenic potentials.

Key words: Food additives, E.coli, Ames spot assay, Mutant strains.

INTRODUCTION

Food additives are substances added to preserve flavour or improve the taste and appearance of food. There has been increasing demand for produced foods due to changes in the life style of the world population, with increasing member of working mothers having less time to feed their families with home made food. Approximately, 70% of the world population consumes food additives one way or the other, everyday (WHO, 1996). The research on toxicological potentials of some food additives has received great attention as people expressed concerns about the mutagenic and carcinogenic potentials of food additive worldwide (Anderson, 1996).

There have been a lot of controversies in the use of potassium bromate in so many parts of world due to the toxicological effects (Michael, 1999). Despite the initiative to ban the use of this agent by the National Agency for food and Drug Administration and Control (NAFDAC) in Nigeria, some bakers still use it as a flour improver because of high costs of available alternatives. Srouf (1989) and Solvay (1996) have reported the use of sodium benzoate as preservatives in soft drink, sodium bicar-

bonate as a leavening agent in baking and ammonium bicarbonate as a raising agent in food industry to be at increasing rate.

The work of Kotsonis et al. (1996) showed that any substance whose dietary concentration is up to 1 ppm should undergo an extensive toxicological assay. Thus, the ability of these chemicals to cause changes in the genetic material in the nucleus of cells in ways that allow the changes to be transmitted during cell division is known as mutagenesis. In view of the above facts, this research intends to assess the mutagenic potentials of potassium bromate, sodium bicarbonate, ammonium bicarbonate and sodium benzoate using the modified Ames spot forward mutation assay.

MATERIALS AND METHODS

Chemicals

Potassium bromate, sodium bicarbonate, ammonium bicarbonate and sodium benzoate were obtained from Nomek Chemical Marchants, Oshodi-Apapa express, Lagos-Nigeria.

Study design

The study was carried out using the modified Ames spot forward mutation assay as described by Ames et al. (1975) and Maron and

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Table 1. Biochemical characteristics of *E. coli* [0157:H791and7] and revertant strains of *E. coli* [0157:H7 (1and7)] with potassium bromate.

Strain	Kliger iron agar (KIA)				Motility indole urea (MIU)			Simmons citrate
	Butt	Slant	H ₂ S	Gas	Motility	Indole	Urease	
Characteristics of <i>E. coli</i> [0157:H791and7]								
0157:H7(1)	GF	LF	-	+	+	+	-	-
0157:H7(7)	GF	LF	-	+	+	+	-	-
Characteristics of revertant strains of <i>E. coli</i> [0157:H7 (1and7)] with potassium bromate								
0157:H7(1)	NGF	NLF	-	-	+	-	-	+
0157:H7(7)	NGF	NLF	-	-	+	-	-	+

Keys: GF-Glucose fermenting, LF- Lactose fermenting, (-)-Negative, (+)-Positive

Ames (1983). The study was performed in the toxicology laboratory of the Pharmacology Department, College of medicine, University of Lagos Nigeria.

Media preparation

The agars used were Macconkey agar, Kliger iron agar (KIA), brain heart infusion agar, Simmons citrate agar, microbiological agar and peptone water. The media were prepared as described by LAB M™ (Topley Home, 52 wash lane, Bury, Lancashire, BL96AU, UK).

Inoculation

The Macconkey agar plate was inoculated with two typed strains of *Escherichia coli* [0157:H7 (1and7)] obtained from Nigeria Institute for Medical Research Yaba, Lagos- Nigeria, which were lactose and glucose fermenting, motile, urease negative, indole positive and citrate negative.

Bacterial mutation assay

The assay was performed using the two typed strains of *E. coli* which have been grown on MacConkey medium to obtain discrete colonies. The experiment was performed in the presence and absence of metabolic activation by phenobarbitone 10 mg/kg day for 3 days which induced wilstar albino rat liver enzyme (S9). The fraction of the liver enzyme was used at a concentration of 10% (v/v) in the S9 mix. The S9 mix was freshly prepared for the experiment according to the method of Maron and Ames (1983).

Test agents and positive control were tested in all strains of the experiment. Ethidium bromide was used as the positive control. Fresh cultures of tested strains obtained from MacConkey plates were grown on LAB M™ nutrient broth. The cultures were incubated for 10 – 12 h at 37°C in order to ensure adequate aeration. A portion of the nutrient broth that contains the organisms was mixed with S9 (mix) and was seeded into the nutrient agar plates using a swab stick, while the other portion without S9 was also seeded on other nutrient agar plates. Whatman paper discs were separately impregnated in 0.2, 0.1 and 0.05 g/ml of each food additives. The discs were then placed on the nutrient agar plates and incubated for 48 h. This was done for the two strains of organisms and the revertant strains produced were re-inoculated into the KIA, MIU and citrate agar to re-examine the organism biochemical characteristics. The results obtained were recorded in tabular form.

RESULTS AND DISCUSSION

The results obtained showed potassium bromate at the maximum concentration of 0.2 g/ml to be positive, while

ammonium bicarbonate, sodium benzoate, and sodium bicarbonate were negative in the Ames assay. The results showed that ethidium bromide and potassium bromate produces revertant strains in the presence of S9 mix, while ammonium bicarbonate, sodium benzoate and sodium bicarbonate produced no revertant strains. Table 1 showed the alteration in the normal biochemical characteristics of the organisms when inoculated with revertant strains produced by potassium bromate.

Bacterial mutation assay are used in a large number of laboratories throughout the world and several large scale trials carried out to test usefulness of this assay in detecting potential carcinogens and mutagens have shown this test to be good at picking out chemicals known to cause cancer (Purchase et al., 1998; McMahon et al., 1979). Bacterial mutation assays fall into three main classes, those that detect forward mutations, those that detect backward mutation and those that rely on a DNA repair deficiency. The forward mutation assay was adopted in this study using a modification of the Ames test.

This study revealed that ammonium bicarbonate gives negative result in the Ames test, although a remarkable zone of inhibition of the organisms was observed which could indicate its antimicrobial characteristics. This result is consistent with the report of Litton Food and Drug research Laboratories (1974) that showed ammonium bicarbonate not to be mutagenic. Sodium bicarbonate and sodium benzoate have been demonstrated to produce no revertant strains in this study. This report is in order with the findings of Baldwin et al. (1995) that showed these agents not to be mutagenic. However, sodium benzoate demonstrated a good antimicrobial effect.

The result of the tested potassium bromate at 0.2 g/ml was positive in the Ames test. This was substantiated with biochemical evidence using a standard procedure as demonstrated by Cowan and Steel (1993) that showed potassium bromate to alter all the natural biochemical characteristics of the organism. This could be the basis why the use of this agent has been controversial and many countries have developed initiatives to ban its use. However, more *in vivo* study should be carried out to investigate the toxicological effects of these agents and all the regulatory body should intensify effort to absolutely

control the use of potassium bromate as a flour enhancing agent.

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