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Induction of Error-free DNA Repair in *Escherichia coli* by Heat Shock and Nutritional Stress in Presence of Formaldehyde

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Heat shock and thiamine deprivation resulting *Escherichia coli* tolerance to formaldehyde stress depend on error-free DNA repair process. Heat shock and thiamine deprivation cause a simultaneous increase in mutation frequency and induce reversion to prototrophy of different nutritional markers as shown in this study through Ames fluctuation tests. Both non-mutagenic stresses in the presence of formaldehyde have now shown reduction in mutation frequency induced to prototrophy. These effects are due to the higher increase in the genotoxicity of formaldehyde in different *E. coli* strains. Both *E. coli* strains, AB1157 and AB1884, exposed to 37°C and 52°C, respectively appeared more resistant to formaldehyde concentrations in preincubation experiments. Formaldehyde appeared different levels of genotoxicity among different *E. coli* strains, in plate tests. The increase of resistance to formaldehyde is due entirely to an increase in the capacity of the cells for DNA repair. The results indicated that heat shock and thiamine deprivation could induce error-free DNA repair process in E. coli as shown herein in the control experiments of Ames-standard and modified fluctuation tests. The latter arises to the positive growth leading to significant reduction in pH than 7.2. Thus, both different types of stress are able to induce error-free DNA repair in *E. coli* cells. Bacterial growth in the Ames-fluctuation tests was negatively correlated with reduce in pH than 7.2. Results obtained from the analysis of variance revealed that; heat shock, formaldehyde concentrations and the interaction between both of them (in some cases) significantly affected on the bacterial growth and also reduced pH below 7.2.

Key words : Ames-fluctuation, Error - free DNA repair, *Escherichia coli*, Formaldehyde, Heat shock, Revertants to prototrophy, Thiamine deprivation.

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The synthesis of a special group of proteins, called stress or heat shock proteins, is induced in all organisms by sudden increase in temperature or many other stresses, including treatment with chemicals or infection by viruses (Craig, 1985; Lindquist, 1986 and Neidhardt *et al.,* 1984). The function of these proteins is unknown, but circumstantial evidence suggests that they may protect cells against the inducing agent (Craig, 1985 and Lindquist, 1986) .Similarly, DNA damage or interference with DNA replication are able to induce DNA repair systems in bacteria (Walker, 1984). Previous work (Fitt and Sharma, 1991; Sharma and Fitt, 1990) has shown that heat shock or defined nutritional stresses cause the induction , in exponential phase of *Escherichia coli* AB1157 or JE1011 , of an error-free DNA repair pathway. In *Escherichia coli,* with which the majority of experiments on inducible DNA repair mechanisms have been performed, two such pathways have been studied in detail and are known as the adaptive and SOS responses, respectively (Walker, 1984). The former is induced by certain alkylating agents and is error-free (non-mutagenic), whereas the latter occurs in response to many treatments that damage DNA or affect its replication, is error-prone, and is thought to play a major role in mutagenesis (Walker, 1984).

The SOS response involves the expression of more than 17 genes, and two of these, *groEL* and *dnaK,* are also induced by heat shock (Walker, 1985), although they are not known to play a role in the DNA repair process itself (Walker, 1985). The similarities between the effects of heat shock (Pardasani and Fitt, 1989a; Sharma and Fitt, 1990) and deprivation of amino acids, glucose phosphate, or thiamine (Fitt and Sharma, 1991; Pardasani and Fitt, 1989a) on the UV resistance of *E. coli* AB1157 prompted .to propose as a general hypothesis (Pardasani and Fitt, 1989a) that they might arise via a single, excision repair-dependent pathway inducible by a wide variety of mutagenic stresses.

The present paper addresses the question of the types of stress, deprivation of amino acids (thiamine, it is a vitamin B_1) on the formaldehyde tolerance of *E. coli* strains through plate tests, that elicit this response by showing that three further unrelated, non-nutritional, chemical or physical treatments also have a similar effect. Accordingly, this investigation studied the effects of combinations of thiamine deprivation and heat shock on exponential stationary phase cultures of *E. coli* using Ames-fluctuation tests.

Material and Methods

Bacterial strains and cultures :Three strains of *E. coli* K12 were kindly provided from *E. coli* Genetic Stock Center, Yale University, New Haven, USA , these strains as follows : (i) AB 1157 (CGSC 1157), genotype : *F–, thr-1, ara-14, leu B6,Δ (gpt - pro A) 62, lac* Y_1, *tsx-33, qsr, supE 44, gal* K_2,χ rac$^-$, *his*G_4 (Oc), *rfb* D_1, *mgl-51, rps* L_{31}, kdg k_{51}, *xyl-5, mtl-1, arg* E_3) Oc), *thi-1;* (ii) AB 1884 (CGSC 1884) the uvrC34 mutant of AB 1157 and does not have qsr of AB 1157; (iii) AB 1886 (CGSC 1886), the uvr *A6* mutant of AB 1157 and does not have qsr of AB 1157.

Cultures were grown at 37°C in a defined synthetic medium (SM) as previously described (Fitt and Sharma, 1989). Sterile non-nutrient dilution buffer was used according to Clowes and Hayes (1968). To study the effect of thiamine deprivation, thiamine-deficient medium (TDM) , i. E. SM without thiamine, was used according to Fitt and Sharma (1991). In the normal or "standard" Ames-fluctuation assay, Davis-Mingioli Medium (DMM) was used, as well as, a modified medium was also developed for use in fluctuation assays. Media were adjusted to pH 7.2 before use according to Arlauskas *et al.* (1985).

Minimal glucose agar medium (MGAM) consisting of 1.5% prolabo agar in Vogel-Bonner medium E (50 x) [magnesium sulfate 10 g/L, potassium phosphate dibasic anhydrous 500 g/L, sodium ammonium phosphate 175 g/L, citric acid monohydrate 100 g/L] with 0.4% D-glucose was used for counting mutant cells reverted to prototrophy. The pH of the medium was adjusted to 7.2 before pouring into plates. All plates were stored at 4°C and warmed to room temperature before use. The top agar also consisted of (g/L), agar 0.6% and sodium chloride 0.5%, according to Maron and Ames (1983).

Chemicals: Formaldehyde (HCHO) packed and distributed by El-Nasr Company, Egypt, according to the methods of Prolabo was used in this study in different concentrations. It consistes from 399 amino acids (M . W . 30.03 KDa) .

Assay of survival with preincubation: The method used was basically that described by Ames *et al.* (1975) for preincubation assays. In this assay, petri plates were filled with 25 ml of autoclave-sterilized minimal glucose agar medium (MGAM) containing 1% below the normal value of each of amino

acids, L-histidine, L-leucine, L-trypotophan, L-threonine and thiamine for which reversion to prototrophy was being studied. To each 2 ml of top agar in the test tube, 0.1 ml of each concentration of formaldehyde, an aliquot of 0.1 ml of 0.2 *M* sodium phosphate buffer (pH 7.4) and 0.1 ml from the suitable dilution of the old culture of the tester *E. coli* strain grown for two weeks at 37°C in the thiamine-deficient medium (TDM) were added . The tubes were vortexed and incubated at 37°C for two hours and shaken at moderate speed during the incubation. Under these conditions, survival was determined by plating no more than 100-200 cells per dish, using appropriate dilution in 0.9% (wt/vol) sterile saline from the old culture. The top agar was mixed and poured over the surface of a minimal agar plate. After the agar had set, plates were inverted and incubated at 37°C for 3-5 days. Colonies on the test and control plates were counted, three replicates in each assay were used (Arlauskas *et al.*, 1985).

Assay of survival without preincubation : The standard assay without preincubation involves adding, in order, the test mutagen, the bacterial tester strain and the buffer (if required, to soft agar) brief mixing and pouring directly onto the minimal agar plates. This are in accordance to Levin *et al.* (1982) with some modification related to Fitt and Sharma (1989). This assay requires somewhat less time and fewer manipulations than the modification with preincubation, but is somewhat less sensitive for some chemicals and mutagens. Viable counts were performed on MGAM medium (Paradasani and Fitt, 1989a). Dilutions were made with non-nutrient buffer, pH 7.0. The dishes were incubated at 37°C and colonies were counted after 1-2 days (Fitt and Sharma, 1991).

Thiamine deprivation : Early exponential phase cultures (O.D. 660 nm of 0.8-1.0 corresponding to approximately 0.5-1 x 10^9 cells / ml) were transferred to the defined medium lacking thiamine and incubation was continued at 37°C for a further 13 day according to Fitt and Sharma (1991).

Heat shock: Cultures were grown at 37°C to an O.D. 660 nm of about 1.0 (mid-exponential phase, 10^9 cells/ml) and were then shifted to the higher temperature of 48 or 52 °C for 45 min. Processing of the control (37°C) and heat-shocked cultures then depended on the purpose of the experiment, as described below. The cultures were grown in the appropriate synthetic medium and subjected to heat shock. The cells were then treated with formaldehyde before plating for survival assays. In the Ames-fluctuation test, heat-shocked cultures were exposed to the test concentration of formaldehyde in a liquid

medium in many replicates (50 test tubes, divided to five replicates each containing 10 tubes). The results reported in this study are the mean of five replicates.

Ames-fluctuation test: The Ames-fluctuation test was performed as described by Hubbard *et al.* (1984). This assay is a modification of the Ames test , the heat-shocked cultures were exposed to formaldehyde under study in a liquid medium in many replicate cultures instead of the agar plate used in the Ames assay. Samples (0.1) ml of the appropriate suspensions from heat-shocked cultures in non-nutrient dilution buffer containing about 10^9 cells/ml were transferred to each concentration of formaldehyde. After 3-day incubation, bromothymol blue (600 µg/ml) was added. Positive tubes (containing prototrophic mutants) turned yellow, whereas negative tubes remained green. For each experiment, spontaneous reversion in response to the heat-shock was included. The significance of the results was statistically determined with the x^2: $P < 0.05$ for $x^2 > 3.84$ and $P < 0.01$ for $x^2 > 6.63$ (Green *et al.*, 1976). A compound is considered as mutagenic: (I) if it induces a statistically significant increase in the number of positive tubes compared to the solvent control, (ii) if a dose-effect relationship is noticeable and (iii) if the result is reproducible.

Statistical analysis: Chi square (x^2) of the Ames fluctuation assays were calculated using the following formulla :-

$x^2 = 2\ n\ (t - c)^2 / (t + c)\ (2n - t - c)$, where n = total number of tubes in the experiment, t = number of positive tubes with each concentration and c = number of control positive tubes . Results were regarded as significant at the 1% level if x^2 values were greater than 6.63 . Each experiment involved 50 control and 50 treated tubes. The data of plate incorporation and preincubation were taken from the linear region of dose-response curves obtained by linear regression analysis (Nielsen *et al.*, 1992). The data were subjected to statistical analysis of variance technique. Treatment means were compared at 0.05 and 0.01 probability levels using the least significant difference (L.S.D.) method as mentioned by Gomez and Gomez (1984).

Results and Discussion

The standard assays with preincubation and plate incorporation:

The effect of thiamine deprivation and heat-shock on the resistance of *Escherichia coli* AB1157 to formaldehyde is shown in Table 1. In the conditions of preincubation at 37°C both non mutagenic stresses induced a

little increase in cell survival. In all other cases, the addition of preincubation resulted in greater survival than plate incorporation, this may due to an increase of prototrophic mutants induced in this case. Heat-shock at 48°C gave a large decrease in cell survival than at 37°C . In all cases shown here, the preincubation addition resulted in greater sensitivity than plate incorporation in respect of reversion to prototrophy. The effect therefore depends on protein synthesis, this are in agreement with the results of Pardasani and Fitt (1989a), who showed that thermal induction of UV resistance in *E. coli* JE 1011 required protein synthesis and/or cell growth. The present results are in accordance with Sharma and Fitt (1990), who found that incubation of the bacteria in minimal medium lacking thiamine caused an increase in UV resistance and a decrease in induced mutation frequency similar to those observed with the parental strain, but changes occurred more slowly.

As shown from the results presented in Table 2 , cell survival at both assays were large decrease gradually than in control experiment. Preincubation experiment at 52°C revealed a large increase in the viability of cells than that of the control and plate incorporation, this are due to a large increase in prototrophy induced. Except at 52°C, cell survival showed a gradually large decrease than that of the control experiment. It can be seen from these studies that thiamine deprivation resulling to induce an error-free DNA repair process in *E. coli* strains, leading to a large increase in tolerance of bacteria to formaldehyde and a corresponding decrease in their formaldehyde-induced prototrophy. The process is dependent on excision repair involving the *Uvr* C_{34} in *E coli* AB 1884 endonuclease and presumably involves an increase in the activity of this enzyme. The simplest hypothesis is that both types of stress lead to the induction of a single regullon or gene network (Gottesman, 1984) involving the Uvr gene. The fact that *Uvr* gene is a part of the SOS regulon appears to be a complicating factor, but it is known that the *Uvr* , at least, is transcribed from the different promoters. The stress-induced increase in *Uvr* C_{34} endonuclease activity in the parental strain would permit a more efficient removal of pyrimidine dimers from its DNA leading to an increased cell survival at 52°C in preincubation assay, together with a decreased lethal mutation frequency owing to the elimination of the signal for SOS regulon induction. Generally, it seems possible that the induction of error-free DNA repair may be a relatively non-specific response to a wide variety of non-mutagenic chemical, nutritional or physical stresses.

TABLE 1. Effect of formaldehyde on prototrophic mutants induced from AB1157 strain after heat shock and thiamine deprivation.

Concentration Of formaldehyde (ppm/L)	No. of prototrophic mutants / ml						Survival %					
	37°C		48°C		52°C		37°C		48°C		52°C	
	a x 10^4	b x 10^4	a x 10^4	b x 10^4	a x 10^3	b x 10^3	a	b	a	b	a	b
0.0	131	127	36	445	1127	964	100	100	100	100	100	100
10	120	130	27	345	1050	954	91	102	75	77	93	98
20	109	134	19	246	974	944	83	105	52	55	86	97
30	99	138	11	147	897	934	75	108	30	33	79	96
40	88	142	3	48	821	925	67	111	8	10	72	95
50	77	146	00	00	744	915	58	114	00	00	66	94

a = Plate incorporation. b = Preincubation.

TABLE 2. Effect of formaldehyde on prototrophic mutants induced from AB1157 strain after heat shock and thiamine deprivation.

Concentration Of formaldehyde (ppm/L)	No. of prototrophic mutants / ml						Survival %					
	37°C		48°C		52°C		37°C		48°C		52°C	
	a	b	a	b	a	B	a	b	a	b	a	b
0.0	1444	1900	4687	1547	11041	5009	100	100	100	100	100	100
10	1253	1697	3929	1273	9826	9371	86	89	83	82	88	187
20	1062	1494	3172	999	8612	13734	73	78	67	64	78	274
30	872	1291	2415	725	7317	18097	60	67	51	46	66	361
40	681	1088	1658	452	6183	22460	47	57	35	29	56	448
50	490	885	901	178	4969	26823	33	46	19	11	45	535

a = Plate incorporation. b = Preincubation.

The results of *E. coli* strain AB 1886 shown in Table 3 revealed that cell survival after heat-shock showed a large decrease than in control experiment at both assays of plate incorporation and preincubation experiments. This appeared to agree partially with the conclusions of Mitchel and Morrison (1983) based on their studies of the thermal induction of UV resistance in yeast, which they found to be dependent on recombinational repair and independent of DNA-excision repair. The mechanism responsible for these effects therefore differs fundamentally from the recombination-dependent increase in UV resistance seen in yeast. In conclusion, the results presented above in the respect of tolerance and or greater survival than those of control experiment when the cells were treated with formaldehyde after heat shock and thiamine deprivation is due entirely to an increase in their capacity for DNA excision repair. The latter arises wholly or partially from an increase in the intracellular level of the key enzyme of the pathway, Uvr ABC endonuclease (Fitt and Sharma, 1993). The real increase in positive protection of the DNA in stressed cells may due to synthesis of a protein by heat shock and nutritional stress that reduced the rate of pyrimidine dimer formation by altering DNA conformation. This would enable the stressed cells to survive better without an increase in excision repair capacity, since a given dose of formaldehyde would produce fewer dimers. These results are in agreement with Goodson and Rowbury (1991), who shown that prior exposure to many non-mutagenic stresses causes a higher increase in the resistance of *Escherichia coli* K 12 to short wavelength (254 nm) UV radiation. Heat-shock (Pardasani and Fitt, 1989b) have also been shown to induce this effect. The results obtained here for all *E. coli* strains tested demonstrate a dose-response for increase or decrease cell survival. Thus, the results with both auxotrophic markers reverted to prototrophy show that the increase of resistance to formaldehyde must arise from an error-free DNA repair process.

It is, therefore, clear that a wide variety of non-mutagenic stresses is able to induce error-free DNA repair, and the present study proposed that this may be a general response to non-mutagenic stress in these bacteria (Sharma and Fitt, 1990). Most of the treatments described above have been shown by others to induce formation of so-called heat-shock or stress proteins (Craig, 1985 and VanBogelen *et al.,* 1987). The function of the latter is still controversial, but they may provide increased resistance to the inducing stresses (Craig, 1985). However, the present study show that the duration of stress needed to give the maximum formaldehyde resistance is longer than required to induce heat shock protein synthesis. The results obtained in this study, together with those obtained previously with DNA repair mutants (Pardasani and Fitt, 1989b),

provide good support for the hypothesis that a common mechanism is involved in producing the increased level of error-free DNA repair in *E. coli* subjected to non-mutagenic stress. *Escherichia coli* has three largely error-free pathways of DNA repair (Friedberg, 1985) : photoreactivation, specific for pyrimidine dimers, excision repair, and recombinational repair. The last two are critical for the survival of cells whose DNA in damaged (Friedberg, 1985). In summary, the available evidence favors the explanation that the increase in resistance of *E. coli* strains to formaldehyde caused by a wide variety of non-mutagenic stresses is due to their common ability to induce a higher level of error-free DNA excision repair in these cells.

Genotoxicity tests:

The tester strain, *Escherichia coli* AB1157 in the absence of formaldehyde (Table 4) appeared a great level of positive tubes containing high rate of mutation frequency induced to prototrophy at both standard and modified fluctuation assays. In addition, at the concentration of 80 µl/L, appeared similar results concerning the high rate of mutation frequency induced to prototrophy using modified fluctuation test alone.

TABLE 3. Genotoxic activity of formaldehyde on prototrophic mutants induced from AB1886 strain through heat shock and thiamine deprivation.

Concentration Of formaldehyde (ppm/L)	No. of prototrophic mutants / ml				Survival (%)			
	37°C		48°C		37°C		48°C	
	A	b	A	b	a	b	A	b
0.0	413	534	284	318	100	100	100	100
10	368	407	263	301	89	76	93	95
20	323	281	242	285	78	53	85	90
30	279	155	222	268	67	29	78	84
40	234	29	201	252	57	5	71	79
50	189	00	181	235	46	00	63	74

a = Plate incorporation b = Preincubation.

TABLE 4. Effect of heat shock and thiamine deprivation on the results of the Ames-fluctuation test of AB1157 strain.

Concentration Of formaldehyde (µl/L)	Number of positive tubes											
	37°C				48°C				52°C			
	SFT	x^2	MFT	x^2	SFT	x^2	MFT	X^2	SFT	x^2	MFT	x^2
00	10		10		10		4		10		10	
80	00	100	10	00	00	100	8	16.6	00	100	10	00
160	00	100	00	100	00	100	00	7	00	100	00	100
240	00	100	00	100	00	100	00	25	00	100	00	100
320	00	100	00	100	00	100	00	25	00	100	00	100
400	00	100	00	100	00	100	00	25	00	100	00	100
								25				

SFT = Standard fluctuation test. MFT = Modified fluctuation test.

In addition , mutation frequency was decreased as a result of genotoxicity of formaldehyde concentrations increased above 80 µl/L. The results indicated that the toxic effect of formaldehyde studied on *E. coli* AB 1157 was shown at 160µl/L using Ames-fluctuation test. This investigation revealed that fluctuation test is the only tests able to demonstrate a genotoxic activity of formaldehyde. Genotoxis : exerting an effect by damaging or otherwise interfering with the action of a gene . This compound was added to some dairy products and it have a health hazards for human populations. The results obtained in Table 5 concerning *E. coli* strain AB1884 revealed that heat shock and defined nutritional stresses (thiamine deprivation) cause lowering induction of an error-free DNA repair pathway using modified fluctuation test than those in standard fluctuation (SFT). Treated cells at 37°C and thiamine deprivation appeared lower mutation frequency induced at 80 µl/L alone using MFT and SFT , while the higher concentrations were more genotoxic against *E. coli* AB1884. In addition, cells treated with 48°C with thiamine deprivation are more tolerant to both formaldehyde concentrations; 80 and 160µl/L alone using a standard fluctuation test and show similar and lower mutation frequency induced than control experiment, respectively. The studies with DNA mutant repair of *E. coli* AB 1884 support the review that these effects of non-mutagenic stress depend on the DNA excision repair pathway or a closely related process. Two possibilities need to be considered.

As shown from the results presented in Table 6, AB 1886 strain appeared high tolerance to formaldehyde concentration than AB 1157 (Table 4) strain using modified fluctuation test (MFT). In addition, the control experiment also revealed that heat shock and thiamine deprivation cause the induction of an error-free DNA repair pathway in exponential phase of *Escherichia coli* AB1886. Treated cells of AB1886 are more tolerance to formaldehyde using MFT and show a lower frequency of mutation induced than control experiment. Detailed studies (Fitt and Sharma, 1989; Pardasani and Fitt, 1989b) with *E. coli* AB 1157 and several of its mutants, all isogenic with the parent strain except for the characters examined, indicated that the effect of non-mutagenic stresses (heat-shock or defined nutritional stresses) was dependent on the *Uvr A*, which present in AB 1886 used in this study, and Uvr *B* genes and, less clearly, on *Uvr C* present here in AB 1884. The similarities between the effects of heat-shock (Sharma and Fitt, 1990) and thiamine deprivation (Fitt and Sharma, 1989) on the formaldehyde tolerance of *E. coli* AB 1886 prompted (Sharma and Fitt, 1990) to

propose as a general hypothesis that they might arise via a single, excision repair-dependent pathway inducible by a wide variety of non-mutagenic stresses. As shown in this work, heat-shock and thiamine deprivation all increase the tolerance of *E. coli* AB1886 to formaldehyde, as well as induced lower percent of mutation frequency. The effects were dependent on protein synthesis (Pardasani and Fitt, 1989a; Sharma and Fitt, 1990), increased to a maximum with time of exposure to the stress (Fitt and Sharma, 1989) and were very similar in all cases.

TABLE 5. Effect of heat shock and thiamine deprivation on the results of the Ames-fluctuation test of AB1884 strain.

Concentration of formaldehyde (µL)	Number of positive tubes							
	37°C				48°C			
	SFT	x^2	MFT	x^2	SFT	x^2	MFT	x^2
0.0	10		4		10		00	
80	2	66.67	2	4.76	10	00	00	0
160	00	100	00	25	6	25	00	00
240	00	100	00	25	00	100	00	00
320	00	100	00	25	00	100	00	00
400	00	100	00	25	00	100	00	00

SFT = Standard fluctuation test. MFT = Modified fluctuation test.

TABLE 6. Effect of heat shock and thiamine deprivation on the results of the Ames-fluctuation test of AB1886 strain.

Concentration Of formaldeyde (µL)	Number of positive tubes											
	37°C				48°C				52°C			
	SFT	x^2	MFT	x^2	SFT	x^2	MFT	x^2	SFT	x^2	MFT	x^2
00	10		10		10		10		10		10	
80	00	100	4	42.86	00	100	8	11.11	6	66.67	4	42.86
160	00	100	6	25.0	00	100	4	42.86	00	100	4	42.86
240	00	100	2	66.67	00	100	2	66.67	00	100	2	66.67
320	00	100	00	100	00	100	2	66.67	00	100	4	42.86
400	00	100	00	100	00	100	00	100	00	100	00	100

SFT = Standard fluctuation test. MFT = Modified fluctuation test.

The results presented in Tables 7, 8 and 9 support the results obtained previously in Table 4, 5 and 6, respectively concerning the assay of Ames-fluctuation test in which the bacteria heat-shocked and thiamine deprivated stresses exposed to formaldehyde in liquid medium in many replicate cultures (50 tube) for each concentration. Positive tubes containing proptotrophic mutants which turned yellow revealed higher optical density measurments for growth and more significant reduction in pH than the initial value of 7.2. Whereas, negative tubes remained green with bromothymol blue (600 μg/ml), appeared significant reduce in the optical density measurments of bacterial growth than control bacteria. The resistance to formaldehyde are due to formaldehyde dismutase-producing strains , this enzyme that catalyze a dismutation of two molecules of formaldehyde to formate and methanol . The functions of this enzyme are to detoxify formaldehyde thus rendering the organism resistant to formaldehyde . Its synthesis is induced by addition of formaldehyde to the medium . This indicated that formaldehyde inducing significant genotoxic activity in *E. coli* cells both with standard and modified fluctuation tests. The genotoxic effect of formaldehyde studied on *E. coli* may explain the hazardous effects of its addition to dairy products on human health. The mutagenic activity of this compound was also shown at the lower concentration with the appearance of higher genotoxic effect. The results obtained here point out that, the fluctuation test is more sensitive than plate tests to study the genotoxic activity of formaldehyde. The infectants and disinfectant by-products is cited by the World Health Organization (WHO) infectious among the chemical found in drinking water that could represent some hazards for public health (WHO, 1994). But, as the data concerning health hazards associated with this compound of chemicals caused health significance in drinking-water the guideline value was 900 μl/L.

Concerning formaldehyde detected, the fluctuation test is the most interesting, as it able to detect the genotoxic activity of this compound. It can be concluded that the available evidence favors the explanation that the decrease in resistance of *E. coli* strains in the fluctuation test to formaldehyde caused by non-mutagenic stresses is due to their common inability to induce a higher level of error-free DNA excision repair in these cells and also to higher genotoxicity related to higher concentrations. In this case, it is accompanied by a large decrease in their mutation frequency induced to prototrophy by formaldehyde. Most of the treatments described above have been shown by Fitt *et al.* (1992 a&b) to induce formation of so-called heat-shock or stress proteins (Jenkins *et al.*, 1988). The function of the latter is still controversial, but they may provide increased resistance to the inducing stresses (Craig, 1985).

TABLE 7. Effect of heat shock and thiamine deprivation on the detailed results of the Ames - fluctuation test of *Escherichia coli* AB1157.

Concentration Of formaldehyde (µl/L)	37°C				48°C				52°C			
	SFT (1)		MFT(2)		SFT		MFT		SFT		MFT	
	O.D. 660nm	PH(3)	O.D. 660nm	pH	O.D. 660nm	PH	O.D 660nm	pH	O.D. 660nm	pH	O.D. 660nm	pH
0.0	0.539	6.34	0.320	6.20	0.376	5.76	0.144	6.47	0.216	5.98	0.288	5.65
80	0.020	6.92	0.248	6.18	0.007	6.99	0.165	5.10	0.007	6.92	0.159	5.83
160	0.021	6.88	0.014	6.72	0.007	6.94	0.048	6.91	0.005	6.91	0.028	6.94
240	0.039	6.98	0.005	6.78	0.012	6.99	0.009	7.07	0.005	6.93	0.008	6.99
320	0.012	7.00	0.003	6.78	0.055	6.96	0.009	7.02	0.005	6.91	0.003	6.90
400	0.005	6.96	0.007	6.80	0.009	6.98	0.007	6.10	0.007	6.96	0.005	6.93
F test	**	**	**	**	**	**	**	**	**	**	**	**
L.S.D. 0.05	0.091	0.07	0.036	0.12	0.039	0.08	0.05	0.28	0.019	0.059	0.046	0.14
0.01	0.123	0.10	0.048	0.16	0.054	0.11	0.06	0.38	0.026	0.080	0.062	0.19

*, ** = P< 0.05 and 0.01, respectively.

(1) SFT = Standard fluctuation test .

(2) MFT = Modified fluctuation test

(3) The initial pH = 7.2

Heat-shock and thiamine deprivation also induces intermediate tolerance of *E. coli* strains to formaldehyde by Uvr AC-independent mechanism. It is, therefore, clear that a wide variety of non-mutagenic stresses is able to induce error-free DNA repair, and it is also proposed that this may be a general response to non-mutagenic stress in the bacteria (Sharma and Fitt, 1990). In almost all kinds of cells subjected to heat shock , sudden rise in the growth temperature , certain proteins (about 17 in *E. coli*) begin to made much faster than usual . If the new temperature is not too high (e.g. 42 C° , *E. coli*) the rate of heat shock proteins synthesis soon declines and the normal pattern of protein synthesis soon resumes (in about 20 minutes in *E. coli*) . If the temperature is too high for growth (50 C°for *E. coli*) synthesis of only heat shock proteins continues . Heat - shock proteins may cause the entire pattern of gene expression to be so abruptly shifed .

TABLE 8. Effect of heat shock and thiamine deprivation on the detailed results of the Ames-fluctuation test of AB1884 strain.

Concentration Of formaldehyde (µl/L)	37°C				48°C			
	SFT		MFT		SFT		MFT	
	O.D. 660nm	PH	O.D. 660nm	pH	O.D. 660nm	pH	O.D. 660nm	pH
0.0	0.282	6.22	0.191	6.90	0.203	4.64	0.097	7.17
80	0.015	6.36	0.084	7.08	0.339	4.85	0.036	7.13
160	0.025	6.78	0.008	7.32	0.154	6.14	0.008	7.19
240	0.032	6.80	0.008	7.30	0.014	6.99	0.006	7.14
320	0.029	6.80	0.007	7.32	0.004	7.01	0.014	7.12
400	0.021	6.80	0.005	7.08	0.004	6.97	0.014	7.07
F test	**	NS	NS	NS	**	**	NS	*
L.S.D. 0.05	0.022				0.117	0.44		0.07
0.01	0.030				0.161	0.60		0.09

SFT = Standard fluctuation test. MFT = Modified fluctuation test
The initial pH = 7.2
*, ** = P< 0.05 and 0.01, respectively NS = Not significant.

Correlation coefficients and regression equations were computed to study the relationships between both the optical density for growth and changes in pH (Table 10). It can be concluded that, in most cases of general, the optical density for growth was negatively correlated with changes in pH. The present results revealed that changes in pH are due to the bacterial growth in fluctuation experiment. This work proved that the thermally and thiamine deprivation affect to increase tolerance of *E. coli* to some formaldehyde concentrations which is due to an error-free repair process, corresponding affect to decrease pH than 7.2, this may be due to the increase in mutations induced of reversion to prototrophy for auxotrophic markers.

TABLE 9. Effect of heat shock and thiamine deprivation on the detailed results of the Ames - fluctuation test of *Escherichia coli* AB1886.

Concentration Of formaldehyde (µl/L)	37°C				48°C				52°C			
	SFT		MFT		SFT		MFT		SFT		MFT	
	O.D. 660nm	pH	O.D. 660nm	pH	O.D. 660nm	pH	O.D 660nm	pH	O.D. 660nm	pH	O.D 660nm	pH
0.0	0.587	5.03	0.252	4.82	0.259	6.70	0.521	5.15	0.404	4.96	0.266	5.94
80	0.011	7.03	0.262	5.26	0.101	6.90	0.628	5.31	0.279	5.84	0.186	6.92
160	0.009	7.00	0.213	5.59	0.003	6.82	0.685	6.06	0.013	6.75	0.154	6.70
240	0.007	6.98	0.193	6.43	0.007	6.86	0.602	6.33	0.022	6.84	0.077	7.28
320	0.004	6.95	0.006	6.77	0.008	6.88	0.467	6.20	0.013	6.83	0.267	6.88
400	0.008	6.96	0.042	6.92	0.009	5.84	0.257	6.84	0.011	6.85	0.017	7.54
F test	**		**		**		**		**		NS	
L.S.D. 0.05	0.057	0.04	0.093	0.63	0.054	0.089	0.137	0.66	0.145	0.45		0.66
0.01	0.078	0.05	0.127	0.86	0.074	0.121	0.187	0.91	0.198	0.61		0.90

The initial pH = 7.2 ** = P< 0.01. NS = Not significant.

TABLE 10. Relationships between both optical density for growth and change in pH for different *E. coli* strains affected by heat shock and thiamine deprivation using Ames - Fluctuation tests

Temperature (°C)	Media	*Escherichia coli* strains					
		AB 1157		AB 1886		AB 1884	
		Regression equation	r	Regression equation	r	Regression equation	r
37	SFT	Y = 6.97 - 1.17 x	-0.98**	Y = 7.01 - 3.37 x	-0.99**	Y = 6.95 - 1.81 x	-0.71^{NS}
	MFT	Y = 6.78 - 2.04 x	-0.98**	Y = 7.08 - 6.92 x	-0.88*	Y = 7.26 - 1.94 x	-0.84*
48	SFT	Y = 6.99 - 3.29 x	-0.99**	Y = 6.63 + 0.54 x	$+0.13^{NS}$	Y = 6.98 - 7.37 x	-0.92**
	MFT	Y = 6.90 - 7.17 x	-0.68^{NS}	Y = 7.19 - 2.29 x	-0.55^{NS}	Y = 7.12 + 0.37 x	$+0.66^{NS}$
52	SFT	Y = 6.95 - 4.49 x	-0.99**	Y = 6.89 - 4.47 x	-0.98**	NT	
	MFT	Y = 6.95 - 5.09 x	-0.99**	Y = 7.59 - 4.44 x	-0.81*	NT	

NT = Not tested. SFT, MFT = Standard and modified fluctuation tests, respectively. r= Correlation coefficient.

Results obtained in the present study concerning the analysis of variance for the Ames-modified fluctuation test (Tables 11 and 12) showed highly significant differences among the treatments of both, changes in pH and the optical density measurments of bacterial growth for both *E. coli* strains, AB1157 and AB1886. The fact that the changes in pH medium are due when the sugar is fermented sufficient acid is produced to the lower pH of the medium . From these observations, there were clear differences between temperatures and there were highly significant differences among the concentrations of formaldehyde for both strains, AB1157 and AB1886. In addition, mean squares of the interaction between the temperatures of heat-shock and formaldehyde concentrations appeared highly significant differences in *E. coli* strain AB1157 alone in comparison with other strains. Thus, it can be concluded that the significant effect of heat shock with thiamine deprivation and the concentrations of formaldehyde on mutation frequency to prototrophy are due in most cases to heat-shock temperatures and also to formaldehyde concentration, with the addition in few cases to the interaction between both of them. All of them were affect on the reversion of auxotrophic markers in *E. coli* strains to prototrophy which revealed in bacterial growth and changes in pH as well.

Results obtained in Tables 13 and 14 appeared the same trend of Ames-standard fluctuation test concerning bacterial growth and changes in pH. In this observations, the significant differences of treatments are due to heat-shock, temperatures, concentrations of formaldehyde and the interaction between both of them, among the three strains of *E. coli* for both bacterial growth and changes in pH. As shown here all of these factors were affect on the reversion of *E. coli* to prototrophy, as well as, on an error-free DNA repair process. It can be concluded that DNA damage can induce either error-free or error-prone DNA repair in *E. coli*. Heat-shock or nutritional deprivation, neither of which is known to damage DNA, can induce error-free repair. Since DNA damage induces both types of repair, it is interesting to speculate that some stresses that do not damage DNA might also be able to induce error-prone as opposed to error-free DNA repair systems. The thermal and nutritional stresses induce tolerance in *E. coli* to formaldehyde has now been shown to depend on an error-free DNA repair process. Both of them cause a simultaneous induction in the formaldehyde-induced frequency of mutation to prototrophy of nutritional markers as shown in the control experiment of the fluctuation tests. In the presence of formaldehyde both of them causes a simultaneous reduction in mutation frequency induced to prototrophy. Thus, formaldehyde is a mutagenic compound has a genotoxic hazards in *E. coli* strains and consequently on human populations as well , whilst larger scale change to genetic material are involved. In conclusion , this study supports the recommendation of prevention of using formaldehyde in food and dairy products preservation .

TABLE 11. Analysis of variance for the detailed of the Ames-modified fluctuation test concerning change in pH responsed to growth after the treatment with heat shock and formaldehyde concentrations

Source	Degrees of freedom (df)	*Escherichia coli* strains						
		AB 1157		AB 1886		df	AB 1884	
		MS	F	MS	F		MS	F
Replicates	4	0.02669	0.66^{NS}	0.53288	1.061^{NS}	4	0.02634	0.23^{NS}
Treatments	17	1.02187	25.39**	3.10267	6.18**	11	0.074	0.65^{NS}
Temperatures	2	0.32138	7.98**	8.15617	16.24**	1	0.01411	0.12^{NS}
Concentrations	5	2.97992	74.03**	6.381627	12.71**	5	0.08279	0.72^{NS}
Temp. x Conc.	10	0.18295	4.54**	0.45249	0.90^{NS}	5	0.07767	0.68^{NS}
Error	34	0.04025		0.50221		22	0.114621	

MS = Mean squares. NS, *, **= P> 0.05, P< 0.05 and P< 0.01, respectively.

TABLE 12. Analysis of variance for the detailed of the Ames-modified fluctuation test concerning optical density of growth at 630 nm in response to heat shock and formaldehyde concentrations

Source	Degrees of freedom (df)	*Escherichia coli* strains						
		AB 1157		AB 1886		df	AB 1884	
		MS	F	MS	F		MS	F
Replicates	4	0.0005	0.25^{NS}	0.00616	0.22	4	0.00536	0.46^{NS}
Treatments	17	0.0599	29.28**	0.22415	8.03**	11	0.01622	1.39^{NS}
Temperatures	2	0.0096	4.70*	1.335	47.82**	1	0.00689	0.59^{NS}
Concentrations	5	0.1808	88.27**	0.14168	5.07**	5	0.03006	2.58^{NS}
Temp. x Conc.	10	0.0096	4.7**	0.04316	1.55^{NS}	5	0.00424	0.36^{NS}
Error	34	0.0020		0.02792		22	0.01163	

MS = Mean squares. NS, *, **= P> 0.05, P< 0.05 and P< 0.01, respectively

TABLE 13. Analysis of variance for the detailed of the Ames-modified fluctuation test concerning change in pH responsed to growth after the treatment with heat shock and formaldehyde concentrations

Source	Degrees of freedom (df)	*Escherichia coli* strains						
		AB 1157		AB 1886		df	AB 1884	
		MS	F	MS	F		MS	F
Replicates	4	0.09655	32.89**	0.041946	0.508^{NS}	4	0.16724	0.34^{NS}
Treatments	17	0.67964	231.56**	2.22802	27.01**	11	2.51458	5.11**
Temperatures	2	0.05879	20.03**	1.03041	12.49**	1	2.73493	5.56*
Concentrations	5	2.42713	724.75**	3.91647	47.48**	5	3.64704	7.42**
Temp. x Conc.	10	0.08006	27.27**	1.62332	19.68**	5	1.33805	2.72*
Error	34	0.00293		0.08248		22	0.49165	

MS = Mean squares. NS, *, **= P> 0.05, P< 0.05 and P< 0.01, respectively

TABLE 14. Analysis of variance for the detailed of the Ames-modified fluctuation test concerning optical density of growth at 630 nm in responsed to heat shock and formaldehyde concentrations

Source	Degrees of freedom (df)	*Escherichia coli* strains						
		AB 1157		AB 1886		df	AB 1884	
		MS	F	MS	F		MS	F
Replicates	4	0.00157	0.42	0.00916	0.83	4	0.00196	0.26
Treatments	17	0.11414	30.73**	0.14380	13.18**	11	0.07193	9.37**
Temperatures	2	0.03203	8.62**	0.02800	2.57^{NS}	1	0.04113	5.36*
Concentrations	5	0.33511	90.22**	0.40350	36.98**	5	0.09364	12.21**
Temp. x Conc.	10	0.02006	5.40**	0.03712	3.401**	5	0.05638	7.35**
Error	34	0.00371		0.01091		22	0.00767	

MS = Mean squares. NS, *, **= P> 0.05, P< 0.05 and P< 0.01, respectively

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حث نظام إصلاح الأخطاء فى الحمض النووى الديؤكسى ريبوزى فى بكتريا القولون من خلال تعريض الخلايا للصدمات الحرارية والإجهادات التغذوية فى وجود الفورمالدهيد

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إهتمت هذه الدراسة بإيضاح مقدرة الإجهادات البيئية غير المطفرة على حث نظم إصلاح الأخطاء فى الحمض النووى الديؤكسى ريبوزى التى لا ينشأ عنها طفرات وأجريت على ثلاثة سلالات من بكتيريا القولون وتمثلت هذه الإجهادات فى الصدمات الحرارية العالية والعالية جدا أو التجويع للثيامين وذلك مع المعاملة بتركيزات مختلفه من الفورمالدهيد بإختبارات أجريت على الأطباق وفى البيئة السائلة وقد تم تتبع المرتدات إلى الطراز البرى والنمو والتغير فى الآس الهيدروجينى لبيئة النمو . وقد أوضحت النتائج مدى علاقة تعرض خلايا بكتريا القولون (*E. coli*) لصدمات حرارية مختلفة مع تجويعها للثيامين بمدى تحمل الخلايا للإجهاد البيئى الناتج عن وجود الفورمالدهيد فى وسط النمو على عملية إصلاح الأخطاء دون إستحداث طفرات فى DNA وإنتاج الطافرات المرتدة من أوليات التغذية. ولقد شوهد فى تجارب الأطباق وإختبارات Ames-fluctuation أن كل من الصدمات الحرارية المختلفة والتجويع للثيامين قد تسببا معاً فى زيادة تكرار الطافرات المستحدثة المرتدة إلى أوليات التغذية بالنسبة للإحتياجات الغذائية المختلفة. ولقد أظهرت الإجهادات البيئية غير الطفرية والتى تشمل كل من الصدمات الحرارية والتجويع للثيامين إنخفاض فى تكرار الطافرات المستحدثة المرتدة لأوليات التغذية فى وجود الفورمالدهيد. وترجع هذه التأثيرات بدورها إلى زيادة كبيرة فى السمية الوراثية للفورمالدهيد على خلايا السلالات البكتيرية المختلفة. فلقد أظهرت خلايا السلالتين AB1157 المعاملة بدرجة حرارة ٣٧ °م و AB1884

المعاملة بدرجة حرارة ٥٢ °م مقاومة مرتفعة لتركيزات الفورمالدهيد المختلفة وذلك فى التجربة التى أجرى فيها تحضين سابق للخلايا البكتيرية فى وجود الفورمالدهيد لمدة ساعتين قبل فردها على أطباق البيئة الحديه. وبالإضافة لما سبق فإنه فى جميع الحالات الآخرى من إختبارات الأطباق أظهر الفورمالدهيد درجات مختلفة من السمية الوراثية على مستوى السلالات المختلفة لبكتريا القولون. ومع ذلك ترجع زيادة مقاومة الخلايا للفورمالدهيد إلى زيادة فى كفاءة الخلايا على إصلاح الاخطاء التى قد تحدث فى محتواها من DNA . ولقد تضمنت النتائج مقدرة ظروف الإجهاد البيئى غير الطفرية على تنبيه حدوث إصلاح لأخطاء DNA فى خلايا بكتريا القولون كما هو مشاهد بتجارب المقارنة القياسية و تلك المحورة من إختبارات Ames-fluctuation . وهذا بدوره يرجع إلى حدوث نمو إيجابى فى جميع تجارب المقارنة لجميع الإختبارات المستخدمة مرتبطاً بحدوث إنخفاض معنوى فى درجة الحموضة فى الوسط عن ٧,٢ . وبذلك فإن هذه الدراسة تعكس مقدرة الإجهادات البيئية على حث نظم إصلاح الأخطاء فى ال DNA و تداخل السمية الوراثية للفورمالدهيد مع مقدرة الإجهادات البيئية فى هذا الإصلاح . لذلك فإن كلا نوعى الإجهاد البيئى المستخدمين فى هذه الدراسة لهما القدرة على حث نظام إصلاح الأخطاء الموجودة بال DNA لخلايا بكتريا القولون. كما أظهرت النتائج وجود إرتباط سالب بين مقدرة الخلايا على النمو فى إختبارات Ames-fluctuation مع إنخفاض درجة حموضة الوسط عن ٧,٢ وقد إتضح أيضاً من نتائج تحليل التباين أن الصدمات الحرارية المختلفة ، تركيزات الفورمالدهيد وفى بعض الحالات التفاعل بينهما قد أثرا جميعاً بدرجة معنوية على نمو الخلايا البكتيرية وإنخفاض درجة الأس الأيدروجينى عن ٧,٢ وكلا النوعين المختلفين من الإستجابة لظروف الإجهاد البيئى التى تعرضت لها الخلايا ترجعان إلى حث نظام إصلاح لأخطاء DNA بهذه الخلايا. وبذلك فإن النتائج المتحصل عليها من هذا البحث تؤكد مدى خطورة الفورمالدهيد على خلايا بكتريا القولون لمقدرته الطفرية وسميته البيولوجية. ولذا فإن منتجات الأغذية والألبان يجب تجنب حفظها بإضافة الفورمالدهيد إليها لما يسببه من أضرار على الصحة العامة للمجتمعات البشرية.