ECOLOGIC BEEMONITORING. STUDY OF DIFFERENT HONEY SAMPLES CAPABILITY OF INDUCING GENETIC MUTATIONS IN *SALMONELLA* MARKER BACTERIA IN AMES TEST

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Abstract

In our trials we used indicatory strains of Salmonella typhimurium *TA98 and TA100, suggested by AMES* et al. *(1971). Trials were achieved without applying the system of metabolic stimulation. Presence of mutagenic effect in the ten samples under investigation was determined by inducing inverse mutations of strains, according to histidine, from auxotrophy to prototrophy. Results were determined by taking into account the presence of the mutagenic effect in all variants of the positive control.*

Results showed 30 investigated honey samples contained mutagenic compounds, inducing mutations of the type of replacing the basic pairs.

Key words: *ecologic beemonitoring/Ames test/mutagenicity of honey/inducing inverse mutations.*

Introduction

It is known the larger part of the chemical compounds that posses a tumor inducing effect in mammals, are mutagenic, that is they are able to cause changes in the DNA structure of living cells. That is why one of the biologic activity markers of the cancerigenic agents effect is their mutagenic activity (BELITSKYI, TURUSOV, 2000). At present, the positive results of testing the mutagenic activity, of chemically contamining the environment, are regarded as markers of the cancerigenic effect, and, consequently, as mutageny related tests, that are included in the list of determining the cancerigenic capacity. Studies of AMES et al., concerning the interactivity between the cancerigenic and mutagenic effects have shown 90% of the cancerigenic matters were also mutagenic. TAMURA *et al.* have shown, for the first time, royal jelly is able of attacking DNA in much higher doses than other medicine preparations. There was established daily doses of 50/200 mg of royal jelly cannot be regarded as cancerigenic. Nonetheless, there was noticed a longer consumption of royal jelly in extreme quantities would be not desirable.

Material and methods

We used in our trials marker strains of *Salmonella typhimurium* TA98 and TA100, proposed by AMES et al.

Experiments were made without using the metabolic activation system.The presence of the mutagenic effect in the investigated honey samples was determined according to inducing reverse mutations in the test-strains of the auxotrophy depending on the histidine at prototrophy.

Honey samples were dissolved in sterile deionized water, then standard concentrations were prepared, of 1,000 and 10,000mg/ml, that were sterilized through a membrane filter, of 0.22 μm diameter (made by the "Millipor" Co., USA).

The selected and semi-enriched (0.7%) agar of the test tubes was treated in a water bath at 100°C, then introduced in a thermostatic water bath at the temperature of 45-46°C.

At the beginning, into the agar test tubes we introduced 0.1 ml of solution of the tested honey sample, then 0.1 ml of bacterial suspension. All additions were made outside the water bath.The contents of the test tube was then rapidly transferred, and poured over a lower agar layer in the 90 mm diameter Petri dish. Dishes were left during 3 to 40 minutes at the room temperature. After it cooled completely, the agar was transferred at 37°C into a thermostat. Estimating the results was made after 48 to 72 hours of incubation.

The experiment was achieved, accompanied by positive controls. As positive controls, matters were used, that induced mutations into the adequate test strains – 2-nitrofluoren (2 NF), for the TA98 strain, and sodium azide, for the TA100 strain. In each control and experimental variant, we used three dishes for each. Experiment was repeated twice. Results were established, taking into account the presence of the mutagenic effect in all variants of the positive control.

Results

The experiment results are shown in tables I and II, as mean of the reverting matters used in the three Petri dishes.

As results from the tables I and II, the honey samples did not present mutagenic activity in the TA98 strain, that registered the mutagenic agents inducing mutations of the calculating frame mutation type. Nevertheless, the samples of buckwheat and polyfloral honey (No.5), eucalyptus (No.7), and buckwheat honey (No. 8) manifested a mutagenic activity in the case of the TA100 strain. In the latter strain, a reversibility from auxotrophy to prototrophy is noticed, as a result of changing the basic pairs.

This way, out of 10 at random examined honey samples, 3 contained mutagenic compounds, inducing mutations of the type of substituting the basic pairs.

Table I

Effect of honey samples on marking bacteria strains, in *Salmonella* Ames test (experiment 1)

Strain variant, Preparation, No. of sample		Preparation dose, mg/dish	Average number of reverting matters/dish	Divisibility of exceeding control
1	2	3	4	5
TA 98	Water	0	27±2.3	1.00
	1	100	31±3.1	1.15
		1000	29±2.3	1.03
	2	100	23±2.0	0.85
		1000	28±2.4	1.03
	3	100	33±3.1	1.22
		1000	32±3.0	1.18
	4	100	25±3.0	0.92
		1000	24±2.9	0.88
	5	100	29±2.7	1.07
		1000	35±3.1	1.30
	6	100	30±3.2	1.11
		1000	26±2.9	0.91
	7	100	29±2.7	1.07
		1000	21±2.4	0.77
	8	100	24±2.3	0.88
		1000	33±3.1	1.00
	9	100	26±3.2	1.06
		1000	33±3.0	1.00
	10	100	31±2.4	0.93
		1000	29±2.3	0.87
	2-NF	5,0	2300±4.6	0.81
	DMSO*	0	25±2.1	92.00
TA 100	Water	0	41±4.1	1.00
HMAC	1	100	149±3.9	1.06
		1000	151±5.1	1.07
	2	100	139±4.1	0.98
		1000	123±5.1	0.87
	3	100	119±4.3	0.84
		1000	153±4.2	1.08
	4	100	145±4.9	1.06
		1000	162±4.8	1.00
	5	100	60±9.9	2.50
		1000	785±33.1	5.57
	6	100	155±5.9	0.96
		1000	143±6.0	0.88
	7	100	435±20.1	3.08
		1000	970±43.5	6.88
	8	100	253±10.4	1.79
		1000	680±19.3	4.82
	9	100	145±5.1	1.02
		1000	137±3.9	0.97
	10	100	141±5.3	1.00
		1000	133±5.1	0.94
	Azide	5,0	1700±55.3	12.06

**DMSO – Solvent (2-NF dimethylsulfoxyde)*

Table II

Effect of honey samples on marking bacteria strains, in *Salmonella* Ames test (experiment 2)

Strain variant, Preparation, No. of sample		Preparation dose, mg/dish	Average number of reverting matters/dish	Divisibility of exceeding control
1	2	3	4	5
TA 98	Water	0	23±2.1	1.00
	1	100	25±2.2	1.08
		1000	19±2.1	0.82
	2	100	23±1.9	1.00
		1000	28±1.9	0.88
	3	100	23±2.1	1.00
		1000	22±2.1	0.95
	4	100	25±1.9	1.08
		1000	24±2.1	1.07
	5	100	29±2.5	1.26
		1000	30±3.0	1.30
	6	100	30±2.9	1.30
		1000	26±2.5	1.10
	7	100	29±2.3	1.26
		1000	21±2.2	0.91
	8	100	24±2.3	1.04
		1000	27±2.1	1.17
	9	100	26±2.1	1.16
		1000	20±2.4	0.86
	10	100	21±1.8	0.91
		1000	29±1.9	1.26
	2-NF	5,0	1880±9.3	75.20
	DMSO*	0	25±2.1	1.00
TA 100	Water	0	131±2.2	1.00
HMAC	1	100	149±2.4	1.13
		1000	150±2.5	1.14
	2	100	139±2.1	1.06
		1000	128±2.1	0.97
	3	100	119±3.1	0.91
		1000	140±3.5	1.07
	4	100	145±3.5	1.11
		1000	125±3.6	0.95
	5	100	410±9.1	3.13
		1000	940±30.2	7.17
	6	100	135±3.1	0.96
		1000	143±4.1	0.88
	7	100	330±6.7	2.52
		1000	845±21.5	6.45
	8	100	293±11.3	2.23
		1000	768±26.4	5.86
	9	100	135±5.1	1.03
		1000	137±4.3	1.04
	10	100	141±4.4	1.07
		1000	133±4.3	1.01
	Azide	5,0	1950±23.7	14.88

**DMSO – Solvent (2-NF dimethylsulfoxyde)*

Discussion

Monitoring the genetic consequences of the environment pollution is now a present day genetic problem. Although during its activity along the human history mankind was confronted to the environment mutagenic agents, the quantity and diversity of the latter substantially increased in the 20th century. At mid-past century, the problem of the anthropogenic mutagenic pollutants was acknowledged as one of the mankind global problems (DUBININ, 2001).

Use of bees and beekeeping products for watching the environmental status, that is to say ecological beemonitoring, can be a useful tool for determining the areas with ecologically pure food products, and for making the state protect those areas.

Conclusions

In the 10 used honey samples – mountain chestnut, citric trees, raspberry, lime-tree, buckwheat and polyfloral, motherwort, eucalyptus, buckwheat, sunflower, 3 samples of floral honey, the samples of buckwheat and polyfloral, as well as those of buckwheat and eucalyptus contained mutagenic compounds, inducing mutations of the substituting the basic pairs in Ames test on *Salmonella.*

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