GLASNIK INSTITUTA ZA BOTANIKU I BOTANIČKE BAŠTE UNIVERZITETA U BEOGRADU
BULLETIN DE L'INSTITUT ET DU JARDIN BOTANIQUES DE L'UNIVERSITE DE BEOGRAD
Tom XXVI-XXVII, 1992 - 1993 (1994), Beograd

UDC 575.6 : 579.25 (497.11)
Original scientific paper

TATJANA STEVIĆ, BRANKA VUKOVIĆ-GAČIĆ, JELENA KNEŽEVIĆ-VUKČEVIĆ, RATKO M. JANKOV*, DRAGA SIMIĆ

DETECTION OF NATURAL BIOANTIMUTAGENS BY BACTERIAL SHORT-TERM TESTS

Botanical Institute and Garden „Jevremovac", Faculty of Biology, Belgrade
*Department of Biochemistry, Faculty of Chemistry, University of Belgrade

Stević, T., Vuković-Gačić, B., Knežević-Vukčević, J., Jankov, R.M. and Simić, D. (1992-1993): *Detection of natural bioantimutagens by bacterial short-term tests.* – Glasnik Instituta za botaniku i botaničke bašte Univerziteta u Beogradu, Tom XXVI-XXVII, 1 - 6.

Antimutagenic activity of CO_2 reextract of the cultivated sage containing terpenoid fraction, detected in *E. coli* repair proficient strain, was tested using *S. typhimurium*/microsome assay (Ames test). There was no effect on spontaneous and UV-induced mutations in TA98 strain. The reduction of UV-induced mutation frequency was observed when tested with TA100 strain with metabolic activation. Possible reasons for the descrepancy between results obtained with *E. coli* and *S. typhimurium* test systems are discussed.

Key words: short-term tests, Ames test, antimutagenesis, sage extract.

Ključne reči: kratkoročni testovi, Ames-ov test, antimutageneza, ekstrakt žalfije.

INTRODUCTION

Evaluation of different biological activities of extracts of various plants is directed to several principles: antimicrobial activities, antimutagenic/anticarcinogenic activities (K u r o d a et al., 1990) and the direct effect on some specific tissues. The present data on mechanisms of inhibitors of mutagenesis/carcinogenesis, although still fragmentary, provides an extremely useful scientific premise for the primary prevention of mutation-related diseases.

According to K a d a et al., (1982, 1986) antimutagenic and anticarcinogenic effect can be achieved by means of three mechanisms: (i) by increasing the fidelity of DNA replication, (ii) by favoring error-free repair of DNA damage, (iii) by inhibiting error-prone repair systems. For some antimutagenic agents mechanisms of mutagenesis inhibition are known: cobaltous chloride increases the fidelity of DNA replication and enhances recombination repair, sodium arsenite inhibits the *umuC* gene expression and enhances error-free repair in bacteria (N u n o s h i b a & N i s h i o k a , 1987). The antimutagenicity of cinnamaldehyde, coumarin, umbelliferone, vanillin and tannic acid is ascribed to promotion of error-free DNA damage repair (K a d a et al., 1986; S h i m o i et al., 1985). There is also considerable evidence for antimutagenic effect of many naturally-occurring compounds in bacterial (K u r o d a & I n o u e , 1988; K u r o d a , 1990) and mammalian test systems (B o o t m a n et al., 1988), although the underlying mechanisms are still obscure.

The bacterial short-term tests, routinely used to detect environmental mutagenes, are recomended for identifying antimutagens. In addition to their rapidity and low costs they provide considerable information about cellular and molecular mechanisms of mutagenesis and antimutagenesis. Combined with activation systems, they can even provide information about the kind of metabolic activation or detoxification that the agent may undergo *in vivo*.

In this work we tested antimutagenic effect of CO_2 reextract of cultivated sage *(Salvia officinalis* L.) containing terpenoids in the *Salmonella typhimurium* mutagenicity test (Ames test). This test is recomended by OECD (Organization for Economic Co-operation and Development, 1986) and is widely used in environmental mutagenicity testing.

MATERIAL AND METHODS

Strains. *Salmonella typhimurium* strains were TA98 *(hisD3052rfaΔuvrB* /pKM101) and TA100 *(hisG46 rfa ΔuvrB*/pKM101) (A m e s et al., 1975).

S. typhimurium mutagenicity assay medium was 1.5% Difco bacto agar and 2% D-glucose in Vogel-Bonner medium E (M a r o n & A m e s , 1983). Top agar containing Difco bacto agar (6 mg/ml) and NaCl (5 mg/ml) was supplemented with 0.05 mM biotine and 0.05 mM histidine.

S9 mix. S9 faction was isolated from the liver of Albino Wister male rats (170-180 g) induced with pheno-barbital/β-naphtho flavone (G a r n e r et al., 1972). S9 mix contained 4% (v/v) S9 fraction, 33 mM KCl, 8 mM $MgCl_2$, 5 mM glucose-6-phosphate and 4 mM NADP in 0.1 M phosphate buffer pH 7.4.

S. typhimurum assay. The overnight culture of *S. typhimurium* strain was washed by centrifugation and resuspendend in 0.01 M $MgSO_2$ giving a similar titer. UV-irradiation conditions were the same as described previously (S i m i ć et al., 1985). Samples

(0.1 ml) of UV-irradiated cells were added in 2 ml of molten top agar with or wihtout S9 mix (0.5 ml), mixed and poured onto minimal glucose agar plates with or without sage exstract. After incubation at 37°C for 48 h the number of His^+ revertants was determined and the presence of the background lawn on all plates was confirmed.

Preparation of sage extract. *Salvia officinalis* L. cultivated in Bački Petrovac (Vojvodina), collected during the flowering period 1992. was dried, ground and subjected to extraction and CO_2 reextraction as described previously (D a r m a t i et al., 1993). The CO_2 extract was dissolved in ethanol just before use and diluted with distilled water.

RESULTS AND DISCUSSION

In our previous work (V u k o v i ć - G a č i ć & S i m i ć, 1993) we designed *Escherichic coli* K12 assay-system for detection of bioantimutagens, factors which reduce the apparent spontaneous and induced mutation frequency by interfering with cellular processes of mutation fixation (K a d a et al., 1985; K u r o d a, 1990). The set of tester strains consists of a) SY252 repair proficient strain for detection of induced reversions, b) isogenic mutator strains deficient in methyl-directed mismatch repair *(mutH, mutL, mutS* and *uvrD)* for detection of spontaneous mutations, 'c) isogenic repair proficient strain carrying *sfiA::lacZ* fusion for measuring the level of SOS induction (induction of mutagenic SOS repair). The inhibition of spontaneous and UV-induced mutagenesis was studied by reversion of *argE3* ochre mutation which can occur by base substitution, mostly at A:T sites (T o d d et al., 1979).

To exclude desmutagens, factors which act directly on mutagens or their precursors and inactivate them (K a d a et al., 1982), we used UV-irradiation as mutagen. The assay-system was validated using model bioantimutagens, cobaltous chloride and tannic acid.

With a set of newly constructed *E. coli* strains we have carried out a comparative screening for natural antimutagens from various medicinal plants. The refractory antimutagenic capacity was obtained with nontoxic concentrations of 9 extracts (St. John's wort, thyme, aloe, camomile, nettle, mint, lime-tree, sage and X-tea), depending on the bacterial strain used and the concentration of the extact applied (V u k o v i ć - G a č i ć & S i m i ć, 1993) illustrating the complex situation which is expected for the whole extract.

Furhter study was performed with CO_2 reextracts of cultivated and wild sage containing terpenoids. Among three extracts tested only extract of cultivated sage, prepared without steam distillation prior to ethanolic extraction, suppressed UV-induced mutagenesis in *E. coli* repair proficient strain (S i m i ć et al., 1994).

To test the sage extract with antimutagenic activity in *E. coli* we used *S. typhimurium* tester strains which detect frameshift mutations (TA98) or base substitutions mostly at G:C sites (TA100) (M a r o n & A m e s, 1983). The experiments were carried out with or without addition of microsomal fraction of rat liver (S9). The presence of metabolic activation enzymes in the test system enables transformation of extract compounds and mimics the situation in mammalia.

Tab. 1. – Effect of sage extract on spontaneous and UV-induced mutations in TA98 strain

Sage extract (μg/plate)	His^+ revertants/plate					
	-S9			+S9		
	-UV	+UV	%I	-UV	+UV	%I
0	41	213		26	307	
50	30	227	-6	34	233	12
75	28	251	-17	36	303	2
100	36	232	-8	38	307	0
150	40	241	-13	34	275	11
200	64	222	-4	29	291	5

UV dose was 6 J/m^2.
The numbers represent the average of duplicate plates.
%I = 1 – (Nt/Nc) x 100
Nt – number of mutants/plate with sage extract;
Nc – number of mutants/plate without extract.

The effect of sage extract on spontaneous and UV-induced His^+ revertant colonies in TA98 strain is shown in Tab. 1. The extract is without significant effect on spontaneous and UV-induced frameshift mutations, neither with S9 fraction nor without.

Tab. 2. – Effect of sage extract on spontaneous and UV-induced mutations in TA100 strain

Sage extract (μg/plate)	His^+ revertants/plate					
	-S9			+S9		
	-UV	+UV	%I	-UV	+UV	%I
0	172	1636		126	2312	
50	134	1744	-7	126	1530	34
75	181	1644	0	133	1684	27
100	171	1560	5	131	1563	34
150	142	1388	15	131	1455	37
200	124	1510	8	132	1320	43

UV dose was 6 J/m^2.
The numbers represent the average of duplicate plates.
%I was calculated as in Table 1.

Moreover, in TA100 strain, detecting base substitution as SY252 stain, UV-induced mutations were not reduced in the absence of S9 fraction (Tab. 2). This result may be due to lack of excision repair in *S. typhimurium* strains which would prevent detection of antimutagenic agents enhancing excision repair. Further study wigh repair proficient *S. typhimurium* strains could validate this hypothesis.

Interestingly, in TA100 strains UV-induced mutations were reduced about 40% when extact was exposed to metabolic activation (Tab. 2). It is already established that this sage extract contains a variety of terpenoids (Đ a r m a t i et al., 1993) and it is possible that in *S. typhimurium* TA100 strain we detect different compounds which require metabolic transformation for antimutagenic activity. The study with purified terpenoids is under the way.

ACKNOWLEDGEMENTS

This work was supported by The Scientific Research Fund of Serbia, Projects Nos. 0323 and 0202.

REFERENCES

A m e s, B.N., M c C a n n, J., Y a m a s a k i, E. (1975): Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test. – Mutation Res. *31:* 347-364.

Antimutagenesis and Anticarcinogenesis Mechanisms II. (1990): Kuroda, Y., Shankel, D.M., Waters, M.D. (Eds.). – Pelnum Press, New York – London.

B o o t m a n, D.A., S c h l e g e l, R., P a r d e e, A.B. (1988): Anticarcinogenic potential of DNA repair modulators. – Mutation Res. *202:* 393-411.

Đ a r m a t i, Z., J a n k o v, R.M., V u j č i ć, Z., C s a n a d i, J., Š v i r t l i h, E., Đ o r đ e v i ć, A., Š v a n, K. (1993): Natural terpenoids isolated from the grown variety of sage. – J. Serb. Chem. Soc. *58* (7-8): 515-523.

G a r n e r, R.C., M i l l e r, E.C., M i l l e r, J.A. (1972): Liver microsomal metabolism of aflatoxin B_1 to a reactive derivative toxic to *Salmonella typhimurium* TA1530. – Cancer Res. *32:* 2058-2066.

K a d a, T., I n o u e, T., N a m i k i, N. (1982): Environmental desmutagens and antimutagens. *In* Environmental Mutagenesis and Plant Biology (E.J. Klekowski, ed.), pp. 133-152. – Preaeger, New York.

K a d a, T., K a n e k o, K., M a t s u z a k i, S., M a t s u z a k i, T., H a r a, Y. (1985): Detection and chemical identification of natural bio-antimutagens. A case of the green tea factor. – Mutation Res. *150:* 127-132.

K a d a, T., I n o u e, T., O h t a, T., S h i r a s u, Y. (1986): Antimutagens and their modes of action. *In* Antimutagenesis and Anticarcinogenesis Mecahnisms (D.M. Shankel, P.E. Hartman, T. Kada & A. Hollaender, eds.), pp. 181-196. – Plenum Press, New York.

K u r o d a, Y., I n o u e, T. (1988): Antimutagenesis by factors affecting DNA repair in bacteria. – Mutation Res. *202:* 387-391.

K u r o d a, Y. (1990): Antimutagenesis studies in Japan. *In* Antimutagenesis and Anticarcinogenesis Mechanisms II (Y. Kuroda, D.M. Shankel & M.D. Waters, eds.), pp. 1-22. – Plenum Press, New York-London.

M a r o n, D.M., A m e s, B.N. (1983): Revised methods for the *Salmonella* mutagenicity test. – Mutation Res. *113:* 173-215.

N u n o s h i b a, T., N i s h i o k a, H. (1987): Sodium arsenite inhibits spontaneous and induced mutations in *Escherichia coli.* – Mutation Res. *184:* 99-105.

O E C D, (1986): Introduction to the OECD guidelines on genetic toxicity and guidance on the selection and application of assays. – Organisation for Co-Operation and Development.

O h t a, T., W a t a a b e, K., M o r i y a, M., S h i r a s u, Y., K a d a, T. (1983): Analysis of the antimutagenic effect of cinnamaldehyde on chemically induced mutagenesis in *Escherichia coli.* – Mol. Gen. Genet. *192:* 309-315.

S h i m o i, K., N a k a m u r a, Y., T o m i t a, I., K a d a, T. (1985): Bio-antimutagenic effects of tannic acid on UV and chemically induced mutagenesis in *Escherichia coli* B/r. – Mutation Res. *149:* 17-23.

Simić, D., Knežević, J., Vuković, B. (1985): Influence of the *recB21* mutation of *Escherichia coli* K12 on prophage lambda induction. – Mutation Res. *142:* 159-162.

Simić, D., Vuković-Gačić, B., Knežević-Vukčević, J., Đermati, Z., Jankov, R.M., (1994): New assay system for detecting bioantimutagenes in plant extracts. – Arch. Biol. Sci. Belgrade, *46* (3-4), 81-85.

Todd, P.A., Monti-Bragadin, C., Glickamn, B.W. (1979): MMS mutagenesis in strains of *Escherichia coli* carrying the R46 mutagenic enhancing plasmid: phenotypic analysis of Arg^+ revertants. – Mutation Res. *62:* 227-237.

Vuković-Gačić, B., Simić, D. (1993): Indentification of natural antimutagens with modulating effects on DNA repair. *In* Antimutagenesis and Anticarcinogenesis Muechanisms III (G. Bronzetti, H. Hayatsu, S. De Flora, M.B. Waters & D.M. Shankel, eds.), pp. 269-278. – Plenum Press, New York-London.

Rezime

TATJANA STEVIĆ, BRANKA VUKOVIĆ-GAČIĆ, JELENA KNEŽEVIĆ-VUKČEVIĆ, RATKO M. JANKOV*, DRAGA SIMIĆ

DETEKCIJA PRIRODNIH BIOANTIMUTAGENA POMOĆU KRATKOROČNIH BAKTERIJSKIH TESTOVA

Institut za botaniku i Botanička bašta „Jevremovac", Biološki fakultet, Beograd
*Katedra za biohemiju, Hemijski faklutet, Univerzitet u Beogradu

U divljem soju *E. coli* otkrivena je antimutagena aktivnost CO_2 reekstrakta kultivisane žalfije koji sadrži frakciju terpenoida. U ovom radu za detekciju antimutagene aktivnosti CO_2 reekstrakta žalfije korišćen je *S. typhimurium*/mikrozom test (Ames-ov test). U soju TA98 nije utvrđen efekat na spontanu i UV-indukovanu mutagenezu, a u TA100 soju utvrđeno je smanjenje frekvence UV-indukovanih mutacija u prisustvu mikrozomalne frakcije ćelija jetre pacova. Navedeni su i diskutovani mogući razlozi koji su doveli do različitih odgovora u *E. coli* i *S. typhimurium* test sistemima.