Bulgarian Journal of Agricultural Science, 14 (No 3) 2008, 271-282
Agricultural Academy

THE Ty1 TRANSPOSITION ASSAY: A SHORT – TERM TEST FOR SELECTIVE DETECTION OF CARCINOGENIC POLLUTANTS IN ENVIRONMENTAL SAMPLES

M. PESHEVA[1], O. KRASTANOVA[1], R. STAMENOVA[2], I. TODOROVA[3], R. SHOEVSKA[3], P. VENKOV[2] and Tsv. TSVETKOV[2*]
[1]*Sofia University Faculty of Biology, BG -1421 Sofia, Bulgaria*
[2]*Institute of Cryobiology and Food Technology, BG -1407 Sofia, Bulgaria*
[3]*National Executive Environmental Agency, BG - 1618 Sofia, Bulgaria*

Abstract

PESHEVA, M., O. KRASTANOVA, R. STAMENOVA, I. TODOROVA, R. SHOEVSKA, P. VENKOV and Tsv. TSVETKOV, 2008. The Ty1 transposition assay: a short – term test for selective detection of carcinogenic pollutants in environmental samples. *Bulg. J. Agric. Sci.*, 14: 271-282

The Ty1 short-term test is based on induction the transposition of the oncogene-like Ty1 retrotransposon in a gene-engineered *Saccharomyces cerevisiae* tester strain. Previous studies have evidenced the positive response of the test to a number of laboratory carcinogens, including such that are undetectable by the other short – term tests and the negative response of Ty1 test to mutagens without carcinogenic potential. In this communication we report the results from the application of Ty1 test to study soil, water and air environmental samples. The same samples were also chemically analyzed for mutagens, carcinogens, heavy metals, polycyclic aromatic hydrocarbons, pesticides and polychlorinated biphenols and the data compared to the responses of the Ty1 test. The Ty1 test was positive in concentration-dependent and kinetics experiments with all samples that contained carcinogens above the ecological standards and gave negative results with samples free of carcinogens, although some of them contained noncarcinogenic mutagens in high amounts. These results evidenced that the property of Ty1 test for selective detection of carcinogens, previously found in experiments with laboratory mutagens and carcinogens, can be extended to environmental studies. It is concluded that the usage of Ty1 test together with another short-term tests for environmental studies is advantageous and will give a more accurate picture of pollution with carcinogenic pollutants.

Key words: short – term test, carcinogens, environment

Introduction

Environment can be polluted with different pollutants, one of the most dangerous for human health being the carcinogens. Presently, environmental pollution is monitored mainly using bacterial tests (Maron and Ames, 1983). The *Salmonella* mutagenicity short-term tests are used to identify chemicals with mutagenic and carcinogenic potential. Although a considerable overlapping of mutagens and carcinogens tested in the

[*]*Corresponding Author: cryobg12@yahoo.com*

Ames assay was first reported (Bartsch et al., 1980), more recent validations have concluded that the correlation between carcinogenicity and mutagenicity is lower than earlier estimations (Ashby and Tennant. 1991; Ramel et al., 1996). Animal and human carcinogens without apparent genotoxic activity are difficult or impossible for detection by the Ames test which provoked the development of other short – term tests, such as alkaline elution, nick-translation, single-cell gel electrophoresis (Leroy et al., 1996), the restriction site mutation assay (Parry et al., 1990), the micronucleous expression in lymphocytes (Krish – Valders and Fenech, 2001). Although some of the assays were evaluated as promising methods for monitoring exposure to genotoxic chemicals, few of them can be applied to large scale environmental studies.

Saccharomyces cerevisiae strains have been used for construction of short-term tests for detection of mitotic gene conversion and crossingover, forward and reverse mutations (Zimmerman et al., 1975) as well as chromosomal missegregation (Alder and Parry, 1993). A system selective for deletions has been constructed in *Saccharomyces cerevisiae* (Schiestl et al., 1989) and has been termed DEL assay. The results obtained showed that the DEL test has a wider detection spector compared to Ames test which was explained by the positive response of the test to deletions inducible by carcinogens (Kirpnick et al., 2005). Although *S. cerevisiae* cells respond to a vast spectrum of mutagens and carcinogens, the short-term tests based on yeasts showed lower sensitivity compared to bacterial tests (Ramel et al., 1996) due to the lower permeability of *S.cerevisiae* cells (Morita et al., 1989, Staleva et al., 1996, Kirpnick et al., 2005).

Recently, the Ty1 transposition assay has been proposed as a short - term test for detection of carcinogens (Pesheva et al., 2005). The test is based on the activation by carcinogens of the *S. cerevisiae* Ty1 retrotransposon whose structure and life cycle are very similar to those of the known oncoviruses. The results obtained evidenced that Ty1 transposition is induced by a number of laboratory carcinogens, including such that are undetectable by Ames, DEL or other short-term tests (Pesheva et al., 2005). In a more recent study (Pesheva et al., 2007) evidence for a selective response of Ty1 test to laboratory carcinogens is provided. The results obtained with carcinogens or mutagens that are not carcinogens, with carcinogenic or non-carcinogenic but toxic heavy metals and with free (carcinogenic) or conjugated (non-carcinogenic) bile acids showed that the Ty1 test responds positively only to carcinogens and gives negative values with non-carcinogenic substances. The sensitivity of Ty1 test to carcinogens was increased by the introduction of the mutation *ts1/sec53* which increases the permeability of *S. cerevisiae* cells (Staleva et al., 1996; Pesheva et al., 2005).

In this report we present evidence that the Ty1 test can be successfully used as a short-term assay for studying environment polluted with carcinogenic substances. The advantages of Ty1 test consist in its high sensitivity and selective detection of environmental carcinogenic pollutants including such that are undetectable with the other short-term tests. The application of Ty1 test together with the Ames test will give a more complete and accurate picture of pollution status for the regions under investigation.

Materials and Methods

Sample collection and processing

Soil (S), water (W) and air (A) samples were collected during 2004-2005 from regions in Bulgaria declared by the National Executive Environmental Agency (NEEA) with low (add number samples) or high (even number samples) pollution levels. Soil samples were from villages around the capital city of Sofia: Yana (polluted) and Lokorsko (clean). Water samples were from Iskar reservoir (clean) and Dolni Bogrov River (polluted). The samples were collected during: July 2004 (S1, S2, W1, W2, A1, A2); December 2004 (S3, S4, W3, W4, A3, A4); April 2005 (S5, S6, W5, W6), June 2005 (S8) and October 2005 (S10-1, S10-4).

Samples collection and extraction were made according to published procedure (Rossi et al., 1995). In summary, whole particular matter from air was collected on glass fiber filters by a low volume sampler

during 24h periods and the daily filters were pooled to obtain the monthly sample. The latter was extracted with toluene in Soxhlet apparatus for 24h and the solvent evaporated with a rotary evaporator. The residual dry matter from each extraction was dissolved in dimethyl sulfoxide (Me_2SO) to obtain a constant ratio ($65m^3$/ml) of air volume to extract volume. Essentially the same extraction method was used for soil samples each one representing an average sample taken in $10m^2$ areas. The water samples were concentrated 10 fold on a rotary evaporator before usage.

Collected and processed samples were studied immediately. The reproducibility of the data obtained in the Ty1 test response was tested by repetitions in the following 15 days with aliquots of samples stored at -20°C. During this period changes in their activity were not observed.

Ty1 transposition test

The Ty1 test was performed as already described (Pesheva et al., 2005) with *Saccharomyces cerevisiae* DG1141ts1 as tester strain. The DG1141ts1 (*MATá ura3-167 his3 Δ 200 Tym HIS3AI ts1*) has a Ty1 element marked with the indicator gene *HIS3AI* developed by Curcio and Garfinkel (1999). The successive transposition of this Ty1 requires transcription and splicing of Ty1-RNA to remove the artificial *AI* intron and encapsulating the spliced RNA in virus-like particles where it is reverse transcribed into Ty1-cDNA with intact HIS3 gene. Every integration of this Ty1-cDNA into new places of the genome gives rise to a HIS^+ colony on a minimal medium lacking histidine. Tester cells that did not undergo Ty1 transposition remain HIS^- because of the *his3 Δ 200* deletion and can not growth on selective medium without histidine. Thus, Ty1 is a quantitative test for determination the carcinogen induced Ty1 transposition. The tester DG1141ts1 strain was obtained from DG1141 strain (Curcio and Garfinkel, 1999) by replacement the *TS1* gene with the temperature sensitive allele *ts1/sec53* which increases cellular permeability to different substances, including mutagens/carcinogens (Pesheva et al., 2005) and increases in this way the sensitivity of the assay.

Tester cells were cultivated at 30°C in YEPD liquid medium to a density of $4\text{-}6x10^7$ cells/ml. Volumes of 200ìl processed samples were added to 3.8ml culture aliquots for 30min, except in the concentration-dependent and kinetics experiments. Cells were washed by centrifugation, suspended in 4ml fresh YEPD and cultivated at 20°C for 12 h to complete the Ty1 transposition events. Dilutions (10^{-3} to 10^{-5} depending on sample-toxicity) were made and cells were plated on YEPD to determine the titer of survived cells. For counting the HIS^+ transposants 200µl plate of undiluted culture were plated on SC-HIS plates (10 plates per sample). After cultivation for 4 days at 30°C the median transposition rates were determined by the Drake (1998) equation. The average value ± S.D. from 5 to 10 repetitions were calculated for each sample. In the supplied tables, results are also presented as "fold increase" of Ty1 transposition (Staleva and Venkov, 2001). The fold increase = Fts/Ftc where:

$$Fts = \frac{\text{Number } His^+ \text{ colonies (SC-His medium) of treated culture}}{\text{Number colonies (YEPD medium) x rate dilution of treated culture}}$$

$$Ftc = \frac{\text{Number } His^+ \text{ colonies (SC-His medium) of control culture}}{\text{Number colonies (YEPD medium) x rate dilution of control culture}}$$

The presentation of results as "fold increasae" of Ty1 transposition takes into account the dilutions of cultures which are different due to the different toxicity and concentrations of the studied chemicals and relates the number of Ty1 transposants of treated to control cells. The Ty1 transposition in the controls was taken as fold increase of 1.0 and by analogy with other short – term tests, a fold increase in treated culture equal or higher than 2.0 is considered as positive response of the Ty1 assay.

In most of the tables results obtained with 200µl processed sample per test are presented, which per-

mits a comparison of the effect of the different samples. The aliquots of 200µl are representative for extracts of 1.5g soil, or particular matter in $6m^3$ of air, or 2ml of water. For highly toxic samples the results obtained with 100µl/test are shown in tables which are indicated by a footnote. Environmental samples were also tested in concentration (= volume) dependent and kinetics experiments.

Preparation of S9 mix

All environmental samples were studied in Ty1 test with external metabolic activation by S9 microsomal hepatic fractions to activate potential promutagens / procarcinogens in the samples. S9 fraction from rat liver was obtained from Microbiological Associates (Rockville, USA) and S9 mix was prepared as described (Maron and Ames, 1983). S9 mix contained per ml: 0.9ml of S9 fraction, 8µmol of $MgCl_2$, 33 µmol of KCl, 5 µmol of glucose-6-phosphate, 4 µmol, of reduced nicotineamideadenine dinucleotide phosphate and 0.5 units of glucose-6-phosphate dehydrogenase with 100 µmol of sodium phosphate buffer, pH=7.0.

Quantitative chemical analysis

Processed environmental samples were analyzed in the National Executive Environmental Agency (NEEA) laboratories according the proposal. NEEA is the National Reference Centre for the European Environmental Agency and the methods used for the quantitative chemical analysis are the internationally recognized analytical methods used in of the EU Environmental Agencies.

Air samples

The basic compounds that are measured include petrol products (â absorption method), sulfur dioxide (ultraviolet fluorescence method), nitrogen dioxide (chemiluminescence method), hydrogen sulfide (UV-fluorescent method) lead aerosols (system MOSW method) ammonia (chemiluminescense method), hydrochloric acid and sulfuric acid, arcenium and other heavy metals.

Water samples

The water samples were studied for nitrites,nitrates, N_2, P, Cl, SO_4, petrol products, cyanides, Pb, Ni, Zn, Cd, Co, Cu. Water samples were also studied for the following indices not shown in tables because their concentrations were below the ecological standards for all studied samples: polycyclic aromatic hydrocarbons such as naphthalene, acenophtalene, acenothylene, fluorine, phenanthrene, anthracene, fluoranthene, pyrene, phenol, benzo(b)fluoranthene, benzo(k)fluoranthene benzo(a)pyrene, benzo(e)-pyrene, indeno(1,2,3,-cd)pyrene, dibenzo(ah)-anthracene, benzo(ghi)perylene**.** Pesticides, such as alpha-HCH, beta-HCH, delta-HCH, epsilon-HCH, heptachlor epoxide, trans-heptachlor epoxide, oxychlodran, cis-chlodran, trans-chlodran, endo sulfanII, a.p.-DDE, p.p.DDE, a.p.DDD, p.p.DDT, a.p.DDT, merix and HCB.

Soil samples

A short excerpt of the chemical analysis of soil samples is given in Table 5 and includes: Pb, Cu, As, Zn, Cd, naphthalene, acenophtalene, acenophtylene, fluorine, phenantrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, crysene,benzo(a)pyrene, benzo(e)pyrene,indeno(1,2,3,-cd)pyrene, dibenzo-(ah)anthracene, benzo (ghi)pyrene and petrol products. The chemical parameters that were analyzed but not included in Table 5 because the concentrations of chemicals were bellow the ecological standards for all samples are: pesticides such as: alpha-HCH, beta-HCH, delta- HCH, epsilon-HCH, heptachlor, aldrin, isodrin, dieldrin, eudrin, cis-heptachlor epoxide, trans-heptachlor-epoxide, oxy-clodran, cis-chlodran, endo sulfanI, endo sulfanII, o.p.DDE, p.p.DDE, a.p.DDD, p.p.DDD, a.p.DDT, p.p.DDT, methoxychlor, merix, ACB, polychlorinated biphenols such as: PCB28, PCB52, PCB101, PCB105, PCB118, PCB138, PCB153, PCB156 and PCB180.

Materials

All chemicals used for samples analysis were analytical grade and obtained from Sigma Ltd. Yeast

media were prepared as described (Sherman et al., 1986) using nutritional components from Difco Chem. Co.

for sample processing is adequate and the Ty1 test does not give false positives with extracts of not polluted soils. Samples collected from polluted regions

Table 1

Ty1 test response to soil samples

Sample	Survival [a], %	Median Rate of transposition x 10^{-7} [b]	Fold increase	Mutagens/carcinogens Found in chemical analysis [c]
Control				
Me_2SO 5% (negative control)	100 (621)	3.4 ± 0.8	1.0	
B(a)P 40µg/ml (positive control)	91(565)	24.1 ± 0.7	7.8	
Clean regions				
S1	95 (590)	5.2 ± 1.1	1.6	Fluoranthene
S3	93 (578)	4.4 ± 1.3	1.4	Fluoranthene
S5	98 (609)	5.0 ± 0.8	1.5	None
Polluted regions				
S2	82 (509)	10.6 ± 1.4	3.8	As,B(a)P, B(a)A, pyrene, crysene, benzo (ghi)perylene, B(e)P, fluorenthene, Pb
S4	59 (366)	8.6 ± 1.0	4.2	As, B(a)P, pyrene, crysene, B(e)P, fluorenthene, Pb
S6	71 (441)	9.5 ± 0.8	3.9	As, B(a)A, pyrene, crysene,fluoranthene, Pb
S8	55 (342)	12.2 ± 1.7	6.5	B(a)P, B(a)A, crysene, petrol products, B(e)P fluoranthene, Pb, A
S10-1 [d]	35 (217)	10.1 ± 0.9	8.5	B(a)P, B(a)A, benzo (ghi)perylene, crysene, petrol products fluoranthene, B(e)P
S10-4	73 (453)	11.9 ± 0.9	4.8	As, Ni, Cu, Pb

[a] Actual number of colonies is given in parenthesis

[b] Average values ± S.D. from 8 experiments

[c] Only substances found in chemical analysis (Table 5) above the ecological norm are given and carcinogens are shown in bold

[d] Tested in 100µl per test

Results

Study of environmental samples in Ty1 test

Results obtained in the study of soil (S) samples are summarized in Table 1. All samples collected from clean regions showed high survival rates and gave negative responses (fold increase <2.0) in Ty1 test. These results also evidence that the method chosen

showed different toxicity with a tendency of higher toxic effect for samples containing petrol products (S-8, S-10). All samples gave positive responses in Ty1 test with a fold increase of Ty1 transposition ranging 4 to 8 for tester cells, treated with 200ìl extract of sample/test for 30min. The positive answer of S samples from polluted regions was confirmed in concentration (= volume) dependent and kinetics experi-

Table 2
Concentration dependence of Ty1 test to soil samples

Sample	Sample extract, µl/test	Survival [a], %	Median rate of transposition x 10^{-7} [b]	Fold increase
Controls				
Me_2SO	50	100 (490)	2.6 ± 1.0	1.0
	100	100 (460)	2.3 ± 0.7	1.0
	200	100 (468)	2.8 ± 0.9	1.0
	300	100 (441)	4.6 ± 1.2	1.0
Clean region [c]				
S1	50	101 (495)	3.4 ± 1.3	1.3
	100	99 (455)	2.1 ± 0.6	0.9
	200	97 (446)	4.0 ± 0.9	1.5
	300	87 (384)	7.2 ± 1.6	1.8
Polluted region [c]				
S2	50	93 (456)	5.1 ± 0.9	2.1
	100	85 (391)	5.7 ± 1.3	2.9
	200	79 (370)	9.1 ± 1.6	4.1
	300	69 (304)	24.1 ± 1.8	7.6

[a] Actual number of colonies is given in parenthesis
[b] Average values ± S.D. from 5 experiments
[c] Median rates and survival values for S1 and S2 were related to the corresponding Me_2SO volume controls

Table 3
Time course of Ty1 test response to soil samples

Sample	Time, min	Survival [a], %	Median rate of transposition x 10^{-7} [b]	Fold increase
Controls				
Me_2SO	15	100 (567)	2.5 ± 0.7	1.0
(200µl)	30	100 (580)	2.6 ± 1.0	1.0
	60	100 (540)	2.5 ± 0.8	1.0
	90	100 (520)	3.1 ± 1.3	1.0
Clean region [c]				
S3 (200µl)	15	93 (527)	3.5 ± 0.6	1.5
	30	98 (568)	3.6 ± 1.1	1.4
	60	95 (513)	4.6 ± 0.8	1.9
	90	91 (473)	5.1 ± 1.3	1.8
Polluted region [c]				
S8(200µl)	15	81 (459)	7.7 ± 1.4	3.8
	30	63 (365)	11.6 ± 0.9	7.1
	60	51 (275)	11.1 ± 1.0	8.7
	90	30 (156)	8.3 ± 1.2	8.9

[a] Actual number of colonies is given in parenthesis
[b] Average values ± S.D. from 4 experiments
[c] Median rates and survival values for S3 and S8 were related to the corresponding Me_2SO volume controls

ments. As shown on Table 2, the positive responses appeared at relatively low doses having moderate killing effect on tester cells and gradually rose with increasing the volume of studied sample. No such volume dependent effect was observed with S samples from the clean regions, except a slight increase in median rate of Ty1 transposition due to the increased concentrations of Me_2SO. It had been shown (Pesheva and Venkov, 2006) and confirmed in this study (Table 2, controls) that higher concentrations of Me_2SO are moderate inducers of Ty1 transposition. For this reason in the standard Ty1 test the concentration of Me_2SO_4 was no more than 5% in the final assay volume using 200 µl processed sample in 3.8ml tester culture. The data on Table 3 also confirm the positive responses of Ty1 test to S samples from polluted regions: with increasing the time of exposure the tester cells responded with an increase in the rate of Ty1 transposition reaching saturation levels at longer periods of treatment. Contrary to these results, treatments with S samples from clean regions did not enhance the rate of Ty1 transposition and background values were obtained even for long exposures. Tables 2 and 3 show results obtained with one S sample and very similar data were found for all other S samples (not shown). The expert of the detailed chemical analy-

sis given in the last column of Table 1 show absence of carcinogens in samples from clean regions and presence of carcinogenic substances and heavy metals in the samples from polluted regions suggesting that the Ty1 test responds positively only to treatment with soil samples polluted with carcinogenic compounds. Special attention should be given to samples S1 and S3 which contain increased amounts of flouranthene and showed negative responses in Ty1 test in concentration dependent and kinetics experiments (Tables 2 and 3). It has consistently been shown fluoranthene to be a potent mutagen in bacterial and many mammalian in vitro test systems, however results from in vivo carcinogenicity studies in rodents indicated it is not carcinogenic (Goldman et al., 2001; Verschueren, 2001). The negative response of Ty1 test to samples S1 and S3 confirmed previous results (Pesheva et al., 2007) evidencing a negative response of Ty1 test to a number of laboratory non-carcinogenic mutagens and further extends the selective response of Ty1 test to carcinogens in environmental studies.

Air samples (A) were collected only in 2004 and represent toluene extracts from powdered dry matter collected by filtration. The analysis of A samples in Ty1 test showed samples A1 and A3 (from clean regions) to give negative results contrary to samples A2 and A4 (from polluted regions) which were positive in volume dependent and kinetics experiments (not shown). The chemical analysis of A samples showed increased amounts of phenol in A2 and A4 which can explain the positive response in Ty1 test. Samples A2 and A4 were taken from one region during summer and winter of 2004 and the positive results obtained in Ty1 test together with the presence of increased concentrations of phenol evidence the existence of a continuous origin polluting the region.

All water samples (W) from clean (W1, W3, W5) and polluted (W2, W4, W6) regions were negative in Ty1 test and did not have a significant toxic effect on tester cells (Table 4) showing absence of toxic or carcinogenic substances in all samples. These negative results coincide well with the chemical analysis of W samples (Table 6). W samples from polluted regions were taken from a small river known to be polluted by previous monitoring studies of NEEA and the absence of pollutants in these samples found in our studies is most likely due to the heavy rainy 2004-2005 years causing flooding in many regions, including the studied one.

Table 4
Ty1 test response to water samples

Sample	Survival [a], %	Median rate of transposition x 10-7 b)	Fold increase	Mutagens/ carcinogens found in chemical analysis
Control(H_2O)	100 (812)	2.5 ± 1.1	1.0	none
Clean regions				
W1	102 (828)	1.0 ± 1.0	0.4	none
W3	99 (804)	2.7 ± 0.7	1.1	none
W5	95 (771)	3.3 ± 0.9	1.4	none
Polluted regions				
W2	97 (788)	3.6 ± 1.1	1.5	none
W4	95 (771)	3.6 ± 0.8	1.7	none
W6	85 (690)	3.4 ± 1.2	1.6	none

[a] Actual number of colonies is given in parenthesis
[b] Average values ± S.D. from 8 experiments

Results obtained in the study of S, W, and A environmental samples in the Ty1 test showed that the assay was positive only with samples polluted with carcinogenic substances. The Ty1 test remained negative in samples free of carcinogens but containing increased amounts of non-carcinogenic mutagens, indicating a selectivity of the test in detection of environmental carcinogens

Chemical analysis of environmental samples

Parts of the detailed protocols for the quantitative analytical study of environmental samples are given in Tables 5 and 6. For the parameters analyzed but not included in tables (indicated in Materials and Methods) values bellow the accepted ecological standards were found. The results obtained in the chemical analysis of soil samples (Table 5) showed a significant pollution of S samples collected from polluted regions. The amounts of some heavy metals (Pb, As, Zn) were

Table 5
Chemical analysis of soil samples

Chemicals	Ecological standard, mg/kg	Pollutants in soil samples, mg/kg								
		Clean regions samples			Polluted regions samples					
		S1	S3	S5	S2	S4	S6	S8	S10-1	S10-4
Pb	50	38	34	39	334	346	317	215	ND	293
Cu	150	29	28	26	73	85	70	169	ND	338
As	25	19	11	9	103	73	70	11	ND	356
Zn	200	78	78	68	342	305	184	215	ND	257
Cd	2	0.4	0.4	0.4	2.2	2.2	1.0	1.0	ND	1.7
Ni	25	ND	ND	ND	ND	ND	ND	ND	ND	29
Naphthalene	0.05	0.05	0.05	0.05	0.12	0.05	0.12	0.48	0.18	ND
Acenophthene	0.03	0.003	0.003	0.002	0.007	0.02	0.01	0.08	0.03	ND
Acenaphthalene	0.03	0.001	0.001	0.001	0.001	0.001	0.009	0.023	0.04	ND
Fluorene	0.03	0.001	0.001	0.006	0.001	0.001	0.017	0.080	0.060	ND
Phenanthrene	0.045	0.042	0.045	0.035	0.145	0.019	0.132	0.840	0.080	ND
Anthracene	0.05	0.001	0.003	0.010	0.021	0.010	0.015	0.120	0.060	ND
Fluoranethene	0.02	0.262	0.136	0.027	0.214	0.053	0.128	0.780	0.080	ND
Pyrene	0.02	0.018	0.021	0.012	0.183	0.047	0.081	0.690	0.090	ND
Benzo(a) anthracene	0.02	0.006	0.005	0.002	0.085	0.020	0.043	0.370	0.130	ND
Chrysene	0.002	0.019	0.002	0.002	0.117	0.057	0.053	0.340	0.100	ND
Benzo(e)pyrene	0.015	0.011	0.015	0.001	0.092	0.176	0.001	0.280	1.000	ND
Benzo(a)pyrene	0.02	0.001	0.001	0.001	0.016	0.160	0.001	0.190	0.060	ND
Indeno(1,2,3-cd)pyrene	0.02	0.001	0.001	0.001	0.716	0.001	0.001	0.002	0.080	ND
Dibenzo(a,h)anthracene	0.02	0.001	0.001	0.001	0.001	0.001	0.001	0.003	0.004	ND
Benzo(ghi)pyrene	0.02	0.001	0.001	0.001	0.692	0.001	0.001	0.007	0.950	ND
Petrol products	50	ND	ND	ND	ND	ND	ND	115	7809	ND

ND- not determined

4 to 6 folds higher compared to the ecological standards. For S10-4 sample taken around a metallurgic works, the values for almost all heavy metals studied were extremely high. Most of the S samples from polluted regions were also polluted with the carcinogenic substances benzo(a)pyrene, benzo(a)anthracene, benzo(hgi)perylene, petrol products and different mutagenic compounds. The samples collected from the clean regions did not contain any carcinogenic substances or heavy metals above the ecological standards. However some of them (S1, S3) contained significantly higher amounts of the potent mutagen fluoranthene.

Air was analyzed automatically 4 times a day for SO_2, NO_2, PB, H_2S and ethanol. In addition, the dry matter was collected on filters to give the monthly sample designated A, which was processed and studied analytically for parameters mentioned in Materials and Methods and in the Ty1 test. The analysis of A samples showed an increase of dust content in A2 and A4 (from polluted regions). Increased amounts of phenol were also found for A2 and 4 samples, having 4.3 μg/m^3 and 3.0 μg/m^3 respectively. For comparison, A1 and A3 (from clean regions) have phenol content of 0.11 μg/m^3 and 0.10 μg/m^3 respectively, which is within the ecological standard. The study of the daily

Table 6
Chemical analysis of water samples

Chemical	Ecological standard, mg/dm^3	Pollutants in water samples, mg/dm^3					
		Clean regions			Polluted regions		
		W1	W3	W5	W2	W4	W6
Nitrites	0.04	0.02	0.03	0.02	0.24	0.49	0.06
Nitrates	10	0.84	1.09	2.84	1.81	2.28	2.35
N2(Kjeldahl)	1	1	1	2.32	4.60	15.45	0.63
P	2	0.16	0.17	0.90	0.29	0.11	2.05
Cl	300	4.1	8.7	3.1	74.5	165.9	132.9
SO_4	300	15.1	16.5	14.9	134.1	274.5	114.4
Petrol products	0.3	0.1	0.1	0.1	0.2	0.1	0.2
Cyanides	0.5	0.002	0.002	0.002	0.004	0.002	0.003
Pb	0.05	0.001	0.004	ND	0.005	0.001	ND
Ni	0.2	0.001	0.003	ND	0.007	0.001	ND
Zn	5	0.003	0.003	0.118	0.410	0.033	0.101
Cd	0.01	0.001	0.001	ND	0.001	0.001	ND
Co	0.01	0.001	0.001	ND	0.001	0.001	ND
Cu	0.01	0.005	0.001	ND	0.005	0.006	ND

ND- not determined

samples showed an irregular pollution with Pb in certain days which is diluted in the monthly samples to amounts below the ecological standard. Other mutagenic/carcinogenic compounds (besides phenol) were not found in any A sample. Starting January 2005 air was monitored by NEEA automatically with analyzers for all necessary parameters and collection of A samples on filters was terminated. Table 6 shows the quantitative chemical analysis of water (W) samples for parameters with values above the ecological norm. Samples W2, W4, W6 collected from polluted regions showed increased amounts of nitrates and N_2. In the same samples the amounts of P, Cl, SO_4 were also higher compared to samples W1, W3, W5 from the clean regions, however the obtained values were in the frames of the ecological standards. Data for pollution with heavy metals, petrol products or mutagenic/carcinogenic substances were not obtained for any W sample.

In summary, the results obtained in the chemical analysis of environmental samples confirmed previous analytical data of NEEA for the significant pollution of some regions (named "polluted" in this study) with different pollutants including carcinogenic substances. Soil and air samples for which the presence of carcinogens over the ecological norm was evidenced, gave positive results in the Ty1 test (Table 1), while samples polluted with non-carcinogenic substances (S1, S3), or samples collected from clean regions were negative in Ty1 test. Based on these results the Ty1 test was implemented into the National System for Environmental Monitoring in Bulgaria as a short-term test for selective detection of environmental pollution with carcinogenic substances.

Discussion

Although evidence for the response of Ty1 test to laboratory mutagenic and carcinogenic compounds has already been presented (Pesheva et al., 2005; Pesheva et al., 2007), its applicability in environmental studies has not been proved. In this communication the Ty1 test was applied to study soil, water and air samples collected from clean and polluted regions

and the results were compared to the data obtained in the analytical chemical analysis of the same samples. The test responded positively in concentration dependent and kinetics experiments to soil samples from polluted regions and gave negative results with samples from the clean regions. The chemical analysis evidenced the presence of carcinogenic substances in all Ty1 positive samples and absence of carcinogens in the Ty1 negative samples. The selectivity of Ty1 test to carcinogens was further supported by the negative responses of the test to samples S1 and S3 free of carcinogens, however containing non-carcinogenic mutagens in high amounts (Table 5).

The positive response of Ty1 test to environmental samples polluted with carcinogenic substances was also evidenced in the study of the air samples: only A2 and A4 samples containing the carcinogenic phenol gave positive results, while samples A1 and A3 lacking any carcinogenic pollutants were negative in Ty1 test. Since all studied water samples were Ty1 negative and free of carcinogens, a conclusion about the selectivity of the test to carcinogens polluting water can not be made at present.

The sensitivity of Ty1 test for detection of carcinogenic pollutants seems very high. All samples containing carcinogens above the ecological norm were positive. The positive answers give a "fold increase" of Ty1 transposition between 4 to 8 which is several folds higher than the "fold increase" of 2 differentiating negative from positive answers of Ty1 test. The concentration of each carcinogenic pollutant per ml of the assay can be easily calculated from the amount of the same carcinogen found in the chemical analysis (Tables 5 and 6) and knowing that 200µl processed environmental sample is added to 3.8 ml assay mixture. It appears that the amount of each carcinogenic pollutant in Ty1 positive samples is about or below the amount of the same carcinogen needed to give positive answer, if tested as a laboratory chemical. For instance, S4 contains calculated 16 µg/ml of benzo(a)pyrene and positive answer was obtained with as low as 20 µg/ml of benzo(a)pyrene tested as a substance (Pesheva et al., 2007); S2 contains calculated 85 µg/ml benz(a)anthracene and positive Ty1 test was obtained with 200µg/ml benz(a)anthracene (Pesheva et al., 2007). Environmental samples usually contain mixtures of carcinogens and mutagens which can significantly alter the effect of individual substances. Since the response of Ty1 test to mixtures of carcinogens has not been studied till now, the results obtained indicate that mixtures of carcinogenic substances in environmental samples does not significantly increase the threshold concentrations of each carcinogen necessary to produce positive answer in the Ty1 test.

The advantages of Ty1 test in environmental monitoring studies consist in its property to detect of carcinogenic pollutants. Ty1 assay is probably the first short-term test for selective detection of the carcinogenic pollutants which are the most dangerous pollutants for human and animal health. Previous studies (Pesheva et al., 2005) evidenced the positive answer of Ty1 test to a number of carcinogens including such that are negative in DEL and the other short-term test, thus increasing the detection spectrum of the test. During last decades industrialization and agriculture in some countries has been done without any care for eventual polluting effects.

The reason for pollution and the exact pollutants are known in few regions only and in most cases environment is polluted with substances of unknown structure and activity.

The detection of pollution with carcinogenic substances in such regions would be the first obligatory step towards re-establishing the balance between nature and human beings. The Ty1 assay is a short-term test with a potential application in this field. It is cheap, fast and sensitive method for detection of carcinogens polluting the environment.

Although average values from 5-10 repetitions are presented in the tables, the response of the test- positive or negative - to an environmental sample was evident from the first experiment, making the Ty1 test a reliable express method for detection of environmental carcinogens. The usage of Ty1 test together with Ames test for monitoring environment will give a better and more complete picture of pollution in the studied regions.

Acknowledgements

We thank Prof. Dimitar Kantardjiev (National Executive Environmental Agency, Sofia, Bulgaria) for the helpful discussion of the results and the critical reading of the manuscript. This research was supported by grand NATO SfP 977977 given to P.V.

References

Alder, I. D. and J. M. Parry, 1993. Development of screening tests for aneuploidy induction by environmental pollutants. *Environ Health Perspect,* **101**: 5-9.

Ashby, J., R.Tennant and W. Definite, 1991. Define relationship among chemical structure, carcinogenicity and mutagenicity for 301 chemicals tested by the U.S. NTP. *Mutat. Res.,* **275:** 229-306.

Bartsch, H., C. Mallaveille, A. M. Camus, G. Martel-Planche, G. Brun, A. Hautefuelle, N. Sabadie, A. Barbin, T. Kuroki, C. Drevon, C. Piccoli and R. Montesano, 1980. Validation and comparative studies on 180 chemicals with *S. typhimurium* strains and V79 Chinese hamster cells in the presence of various metabolizing systems. *Mutat. Res.,* **76:** 1-50.

Curcio, M. J. and D. J. Garfinkel, 1999. New lines of host defense Inhibition of Ty1 retrotransposition by Fus 3p and NER / TFII H. *Trends of Genet.,* **15:** 43-45.

Drake, J. W., 1998. The molecular bases of mutation. *Holden Day, Ocland*, CA pp. 382.

Goldman, R., L. Enwold, E. Pellizzari, G. Beach, E. Bowman, S. Krishnan and P. Shields, 2001. Smoking increases carcinogenic polycyclic aromatic hydrocarbons in human lung tissue. *Cancer Res.,* **61:** 6064-6371.

Kirpnick, Z., M. Homiski, E. Rubitski, M. Repnevskaya, N. Howlett, J. Aubrecht and R. H. Schiestl, 2005, Yeast DEL assay detect clastogens. *Mutat. Res.,* **582:** 116-134.

Kirsch-Volders, M. and M. Fenech, 2001. Induction of micronuclei in non-dividing mononuclear lymphocytes and necrosis/apoptosis may provide a more comprehensive cytokinesis block micronucleous assay for biomonitoring purposes. *Mutagenesis,* **16:** 51-58.

Lerry, T., P. van Hummelen, D. Anard, P. Castelian, M. Kirsh-Volders, R. Lamveris and D. Lison, 1996. Evaluation of three methods for the detection of DNA single-stranded breaks in human lymphocytes. *J. Toxicol. Environ Health*, **47:** 409-442.

Maron, D. M. and B. Ames, 1983. Revised Methods for the *Salmonella* mutagenicity test. *Mutat. Res.,* **113**:173-215.

Morita, T., Y. Iwamoto, T. Shimizu, T. Maswazawa and Y. Yanagihara, 1989. Mutagenicity test with a permeable mutant of yeast on carcinogens showing false negative *Salmonella* assay. *Chem. Pharm. Bull.,* **37:** 407-409.

Parry, J. M., M. Shamsher and D. O. Skibinski, 1990. Restriction site mutation analysis, a proposed methodology for the detection and study of DNA base changes following mutagen exposure. *Mutagenesis,* **5:** 209-212.

Pesheva, M., O. Krastanova, L. Staleva, V. Dentcheva, M. Hadzhitodorov and P. Venkov, 2005. The Ty1 transposition test: a new short-term test for detection of carcinogens. *J. Microbiol. Methods,* **61:** 1-8.

Pesheva, M., O. Krastanova, R. Stamenova and P. Venkov, 2007. Response of Ty1 transposition test to carcinogens. *Bulg. J. Agric. Sci*., 14: 1-15.

Pesheva, M. and P. Venkov, 2005. Dimethylsulfoxide has a recombinogenic effect on *Saccharomyces cerevisiae* cells. *Biotechnol. and Biotechnol. Equip.,* **19:**38-42.

Ramel, C., H. Cederberg, J. Magmusson, E. Vogel, A. T. Natarajen, L. H. Mullander, J. M. Nivard, J. M. Parry, A. Leyson, M. A. Comendator, L. M. Siera, J. A. Ferreiro and S. Consuegra, 1996. Somatic recombination gene amplification and cancer. *Mutat. Res.,* **353:** 85-107.

Rossi, C., P. Poli, A. Buschini, F. Cassoni, F. Magnani, Luartini, S. Tolomei and C. Girbelli, 1991. Genetic activity of samples collected from a waste incinerator and its neighboring areas. *Toxic. Environ. Chem.,* **30**: 51-61

Sherman, F., G.,R.,Fink and R. Hicks, 1996. Laboratory Course Manual for Methods in Yeast genetics. *New York: Cold Spring Harbor Laboratory Press*

Schiestl, R. H., R. D. Geitz, R. D. Mehta and P. J. Hastings, 1989. Carcinogens induce intrachromosomal recombination in yeast. *Carcinogenesis,* **10**: 1445-1455.

Staleva, L. S. and P. V. Venkov, 2001. Activation of Ty1 transposition by mutagens. *Mutat. Res.,* **474**: 93-103.

Staleva, L., L. Walcheva and P. Venkov, 1996. Enchanced permeability increases the sensitivity of a yeast test for mutagens. *Mutat. Res.,* **370**: 81-89.

Verschueren, E., 2001. Fluoranthene, Handbook at Environmental data on Organic Chemicals, *NY, John-Willey and Sons, Inc 4th Ed.* Vol1, pp. 1158-1161.

Zimmerman, F. K., R. Kern and H. Rassenberg, 1975. A yeast strain for simultaneous detection of induced mitotic crossingover, mitotic gene conversion and reverse mutation. *Mutat. Res.,* **28**: 381-338.

Received September, 12, 2007; accepted for printing February, 3, 2008.