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Comparison of the sensitivity of strains of *Salmonella enterica* serovar Typhimurium in the detection of mutagenicity induced by nitroarenes

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ABSTRACT. The use of strains of *Salmonella enterica* serovar Typhimurium with different metabolic capacities can indicate the class or classes of compounds present in an environmental sample and enable the diagnosis of the mutagenic activity of these pollutants adsorbed on particulate matter (PM) in the air. In the present study, the sensitivity of *Salmonella* strains TA98NR, TA98/1,8-DNP_6, YG1021, and YG1024 to detect nitro compounds adsorbed on samples of PM 2.5 was compared from three sites in Rio de Janeiro city. Samples were collected using a

high-volume sampler at three sites: one with light traffic and two with heavy traffic. The assays were performed in the presence of 10-50 μg/plate organic extracts with and without exogenous metabolization. The YG1021 and YG1024 strains showed the highest rev/m^3 values, confirming their enhanced sensitivity. As YG1024 also demonstrated sensitivity to nitro and amino compounds, we suggest its use in research into environmental contamination.

Key words: Sensitivity; *Salmonella enterica* serovar Typhimurium; Nitroarenes; Mutagenicity

INTRODUCTION

The *Salmonella*/microsome microsuspension assay has been used for large, multisite, and/or time series studies, for bioassay-directed fractionation studies, for identifying the presence of specific classes of mutagens, and for site or source comparisons of relative levels of airborne mutagens (Claxton et al., 2004). The use of strains of *Salmonella enterica* serovar Typhimurium with different metabolic capacities can indicate the class or classes of compounds present in an environmental sample (DeMarini et al., 2004; de Aragão Umbuzeiro et al., 2008). The *Salmonella*/microsome assay identifies the presence of organic compounds and enables diagnosis of the mutagenic activity of these pollutants adsorbed on particulate matter (PM) in the air. The responses are related to the presence of a variety of compounds, including polycyclic aromatic hydrocarbons (PAHs) and nitroarenes (Menck et al., 1974; Ducatti and Vargas, 2003).

Nitroarenes are a class of environmentally hazardous compounds (Watanabe et al., 1990). These nitro compounds have been detected in the extracts of diesel and gasoline emissions, fly ash, cigarette smoke condensates, and home heater emissions (Rosenkranz and Mermelstein, 1983; Watanabe et al., 1990). Typical nitroarenes, such as 2-nitrofluorene, 1-nitropyrene (1-NP), and 1,8-dinitropyrene (1,8-DNP), are potent mutagens (Wang et al., 1980; Watanabe et al., 1990). The contributions of nitroarenes to direct mutagenic activity are commonly investigated through the *Salmonella*/microsome assay with the following *S. enterica* serovar Typhimurium strains: TA98 (frameshift strain) and the derivative strains TA98/1.8-DNP_6 (*O*-acetyltransferase-deficient), TA98NR (nitroreductase-deficient) (Rosenkranz and Mermelstein, 1980), YG1021 (nitroreductase-overproducing), and YG1024 (*O*-acetyltransferase-overproducing) (Watanabe et al., 1989, 1990). Genotoxic evaluation studies of particulate matter using these strains have been conducted previously (Sato et al., 1995; Vargas et al., 1998; Ducatti and Vargas, 2003; de Aragão Umbuzeiro et al., 2008; Coronas et al., 2009; Pereira et al., 2010; Rainho et al., 2012, 2013a,b).

In the present study, comparisons were made of the sensitivity of strains of *S. enterica* serovar Typhimurium to detect nitro compounds adsorbed on samples of PM 2.5 collected at three sites in the city of Rio de Janeiro.

MATERIAL AND METHODS

Sampling sites

The samples were collected between August and October 2010 at three sites in Rio

de Janeiro: the campus of Universidade do Estado do Rio de Janeiro (site 1), Avenida Brasil (site 2), and Rebouças tunnel (site 3). Site 1, with light traffic, is located in a residential area of the city northern zone. Site 2 has heavy traffic (~250,000 vehicles/day) and is the city longest expressway, covering 58 km in length and crossing 27 neighborhoods. Site 3 also has heavy traffic (~190,000 vehicles/day) and is a 2.8 km-long tunnel that connects the northern and southern zones of the city (Rainho et al., 2012, 2013a,b).

Airborne PM 2.5 samples were collected on fiberglass filters (E558 X 10IN; 254 x 203 mm) using a high-volume collector (AVG MP 2.5; 1.13 m^3/min) for 24 h for sites 1 and 2, and for 6 h for site 3. Four monthly samplings were performed at each site. At the end of the sampling period, the filters were combined to form a pooled sample (Rainho et al., 2012, 2013a,b).

Extraction of organic compounds

Half of each filter was sonicated in three rounds of 10 min each using 99.9% pure dichloromethane (CASRN. 75-09-2; TediaBrazil; Brazil). The extracts were concentrated to 15 mL in a rotating evaporator and filtered through a 0.5 µm Teflon membrane. The concentration of extractable organic matter (EOM) was calculated and expressed as µg/m^3. Prior to the bioassays, the organic extract was dried at 4°C and resuspended in 5 µL 99.9% pure dimethyl sulfoxide (DMSO; CASRN. 67-68-5; Synth; Brazil) (Vargas et al., 1998).

Salmonella/microsome assay

The organic extracts were assayed for mutagenicity using the microsuspension version (Kado et al., 1986) of the *Salmonella*/microsome assay (Maron and Ames, 1983). *S. enterica* serovar Typhimurium TA98 (frameshift strain) and the derivative strains TA98/1.8-DNP_6 (*O*-acetyltransferase-deficient) and TA98NR (nitroreductase-deficient) (Rosenkranz and Mermelstein, 1980) were used, with and without metabolic activation (S9 mix fraction). Five concentrations of each sample (10, 20, 30, 40, and 50 µg/plate) were tested in triplicate. The samples were pre-incubated for 90 min. All assays were carried out under yellow light and in the presence of negative (5 µL/plate DMSO solvent) and positive (0.5 µg/plate 4-nitroquinoline oxide; CASRN. 56-57-5; Sigma Chemical Company; St. Louis, MO, USA) controls. Plates were incubated in the dark at 37°C for 72 h, after which time revertants were counted. The sample was considered to be positive when the mutagenesis value was at least twice the negative value, and when a significant ANOVA ($P < 0.05$) and a positive dose-response rate ($P < 0.05$) were observed. The results of the different assays were analyzed using the SALANAL program (Salmonella Assay Analysis, version 1.0; Integrated Laboratory Systems; Research Triangle Park, NC, USA). The choice between linear regression and the Bernstein model (Bernstein et al., 1982) was made to allow the elimination of data for doses outside the linear portion of the dose-response curve. Positive results were interpreted as presenting significant mutagenicity. Positive responses were expressed as the number revertants per volume of air sampled (rev/m^3), i.e., rev/µg multiplied by EOM in µg/m^3. In the cytotoxicity test, the solutions containing the sample and the bacterial culture (100-200 cells) were plated on nutrient agar plates and incubated at 37°C for 24 h, and the surviving colonies were counted. The sample was considered to be cytotoxic if the percentage of surviving cells was less than 60% of the negative control at one or more doses (Coronas et al., 2009).

RESULTS AND DISCUSSION

Salmonella/microsome assay

Table 1 shows the mutagenic activity, expressed as rev/m^3, analyzed using the classic nitroreductase- and *O*-acetyltransferase-deficient derivate strains TA98NR and TA98/1,8-DNP$_6$.

Table 1. Induced mutagenic by airborne particulate matter organic extracts (rev/m^3).

Site	Month	TA98		TA98NR		TA98/1,8-DNP$_6$	
		-S9	+S9	-S9	+S9	-S9	+S9
1	August	n.d.	15.80 ± 1.40	n.d.	6.50 ± 1.00	6.76 ± 2.12	n.d.
	September	n.d.	13.00 ± 2.10	1.42 ± 0.63	2.50 ± 0.50	n.d.	n.d.
	October	n.d.	n.d.	4.54 ± 0.60	2.20 ± 0.40	n.d.	2.60 ± 0.30
2	August	17.00 ± 1.90	1.50 ± 0.50	9.85 ± 1.70	10.20 ± 1.00	12.26 ± 0.93	4.30 ± 1.70
	September	5.70 ± 0.90	n.d.	7.16 ± 2.67	6.00 ± 0.50	1.44 ± 0.07	n.d.
	October	n.d.	n.d.	n.d.	2.40 ± 0.30	1.80 ± 0.65	n.d.
3	August	39.60 ± 11.90	13.00 ± 2.30	2.30 ± 1.25	7.10 ± 0.80	10.67 ± 2.93	7.70 ± 0.80
	September	56.40 ± 20.60	58.70 ± 11.80	2.78 ± 1.71	4.30 ± 2.80	35.37 ± 6.21	6.90 ± 1.70
	October	9.30 ± 1.80	46.50 ± 4.20	n.d.	5.80 ± 2.50	n.d.	19.00 ± 4.00

1 = Universidade do Estado do Rio de Janeiro; 2 = Avenida Brasil; 3 = Rebouças tunnel. n.d. = not detected. Negative controls: DMSO for the mutagenicity assay without S9 mix were: TA98, (28 ± 5); TA98NR (23 ± 3); TA98/1.8-DNP$_6$, (17 ± 2). DMSO for the mutagenicity assay with S9 mix were: TA98, (45 ± 8); TA98NR (33 ± 7); TA98/1.8-DNP6, (25 ± 4). Positive controls for the mutagenicity assay without S9 mix were: 4-nitroquinoline oxide (0.5 µg/plate) for TA98, (853 ± 72); TA98NR (120 ± 2); TA98/1,8-DNP$_6$ (114 ± 35). Positive controls for the mutagenicity assay with S9 mix were: 4-nitroquinoline oxide (0.5 µg/plate) for TA98, (198 ± 50); TA98NR (121 ± 1); TA98/1,8-DNP6 (95 ± 33).

The EOM values ranged from 5.54 to 9.66 µg/m^3 at site 1, from 5.48 to 7.76 µg/m^3 at site 2, and from 20.93 to 25.05 µg/m^3 at site 3 in the three months of study, as calculated elsewhere (Rainho et al., 2013b). No cytotoxic effects were detected for any of the samples analyzed.

The contribution of nitro compounds to the mutagenic activity of air samples from urban areas is associated with the presence of PAH derivatives. Among these, the mono- and dinitro-PAHs associated with oxygenated PAH have been correlated with direct frameshift mutagenic activity (Sato et al., 1995; Vargas et al., 1998; Ducatti and Vargas, 2003). In the present study, the contribution of mono- and di-nitroarenes to direct mutagenic activity was investigated though the *Salmonella/*microsome assay with strains TA98NR and TA98/1,8-DNP$_6$, respectively. Nitroarenes are dependent upon this 'classic' nitroreductase to express their mutagenicity, as evidenced by their greatly decreased activity in the nitroreductase-deficient strain (Rosenkranz and Mermelstein, 1983). However, some nitroarenes express all or a major fraction of their activity even in the absence of the 'classic' nitroreductase (e.g., 1,8-DNP). The fact that there is residual activity expressed in TA98NR (the microorganism deficient in the 'classic' nitroreductase), and the finding of the full expression of the mutagenicity of other chemicals in TA98NR has led to the conclusion that *S. enterica* serovar Typhimurium may contain additional nitroreductases as well as other specific enzymes. Indeed, it has been possible to construct bacterial strains lacking the enzyme that recognizes 1,8-DNP (e.g., TA98/1,8-DNP$_6$) (Rosenkranz and Mermelstein, 1983). The lower mutagenic activity in nitroreductase-deficient and *O*-acetyltransferase-expressing strains, TA98NR and TA98/1,8-DNP$_6$, compared to the parental strain TA98, indicated the participation of mono- and di-nitroarenes in the total mutagenicity of the extracts. The presence of mono-nitroarenes was detected at site 2 (August) and site 3 (August and September). A lower mutagenic response was

observed for TA98NR (42% at site 2 and 94-95% at site 3) than was observed for TA98. At site 2 (August and September) and site 3 (August and September), a lower mutagenic response was observed for the TA98/1,8-DNP_6 strain (28-75% at site 2 and 37-73% at site 3) than for TA98, indicating the presence of di-nitroarenes. The extent of the reduction in the mutagenic response detected at sites 2 and 3 can be attributed to the intense traffic in these areas of the city. Studies performed with TA98NR and TA98/1,8-DNP_6 in areas contaminated by vehicular emissions and industrial activity in Rio Grande do Sul State of Brazil showed similar results: a reduction of 10-95% for TA98NR and a reduction of 61-85% for TA98/1,8-DNP_6 (Vargas et al., 1998; Ducatti and Vargas, 2003). Similar results were also detected in the industrial city of Cubatão, São Paulo (Sato et al., 1995). Meanwhile, an absence of mutagenicity was observed at site 1, which is characteristic of residential areas with a low flow of vehicles.

Mutagenic results were detected in the presence of metabolic activation for TA98NR and TA98/1,8-DNP_6 for all sites. However, the decrease in revertants after metabolization was only observed in a few months. The effects of the S9 mix preparations on the mutagenicity of nitroarenes in the *Salmonella* strains revealed a broad spectrum of responses ranging from the absence of mutagenicity to an absolute requirement for mutagenic activation. In some instances, the presence of S9 mix permitted the expression of mutagenicity by nitroarenes even in nitroreductase-deficient microorganisms, indicating the presence of nitroreductase activity in the S9 mix (Rosenkranz and Mermelstein, 1983). However, it must be noted that, in general, arylamines exhibit much lower mutagenicity in the presence of S9 mix than do corresponding nitroarenes in the absence of S9 mix.

Strains YG1021 and YG1024 are also commonly used in environmental studies (Coronas et al., 2009; Pereira et al., 2010; Rainho et al., 2013b). The higher mutagenic activity seen in over-expressing strains YG1021 and YG1024 compared to the parental strain TA98 indicates the participation of nitro and amine compounds, respectively. The extracts from sites 1, 2, and 3 were therefore also tested with strains YG1021 and YG1024, and showed the following results: increase in reversion for YG1021 (6-14% at site 1; 2-144% at site 2; 12-281% at site 3), and increase in reversion for YG1024 (3-38% at site 1; 105-155% at site 2; 1-145% at site 3) (Rainho et al., 2013b). Two studies conducted in different parts of Rio Grande do Sul also detected the presence of nitro and amino compounds through the high activity of strains YG1021 (23-264%) and YG1024 (554-1821%) (Coronas et al., 2009; Pereira et al., 2010). This increase in the percentage of reversal shown by YG1021 and YG1024 is attributed to plasmids pYG216 and pYG219, which increase the sensitivity of these strains to nitro and amino compounds, respectively (Watanabe et al., 1990).

CONCLUSION

The *Salmonella*/microsome assay has been used for large, multi-site and/or time serie studies to identify the presence of specific mutagens and classes of mutagens. All the strains used in the present study are sensitive to nitroarene, although strains YG1021 and YG1024 showed the highest rev/m^3 values, thus confirming their enhanced sensitivity. YG1024 also demonstrated sensitivity to nitro and amino compounds. We recommend the use of strains YG1021 and YG1024 in research into environmental contamination by these pollutants.

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