MUTAGENECITY EFFECT OF SURFACE WATER IN THE LOWER STREAM OF THE MISSISSIPPI RIVER

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INTRODUCTION

The aquatic systems all around the world have been found to be seriously polluted with toxic organic and inorganic chemicals, and waste products (Callahan et al. 1979; Edward et al 1979; Bakre et al.1990; Rehana et al. 1996). Civilian activities along the river contaminate the surface water with huge amount of waste materials and products coming from humans, animals, industrial plants, housing establishments, and factories. Increased usage of fossil fuels in the process of transportation, heating, and power generation resurface the accumulated old pollutants in the fossil fuels. Lately, the chemical industries added a variety of new synthetic compounds, including insecticides to the environment pollution. As the Mississippi River runs through the highly active industrial, agricultural, and populated areas in this country, the constant survey of the toxicity of the surface water of this river appears to be very important for the safety of population along the river. This study intends to apply the Salmonella bacterial mutagenic test for evaluation of the most basic genotoxic effect of the Mississippi River water in its down stream areas. By application of the estimated correlation coefficient for phase analysis and the statistical mean value for significant analysis in the bacterial mutagenicity assay, this study intends to give a more detailed and accurate assessment of the genotoxicity potential of the Mississippi riverwater from Memphis to Greenville, Vicksburg to St. Francisville, and from Baton Rouge to the Marine Transportation Center.

MATERIALS AND METHODS

Salmonella Mutagenesis Test Strains TA98 was obtained from Dr. Ames Laboratory, University of California at Berkeley. Mutagenic assay was performed according to the method of Ames et al. (1975). A *Salmonella* mutant test strain, TA98 was streak cultured in MG agar plate (1,5% agar, 2% dextrose, 0.00978% $MgSO_4$, 0.183% citric acid,1% K_2PO_4, and 0.229% $NaHNH_4PO_4$) with the addition of histidine (260 μM), biotin (3 μM), and ampicillin (25 μg/ml) for maintenance. For preparation of frozen stocks, liquid culture of TA98 strain was made by shaking the culture of a single colony of tester strains to early stationary phase in 40 ml of the Oxoid Nutrient broth #2. The 40 ml culture was mixed with 7 ml glycerol and dispensed 1 ml per microtube for storage at -80°C in a deep freezer. For the mutagenecity test, one volume of frozen stocks was diluted into 80 volumes of fresh Nutrient broth #2 for culture in a shaker incubator. Following 5 to 6 hours of incubation at 30°C and 200 rpm in a shaker incubator, mid-logarithmic phase of the growth was obtained. 600 μl of the culture was mixed with 600 μl of sample (without dilution), with 600 μl of 2 times diluted sample (300 μl sample + 300 μl distilled water), and with 600 μl of 4 times diluted sample (150 μl sample + 450 μl of distilled waters), respectively. Following 60 minutes of incubation with occasional shaking at room temperature, 3.0 ml of solution containing 0.1 mM phosphate buffer (pH 7.4), 33 mM potassium chloride and 8 mM magnesium chloride was added to each incubation tube. After vortex for 20 seconds, the mixture was dispensed 0.7 ml per tube into 5 tubes containing 2 ml top agar for vigorous mixing before coating on the MG agar plate. The top agar was prepared to include 0.5% NaCl, 0.6% agar, 0.5 mM histidine and 0.5 mM biotin, so that test bacteria can divide a few times to develop reverse mutation for formation of visible colonies on MG plate. The number of colonies (revertants) formed by reverse mutation were recorded after 48 hours and 72 hours of incubation at 37°C.

The mean numbers and standard deviations of revertants for each sample dilutions were calculated from 5 plates. Also the three mean numbers obtained from 3 sample dilutions in each sample were used for calculation of correlation coefficient and the best fit regression (linear, exponential, logarthmic, or power regression). The correlation coefficient was used for further significant test analysis using the following formula:

$$t_\gamma = \gamma/[(1-\gamma^2)/(N-2)]^{1/2}$$
(n=3 in this case)

The critical value for significant result is $t_\gamma = 0.985$ according to the t-table with the degree of freedom (DF= 3-2). The result of correlation coefficient (γ) in each best fit regression was used for comparison with the critical value. The positive correlation coefficient indicated that the genotoxicity of the water is at the early stage of mutagenic phase. There is a proportional increase of revertants appearing on the test plates to the proportional increase in the concentration of sample water in the test mixture. The negative correlation coefficient indicates that the

genotoxicity of the water is at the late or terminal stage of lethal phase. There is a proportional reduction of revertants appearing on the test plates to the proportional increase in the concentration of sample water in the test mixture.

RESULTS

Table 1 contains the list of test results obtained from Memphis and Greenville. Three samples obtained from the Memphis area demonstrated that the genotoxicity of the river water were all at a lethal phase. On the contrary, two samples collected from the Greenville area demonstrated the mutagenic phase of genotoxicity. The result indicated that the genotoxicity of the river water will be seriously affected near a larger city with an extremely large population. Nevertheless, when such water runs through a rural area to another smaller city like Greenville, it may be remediated.

Table 2 contains the list of test results obtained from Vicksburg, Natchez, and the St. Francisville area. The result also demonstrated that the contaminated water was at the lethal phase near the larger city and industrialized area of Vicksburg. On the contrary, the river water samples obtained in smaller cities, like Natchez or St. Francisville, showed early stage of genotoxicity at the mutagenic phase. These test results indicated again the remediation effect of the river environments in the rural area compared to the contaminated water from a larger city.

Table 3 contains the list of test results obtained from the Baton Rouge, New Orleans, Belle Chase, and the Marine Transportation areas. These samples were all collected on the same day and run in one experiment for better comparison. All results indicated strongly lethal effects with negative signs in the correlation coefficient and a much reduced mean numbers in the colony formation in the bacterial mutagenecity test. Also, no clearly visible remediation effect, except a small degree of improvement, was seen at Belle Chase. Finally, the small improvement of the water genotoxicity was possibly caused by the merger with the canal water coming from Lake Pontachella.

DISCUSSION

There are two distinctly different responses in the formation of bacterial colonies to the increase of genotoxic materials in the bacterial mutagenecity test. One, the early stage is the increase of bacterial colonies in response to the increase of lower concentration of genotoxic material to express the mutagenic effect. Two, the late phase is the reduction of bacterial colonies in response to the increase of higher concentration of genotoxic material to express the lethal effect. Combined together, the bell-shaped curve will be formed to the continual increase of concentration to include the critical concentration of genotoxic material to have phase change in response. To find whether the genotoxic effect of the water is at mutagenic phase or at the lethal phase is important before quantitative analysis and interpretation can be completed. It is, therefore, the primary intention of the current experiment to identify the phase of water toxicity before further analysis of the data within that phase. By two sequential dilutions of sample water to assay the response of tester strain bacteria at three concentration levels for correlation coefficint analysis, this study was able to locate the phase of genotoxicity. Subsequently, more adequate assessment of the assay result could be performed. The early stage of mutagenic phase is characterized by an increase of revertants to increasing amount of mutagen. Increase of mutagen is reflected in increase of revertants. On the contrary, the late stage of lethal phase is characterized by the decline in the number of bacterial colonies to the increasing amount of mutagen. Increase of mutagen is reflected in the reduction of bacterial colonies in the test. The correlation coefficient will form between two variables, x variable for the sample concentrations (25, 50, or 100) and y variable for the numbers of revertants formed either on Day 2 (y_1) or Day 3 (y_2). The degree of correlation are expressed in the positive value between 1 to 0 for the mutagenic phase and in the negative value between -0 to -1 for the lethal phase. Based on this classification, few cities along the river are still at early mutagenic phase [Greenville (#1), Natchez (#2), St. Francisville (#3)], with the severity rank quoted in the parenthesis. The genotoxicity of the surface water in other big city areas or industrialized areas along the Mississippi River appear to be in the most serious lethal phase [New Orleans (#1), Belle Chase (#2), Vicksburg (#3), Memphis (#4), Baton Rouge (#5), and Marine Transportation Center (#6)], with the rank of severity in lethality quoted in the parenthesis.

CONCLUSION

Mutagenecity of the surface water of the Mississippi River down stream from Memphis to the Marine Transportation has been studied by application of bacterial mutagenecity test using TA 98 with the application of the estimated correlation coefficient for phase analysis and the statistical mean values of water samples for comparison. Subsequently, the ranks of severity in genotoxicity can be established for various location areas. For lower genotoxicity with positive sign in the correlation coefficients, genotoxicities of different samples were compared at the mutagenic phase. Subsequently, the increase in the number of revertants in the test was evaluated as severity of genotoxic effect (Greenville, Natchez, and St. Francisville). For higher genotoxicity with negative sign in the correlation coefficients, the genotoxicities of different samples were compared at lethal phase. Subsequently reduction in the bacterial colonies in

the test was evaluated as severity of genotoxic effect (New Orleans, Belle Chase, Vicksburg, Memphis, Baton Rouge, and Marine Transportation). The ranks of order of severity in genoxicity was estimated by combination of estimates of two phases in the whole areas. New Orleans was ranked as #1 in its genotoxicity of the water, with Belle Chase, Vicksburg, Memphis, Baton Rouge, Marine Transportation, Greenville, Natchez, and St. Francisville ranked as #2, #3, #4, #5, #6, #7, #8 and #9 after New Orleans in the rank of toxicity in the water of the Mississippi River.

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Table 1. Correlation coefficient analysis between water sample amounts (X) and numbers of revertants (Y_1, Y_2) on the bacterial mutagenic test at Memphis and Greenville.

Location-No (Date of coll.)	Sample vol. (µl, X)	Numbers of revertants Day 2 (Y_1)	Day 3 (Y_2)
Memphis-1	100	13.4± 5.0*	25.8 ± 6.1*
(9/22/96)	50	17.5± 6.8	28.0 ± 5.9
	25	21.3± 6.2	33.5 ± 7.7
Correlation	(Best fit reg.) &	γ=-0.999(Logar.)#	γ=-0.977(Power)
Memphis-2	100	12.8± 3.3	23.8 ± 5.9
(6/7/97)	50	21.0± 8.3	35.0 ± 6.2
	25	18.0± 3.5	27.3 ± 3.9
Correlation	(Best fit reg.)	γ=-0.762(Linear)	γ=-0.521(Linear)
Memphis-3	100	15.6± 2.4	27.2 ± 2.5
(7/13/97)	50	16.8 ± 2.5	31.0 ± 6.8
	25	18.0± 5.6	27.3 ± 3.9
Correlation	(Best fit reg.)	γ=-0.994(Power)	γ=-0.972(Linear)
Greenville-1	100	16.6± 3.2	36.0 ± 3.3
(9/13/96)	50	13.3± 3.0	32.3 ± 6.2
	25	15.2± 3.5	36.0 ± 4.2
Correlation	(Best fit reg.)	γ=+0.586(Linear)	γ=+0.189(Linear)
Greenville-2	100	19.6 ± 7.0	36.0 ± 6.1
(9/22/97)	50	17.8± 5.0	30.5 ± 6.6
	25	17.3± 3.9	33.0 ± 3.7
Correlation	(Best fit reg.)	γ=+0.994(Expon.)$	γ=+0.693(Linear)

* Mean ± standard deviation.
& Best fit regression.
# Lograrithmic regression for the best fit regression.
$ Exponential regression for the best fit regression.

Table 2. Correlation coefficient analysis between water sample amounts (X) and numbers of revertants (Y_1, Y_2) on the bacterial mutagenic test at Vicksburg, Natchez, and St. Francisville.

Location-No. (Date of coll.)	Sample vol. (µl, X)	Numbers of revertants Day 2 (Y_1)	Day 3 (Y_2)
Vicksburg-1	100	14.0 ± 3.7*	31.2 ± 4.5*
(6/17/97)	50	9.0 ± 4.4	22.3 ± 8.6
	25	11.0 ± 4.9	26.8 ± 7.8
Correlation	(Best fit regr.)&	γ=+0.737(Linear)	γ=+0.65(Linear)
Vicksburg-2	100	13.5 ± 1.3	22.0 ± 2.0
(7/20/97)	50	20.0 ± 5.4	32.5 ± 7.3
	25	23.0 ± 8.1	34.0 ± 8.9
Correlation	(Best fit regr.)	γ=-0.999(Linear)	γ=-0.976(Linear)
Natchez-1	100	16.6 ± 5.9	30.0 ± 7.9
(9/13/96)	50	16.8 ± 4.3	32.5 ± 6.6
	25	12.5 ± 2.4	23.8 ± 1.7
Correlation	(Best fit regr.)	γ=+0.721(Linear)	γ=+0.543(Linear)
Natchez-2	100	17.2 ± 3.8	28.± 3.9
(8/11/97)	50	21.3 ± 5.6	4.5 ± 7.8
	25	17.3 ± 2.8	30.3 ± 8.3
Correlation	(Best fit regr.)	γ=-0.209(Linear)	γ=-0.478(Linear)
St. Francisville-1	100	15.8 ± 5.6	29.2 ± 7.6
(9/22/96)	50	15.3 ± 3.8	24.5 ± 2.4
	25	14.0 ± 4.1	27.5 ± 7.6
Correlation	(Best fit regr.)	γ=+0.904(Linear)	γ=+0.527(Linear)

* Mean ± standard deviation.
& Best fit regression.

Table 3. Correlation coefficient analysis between water sample amounts (X) and numbers of revertants (Y_1, Y_2) on the bacterial mutagenic test at Baton Rouge, New Orleans, Belle Chase, and Marine Transportation Center.

Location-No (Date of coll.)	Sample vol. (µl, X)	Numbers of revertants Day 2 (Y_1)	Day 3 (Y_2)
Baton Rouge-1	100	15.0 ± 5.5*	27.0 ± 5.1*
(8/30/97)	50	17.3 ± 5.1	31.5 ± 2.5
	25	21.3 ± 6.2	31.8 ± 4.9
Correlation	(Best fit regr.)&	γ=-0.998(Power)	γ=-0.961(Expon.)#
New Orleans-1	100	9.6 ± 3.0	21.6 ± 7.0
(8/30/97)	50	15.0 ± 3.6	28.0 ± 6.6
	25	17.0 ± 3.5	29.0 ± 3.9
Correlation	(Best fit regr.)	γ=-0.997(Linear)	γ=-0.978(Linear)
Belle Chase-1	100	13.0 ± 2.3	27.0 ± 4.1
(8/30/97)	50	21.5 ± 6.1	35.8 ± 7.1
	25	14.0 ± 3.5	30.7 ± 0.6
Correlation	(Best fit regr.)	γ=-0.321(Expon.)	γ=-0.582(Linear)
Marine Transp.-1	100	17.2 ± 5.6	29.8 ± 7.8
(8/30/97)	50	17.8 ± 3.8	33.8 ± 6.3
	25	24.3 ± 5.6	37.3 ± 6.5
Correlation	(Best fit regr.)	γ=-0.907(Power)	γ=-0.999(Logar.)
Distilled H_2O	100	18.4 ± 4.5	28.8 ± 3.6
(Negative control)	50	17.3 ± 1.0	26.5 ± 2.6
	25	17.8 ± 3.5	29.0 ± 4.3
Correlation	(Best fit regr.)	γ=+0.693(Linear)	γ=+0.118(Linear)
Daunomycin(5 ug)	100	55 ± 18	64 ± 16
(Positive control)	50	166 ± 63	173 ± 63
	25	227 ± 75	240 ± 78
Correlation	(Best fit regr.)	γ=-0.999(Linear)	γ=-0.998 (Linear)

* Mean ± standard deviation.
\# Exponential for best fit regression.
& Best fit regression.