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Mutagenicity of River Water in Korea

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Though various chemical substances are involved in pollution of the environment, the effects of pollutants in the air, water, and soil on the ecosystem including humans are still unclear. The pollution of river water has been evaluated based on the levels of noxious substances, namely heavy metals and organophosphorus, biochemical oxygen demand (BOD), chemical oxygen demand (COD), dissolved oxygen (DO), or the values of nitrogen, phosphorus and suspended solid (SS). In recent years, complex pollution of river water, especially its mutagenicity and carcinogenicity, has become a problem (Kinae *et al.* 1992). This is due to small amounts of organic substances such as organic solvents discharged from metal processing factories and semiconductor factories or agricultural chemicals discharged from farmland and golf courses. However, individual analysis of various chemical substances in the environment is very difficult. The Ames test (Ames *et al.* 1975) is useful for evaluating the state of complex pollution by these chemicals and the safety of the environment. Investigation of the distribution of mutagens and their serial changes by this method may allow identification of the causes of and sources of these mutagens.

We investigated rivers in Korea by the Ames test using the blue rayon (BR) method in order to clarify the distribution and characteristics of mutagens in river water and the pollution source.

MATERIALS AND METHODS

To evaluate the mutagenicity of river water in the entire course of the river from the upper to lower stream, 3 rivers that flow through the south of Korea were used as major sampling sites, considering the points for water supply and sewerage and the points of river branching and confluence. The 3 rivers are branches of the same source. The NakDong River flows through an industrial district, the East NakDong River through a residential district, and the West NakDong River through an agricultural district. In the lower stream, the East NakDong River joins the NakDong and West NakDong Rivers, flowing into the sea.

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Sampling was performed by the BR suspension method (Hayatsu 1992) between August, 1994 and September, 1995 during fine weather to exclude the effects of rain water. Blue rayon was purchased from Funakoshi Pharmaceutical Co., Ltd. This BR contained 25 μmol/g of the copper phthalocyanine derivative. Six nylon nets (20 cm in length) containing 0.5 g BR were attached to a wooden plate and suspended in the river.

After 24 hrs, the blue rayon was recovered and washed with distilled water. Water was removed using a paper towel, and elution was performed twice with 80 ml of methanol-concentrated aqueous ammonia (50:1). The extracts obtained were twice mixed and dried under reduced pressure. The residue was dissolved in a small amount of methanol, placed in a test tube for the mutagenicity test, and after evaporation of methanol, re-dissolved in 0.1 ml of dimethyl sulfoxide (DMSO) as specimens for the mutagenicity test.

Mutagenicity was evaluated by the Ames test using *Salmonella* typhimurium TA98 and 100, and YG1041 (Watanabe *et al.* 1989) and 1042 (Watanabe *et al.* 1990) with and without metabolic activation by S9 mix (+S9 and -S9, respectively). After culture at 37°C for 48 hrs, revertant colonies were counted. S9 mix was prepared using S9 and cofactor (Oriental Yeast, Co., Ltd.). A positive control test was performed for each test using Furylfuramide (AF2) as a positive control in the direct method and Benzo(a)pyrene (BaP) in the indirect method.

RESULTS AND DISCUSSION

Since the sensitivity of the cell strains differs according to the test, it is difficult to directly compare the counts of net revertant colonies obtained in different tests performed at different times. However, the mutagenicity of river water seemed to be increased downstream in the course of the river. Among the 3 branches, the NakDong River flowing through an industrial district was markedly mutagenic, while the East Nakdong River flowing through a residential district and the West NakDong River flowing in an agricultural district were not mutagenic (Tables 1 and 2). For the West NakDong River, since different agricultural chemicals are sprayed at 6-month intervals over this district, samples were obtained at each time of chemical spraying and analyzed. However, the mutagenicity of the river water was not detected *(data not* shown). For the NakDong River flowing in an industrial district, sampling was performed in summer and winter. Both in summer *(unpublished data*) and winter, high mutagenicity and marked dose-dependency was observed (Figs. 1 and 2). In this study, a high positive rate was observed in the TA98 with S9 mix (+S9) strain (Table 1), suggesting that the primary mutation type is frame shift. Comparison among the cell strains showed higher sensitivity of YG strain (Table 2), which is highly sensitive to nitroarene and aromatic amine. However, since its sensitivity was not significantly higher than that of TA, the characteristics of the mutagen could not be clarified. The characteristics of the mutagenicity will be clarified by further monitoring at each investigation point with

Table 1. Mutagenicity of Korean river extracts for Salmonella tester strains TA98 and TA 100 (revertants/plate)

Sites	Dose (μg/plate)	TA98		TA100	
		-S9	+S9	-S9	+S9
West NokDong					
Aug. 1994	683	9	15	12	37
	342	0	13	22	31
	170	0	0	17	30
	85	3	2	14	11
East NokDong					
Aug. 1994	278	3	16	13	3
	139	5	7	0	13
	69	0	7	13	26
	35	0	0	3	0
NokDong					
Feb. 1995	125.0	35	2082	61	214
	62.5	10	643	10	83
	31.3	0	147	21	52
	15.6	0	79	19	10
	7.8	0	34	0	2

Table 2. Mutagenicity of Korean river extracts for Salmonella tester strains YG1041 and YG1042 (revertants/plate)

Sites	Dose (μg/plate)	YG1041		YG1042	
		-S9	+S9	-S9	+S9
NokDong					
Feb. 1995	250.0	59	2228	126	410
	125.0	34	2374	77	252
	62.5	16	1138	64	300
	31.3	8	538	43	309
	15.6	1	246	35	170
West NokDong					
Feb. 1995	250.0	0	27	45	84
	125.0	0	14	15	62
	62.5	0	13	30	37
	31.3	0	12	32	13
	15.6	0	4	0	10

consideration to the effects of the daily periodicity, rainfall and seasonal variation. In addition, the association between the degree of mutagenicity and the conventional parameters of water pollution such as COD (Table 3) should be evaluated.

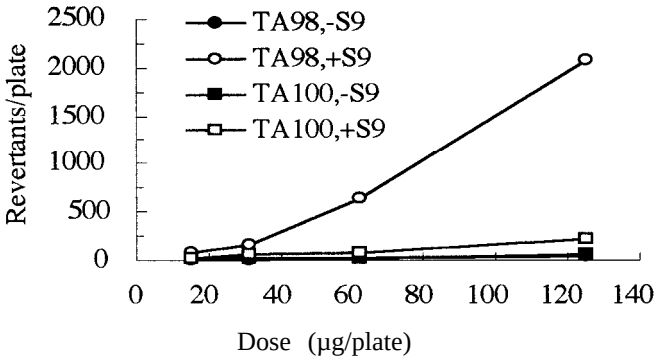

Figure 1. Mutagenicity of the NokDong River extract for Salmonella tester strains TA98 and TA 100

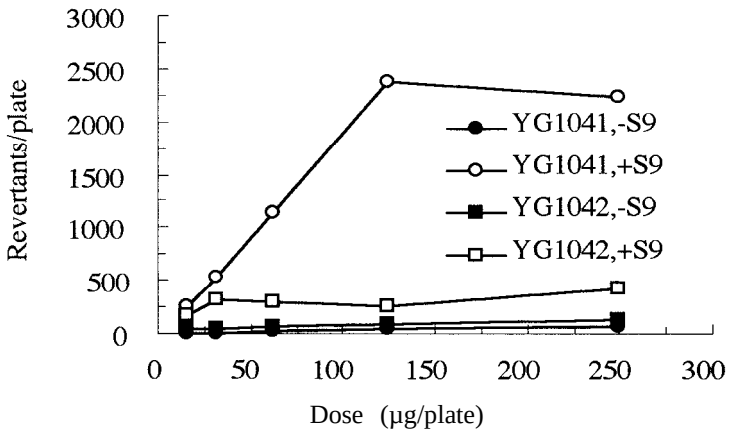

Figure 2. Mutagenicity of the NokDong River extract for Salmonella tester strains YG1041 and YG1042

Unlike the conventional examination methods of the water quality, the Ames test by the BR method evaluates the water quality not using samples obtained at a certain site at a certain time but using the amount of daily exposure. Therefore, quantitative expression such as the amount of a mutagen/l water can not be used. This method also differs from the examination method for long-term pollution using biological concentration by aquatic organisms. However, the state of pollution by mutagens can be readily evaluated by the Ames test using the BR method. Our results also suggested that the BR method is a simple useful method of sampling of polycyclic mutagens.

In the mutagenicity test of water pollutants, there are no established methods of extraction, concentration, and evaluation of mutagenicity. Our study showed

Table 3. Chemical characterization of raw water samples

Sites	Date	DO (mg/l)	COD (mg/l)	SS (mg/l)
East NokDong	Aug. 1994	10.59	2.82	11.19
West NokDong	Aug. 1994	5.89	5.01	10.25
NokDong	Feb. 1995	1.36	6.77	20.00

very high mutagenicity of the river water flowing through an industrial district, suggesting serious pollution of river water in such districts. The source of the pollution appears to be waste fluid from surrounding factories. Though its direct effects on human health are unclear, long-term exposure to the pollutants via fishes and drinking water may have toxic effects on human health. Further studies are needed on the structure, characteristics, and derivation of the mutagens, the amount of pollution, and the risk to humans.

We carried out a mutagenicity study using samples obtained by the blue rayon (BR) suspension method from 3 rivers in Korea. The river water into which waste fluid of daily living and agriculture flows was not mutagenic. On the other hand, the river water into which industrial waste fluid flows was highly mutagenic in TA98 with S9 mix (2082 revs/plate), suggesting that the primary mutation type is frame shift. The mutagens should be identified as soon as possible, and the risk to humans should be evaluated.

REFERENCES

Ames BN, McCann J, Yamazaki E (1975) Methods fordetecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mut Res 31: 347-364

Hayatsu H (1992) Cellulose bearing covalently linkedcopper phthalocyanine trisulphonate as an adsorbent selective for polycyclic compound and its use in studies of environmental mutagens and carcinogens. J Chromatog 597: 37-56

Kinae N, Sugiyama C, Nasuda MY, Goto K, Tokumoto K,Furugori M, Shimoi K (1992) Seasonal variation andstability of chlorinated organic mutagens in drinking water. Wat Sci Technol 25: 333-340

Watanabe M, Ishidate M Jr, Nohmi T (1989) A sensitive method for the detection of mutagenic nitroarenes: Construction of nitroreductase-overproducing derivatives of *Salmonella* typhimurium strains TA98 and TA100. Mut Res 216: 211-220

Watanabe M, Ishidate M Jr, Nohmi T (1990) Sensitive method for the detection of mutagenic nitroarenes and aromatic amines: new derivatives of *Salmonella* typhimurium tester strains possessing elevated *O*-acetyltransferase levels. Mut Res 234: 337- 348