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Detection of Mutagenicity in Mussels and Their Ambient Water

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Mussels provide an excellent system for monitoring marine pollutants; the system is often called "mussel watch" (Goldberg 1975). Investigators have reported the susceptibility of this organism to petroleum hydrocarbons (Lee et al. 1972) and polynuclear aromatic hydrocarbons (Dunn and Young 1976). We also showed the applicability of this organism to monitor the oil pollutions, by detecting organosulfur compounds in field samples (Kira et al. 1983). In the present study, we measured the mutagenicity of mussel bodies and that of their ambient water, and investigated the correlation between the mussel- and water-mutagenicities. Mutagenic compounds being detected here are those adsorbable to blue cotton (Hayatsu et al. 1983) or blue rayon and are extractable with a methanol-ammonia solution, and the Ames assay was used for the detection of mutagenicity, with Salmonella typhimurium TA98 as the tester strain and with S9-mix for metabolic activation.

MATERIALS AND METHODS

Mussels were collected during August 11-13th, 1988, in the Seto Inland Sea of Japan shown in Figure 1. Site A is near an agricultural area, B and C are ports surrounded by large industrial areas which accommodate petrochemical operations, oil refineries, a power plant and other manufacturers. Sites D, G, H, and I are small fishery ports. Sites E and F are commercial ports where ferryboats and other ships move in and out frequently. There are several cultivation stations for sea weeds and oysters in areas offshore from sites G, H and I. Mussels were harvested with a small rake attached to a collecting net (see the tool in Figure 2). We collected 18-40 mussels per site and the flesh was

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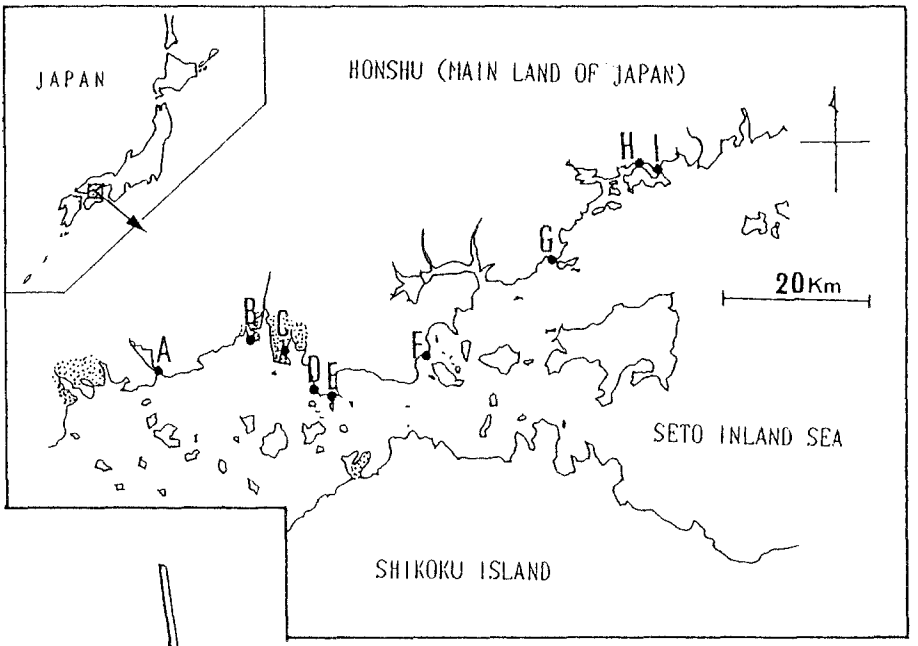

Figure 1. Sampling sites in the Seto Inland Sea

A - I: sampling sites (see text).
Dotted area: industrial area

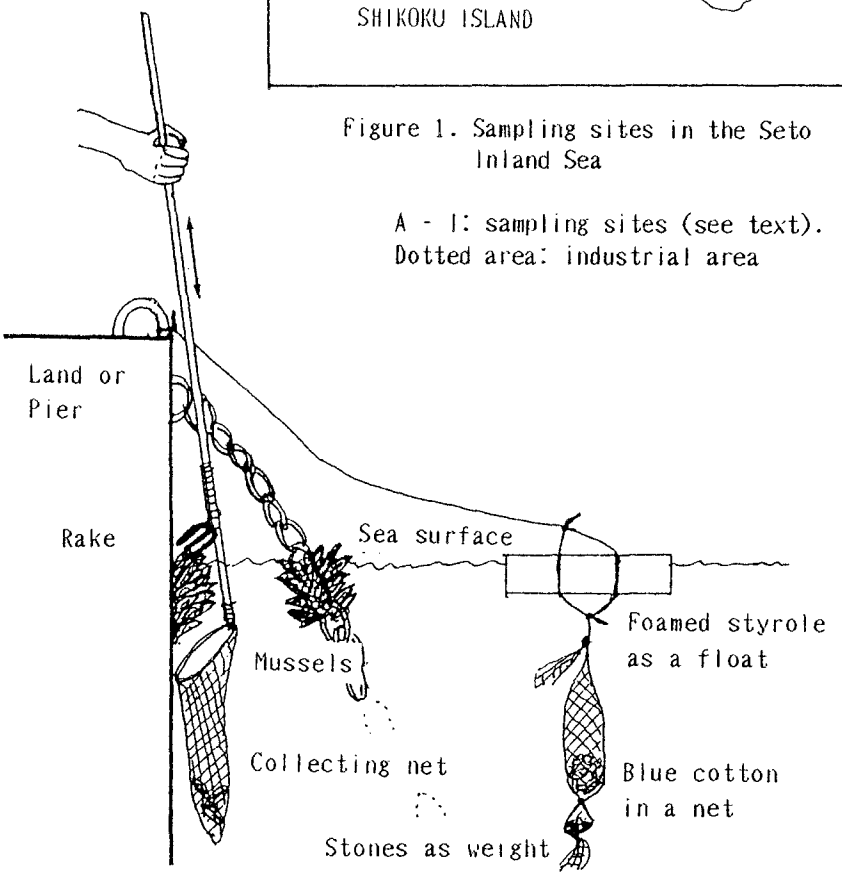

Figure 2. Collection of mussels and immersion of blue cotton in the ambient water.

Scheme 1. Extraction of mutagens from blue rayon (or cotton)

1. Take out the blue rayon and wash with 50 mL distilled water.
2. Remove the water with aspiration and wipe out the residual water with paper towel.
3. Transfer the blue rayon into a flask and extract the rayon with 150 mL methanol/concentrated ammonia (50:1) by shaking for 30 min at room temperature.
4. Evaporate the extract under reduced pressure to dryness.
5. Dissolve the residue in a small amount of methanol and transfer the solution into a test tube.
6. Remove the methanol by evaporation under reduced pressure and store the residue at -20° for mutagenicity assay.
7. Dissolve the sample in 0.1 mL dimethylsulfoxide for the mutagenicity assay.

taken out and weighed. One gram of blue cotton in a net was allowed to stand at a depth 30-50 cm from the water surface as shown in Figure 2, and the cotton was recovered after 24 hr standing. Blue cotton was purchased from Funakoshi Chemicals (Kanda Surugadai 2-3, Chiyoda-ku, Tokyo 101); the same material is available also from Sigma (St. Louis, MO) and Pierce (Rockford, IL).

Thirty grams of mussel flesh was homogenized with 350 mL of acetone in a Waring blender and the homogenate was centrifuged at 13000 x g for 30 min. The acetone layer was collected and evaporated under reduced pressure to give a thick brown oil. To the oil was added 4 mL acetone and the solution obtained was poured into 500 mL distilled water. To the resulting suspension, blue rayon, 1 g, was added and the mixture was gently shaken for 30 min at room temperature. Blue rayon is a recently developed, improved preparation of blue cotton, having greater content of the copper-phthalocyanine-ligand than blue cotton, and was obtained from Funakoshi Chemicals (for address see above). Extraction of polycyclic compounds adsorbed to the blue-rayon and -cotton was carried out as described in Scheme 1. In the case of mussel flesh, the extract thus obtained was again dissolved in water (200 mL) and extracted with blue rayon (1 g) to ensure complete removal of histidine, which can interfere the mutagenicity assay.

The tester strain used was Salmonella typhimurium TA98, which is a kind gift of Dr. B.N. Ames of the University of California, Berkeley. The bacteria were preincubated with the test sample and S9-mix for 20 min at 37°C. The S9 was prepared from livers of SD-rats treated with polychlorinated biphenyl (PCB-54, Tokyo Kasei Co.,

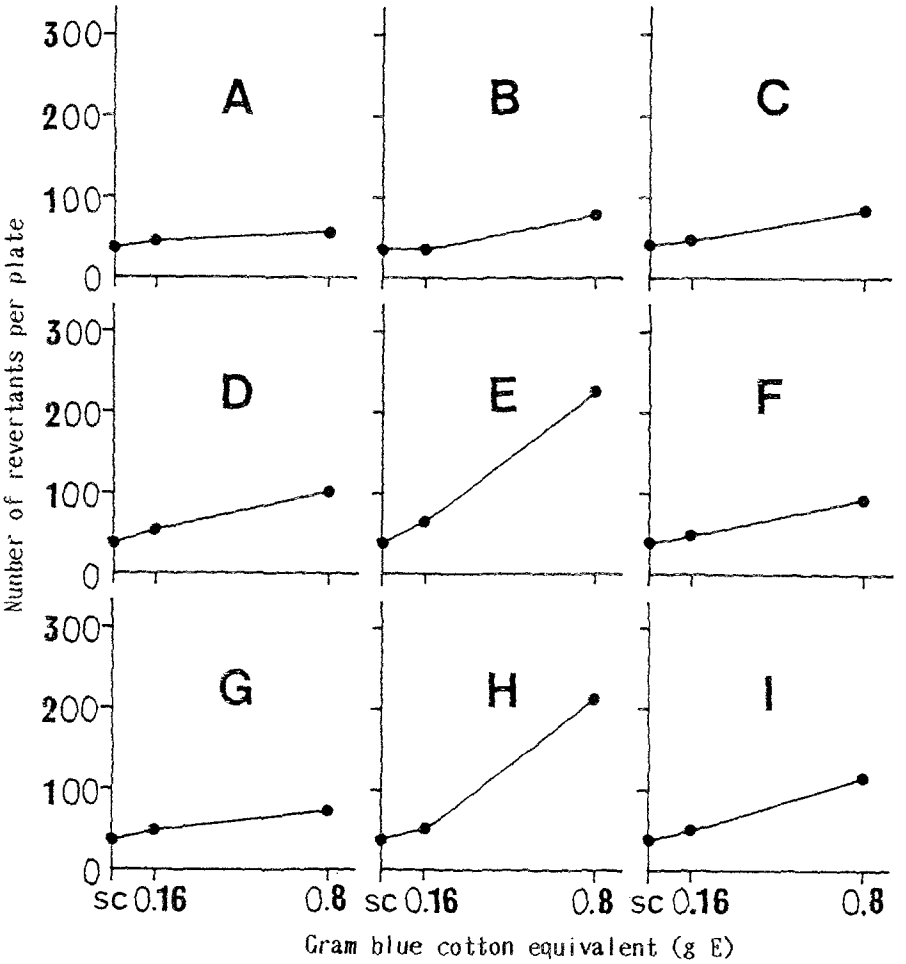

Figure 3. Mutagenic activity of ambient sea water

A - I : sampling sites (see text and Figure 1)
SC : solvent control

Nihonbashi-honcho, 3-1-13, Chuo-ku, Tokyo 103; the chlorine content, 54%). These procedures were worked out according to the techniques previously reported (Ames et al. 1975; Yahagi et al. 1977).

RESULTS AND DISCUSSION

In the preparation of samples for mutagenicity assays, we used blue cotton and blue rayon. These adsorbents, the chemical structure of which is cellulose bearing covalently linked copper phthalocyanine trisulfonate, have a strong affinity to polycyclic compounds, such as polycyclic aromatic hydrocarbons and mutagenic hetero-

Table 1. Mutagenicity of mussel bodies and ambient sea water

Sites[b]	Mutagenicity (Number of revertants per plate)[a] Mussel (10 g mussel equivalent;A)	Sea water (0.8 g blue cotton equivalent;B)[c]	Ratio (A/B)
A	407	73	5.6
C	414	94	4.4
D	365	130	2.8
F	220	104	2.1
G	104	88	1.2
H	275	187	1.5
Solvent control[d]	35	32	
Blue rayon (cotton) control[d]	45	38	

a) The assay was done with Salmonella typhimurium TA98 in the presence of S9-mix. The preincubation method (Yahagi et al. 1977) was used. Values other than those for controls represent net increase over the solvent control.
b) No mussels were found at sites B, E, I.
c) Values taken from results illustrated in Figure 3.
d) Dimethylsulfoxide (0.1 mL) only was used for the solvent control. Blue-rayon- and blue-cotton-controls were prepared using distilled water in place of mussel homogenate and sea water.

cyclic amines (Hayatsu et al. 1983). Blue cotton has been extensively used for isolating polycyclic mutagens from crude samples such as cigarette smoke condensates (Yamashita et al. 1986) and opium pyrolysates (Friesen et al. 1987). Blue rayon has recently been used for adsorption of mutagens from river water (Sakamoto and Hayatsu 1988) and shell-fish meats (Hayatsu and Hayatsu 1988).

Figure 3 shows the mutagenic activities of extracts from ambient waters. Every sample showed dose-dependent increase in the number of revertant colonies, and the numbers found for 0.8 g E blue cotton were all over 2 times the value for the solvent control. Thus at all the sites examined, the sea water contained mutagens. The samples from site E and H gave colonies greater than 200. It should be noted that our previous work indicated that some river-water can give rise to formation of greater than 1000 colonies with the same monitoring technique (Sakamoto and Hayatsu 1988).

Table 1 shows the results of the mutagenicity assay for mussels, together with those for the corresponding sea water. At every site, the mussel sample gave a greater number of revertant colonies than the water sample. The ratio between the activities of these two samples

differ significantly among the sites, indicating that there is no immediate parallelism between these mutagenic activities.

The 24 hr hanging of blue cotton in the ambient water would adsorb polycyclic compounds that have made contact with this adsorbent; the mutagenicity of the blue cotton extract may thus be regarded as a semi-quantitative indication of short-term pollution with mutagens bearing polycyclic structures. On the other hand, the mutagenicity detected in mussels may be a result of long-term exposure of this organism to such pollutants. It is also possible that the mutagens in mussels include those that are metabolites of non-mutagens. Therefore it is not surprising to find discrepancies between the mussel-body mutagenicity and ambient-water mutagenicity. The high mussel-mutagenicity may be related to the presence of a heavily industrialized area in vicinity. Site G, where both the mussel-mutagenicity and water- mutagenicity were low, is relatively far from either an industrial area or a large city.

Our recent studies have shown that oysters in the Seto Inland Sea area are mutagenic (Hayatsu and Hayatsu 1988), as detected with the technique used in the present work. A preliminary analysis indicates that the components in the blue-rayon extracts of oysters are polycyclic aromatic hydrocarbons. An analytical study has shown that the oysters from this area are contaminated with polycyclic aromatic hydrocarbons including benzo(a)pyrene (Obana et al. 1981), which is mutagenic in the Salmonella assay (McCann et al. 1975). The mutagenic components in the mussels we examined are probably similar to those in oysters, although they are to be identified by further studies.

The results we present here show that mussels may be useful for detecting long-term pollution of sea water with mutagens.

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