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Microbial Influences on the Mobility and Transformation of Radioactive Iodine in the Environment

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Long-lived radioactive iodine (^{129}I, half-life: 1.57×10^7 y) has been released into the environment from nuclear fuel reprocessing plants. ^{129}I may also be released from ground storage of nuclear waste. Given its long half-life, a better understanding of the behavior of iodine in the environment is necessary to ensure the safety of humans and the health of the environment. In this report, we summarize our recent results and new experimental data about microbial influences on the mobility and transformation of iodine. Microbial volatilization of organic iodine was observed in soil slurries and seawater samples, and various species of aerobic bacteria were considered to play a significant role through methylation of iodide (I^-) to form methyl iodide (CH_3I). The volatilization of iodine was also found in iodide-rich natural gas brine water, where iodide concentration is approximately 2,000 times higher than that in seawater. In this case, however, a significant amount of molecular iodine (I_2) was produced together with organic iodine compounds. Iodide-oxidizing bacteria, which oxidize iodide to I_2, were isolated from seawater and natural gas brine water. Phylogenetically, they were divided into two groups within the α-subclass of the *Proteobacteria* (*Roseovarius* sp. and unidentified bacteria), and they produced not only I_2 but also diiodomethane (CH_2I_2) and chloroiodomethane (CH_2ClI). Iodide-accumulating bacteria, which accumulate iodide to concentrations 5,500-fold over that of the medium, were also isolated from marine sediment. They were closely related to *Arenibacter troitsensis*, and iodide uptake was mediated by an active transport system. Our results suggest that the fate of iodine can be affected by microorganisms, particularly by bacteria, through processes such as volatilization, oxidation, and accumulation.

1. Introduction

Iodine-129 (^{129}I, half-life: 1.57×10^7 y) is one of the most persistent anthropogenic radionuclides, and it has been released into the environment from nuclear weapons testing and nuclear fuel reprocessing plants.[1, 2] ^{129}I has the same geochemical properties as stable iodine (^{127}I), and is rapidly dispersed in the environment since its predominant chemical forms iodide (I^-) and iodate (IO_3^-) are highly soluble and mobile. In the UNSCEAR-2000 Report,[3] ^{129}I is regarded as one of the most critical radionuclides to be assessed from the viewpoint of global circulation. Therefore, a better understanding of the behavior of iodine throughout the biosphere is needed to ensure the safety of ^{129}I. However, the biogeochemical cycling of iodine is not fully understood. Since microorganisms affect the mobility and speciation of many trace elements in the environment,[4] it is possible that the chemical behavior of iodine is also influenced by microbial activities. In this paper, we summarize our recent investigations and present new experimental data in relation to microbial effects on the chemical behavior of iodine in the environment.

2. Experimental

Volatilization of Iodine from Environmental Samples. Soil slurries, fresh seawater, and natural gas brine water were dispensed into 120-mL serum glass bottles, and radioactive iodide tracer ($^{125}I^-$) was added to give a final concentration of approximately 30 kBq/mL. In order to change the composition and the level of microbial populations, yeast extract, cycloheximide (as a eukaryotic antibiotic), or a mixture of streptomycin and tetracycline (as prokaryotic antibiotics) was added to some samples to give final concentrations of 1 g/L, 0.15 g/L, and 0.025 g/L each, respectively. The final volume of the samples was adjusted to 20 mL. The bottles were sealed with butyl rubber stoppers, and incubated for 2 to 7 days with shaking. Volatile organic and inorganic iodine compounds were collected with activated charcoal and silver wool traps, respectively.[5] The activity of ^{125}I was measured with a NaI scintillation counter (Aloka ARC-370M). In some cases, chemical formulas of the organic iodine compounds were identified by a gas chromatograph equipped with an electron-capture detector (GC-ECD) and GC coupled to a mass-selective detector (GC-MS).

Experiments with Bacterial-Pure Cultures. Bacterial capacities for volatilizing iodide were determined by radiotracer techniques. Bacterial strains, isolated from soil, seawater, marine sediment, and marine algae, were cultured with $^{125}I^-$ in serum glass bottles. Volatile organic iodine compounds were collected and determined as described above. The strains were judged to have abilities to volatilize iodide when their percentages of volatilization were more than 0.01%. In some cases, chemical formulas of the organic iodine compounds emitted from the cultures were identified by GC-ECD and GC-MS.

To isolate iodide-oxidizing bacteria, soil, seawater, marine sediment, and natural gas brine water were diluted and spread on agar media containing stable iodide (KI) and soluble starch.[6] In some cases, environmental samples were enriched with 1 mM of iodide for 7 to 180 days before they were spread on the media. If iodide was oxidized to I_2 by bacteria, the color of the bacterial colonies became purple since I_2 forms a purple complex with starch. Bacterial production of I_2 and organic iodine compound was determined by radiotracer techniques. Chemical formulas of the organic iodine compounds were also identified by GC-MS. The iodide-oxidizing enzyme

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activity was assayed spectrophotometrically using the culture supernatant as a crude enzyme.

For isolation of iodide-accumulating bacteria, two types of screening were carried out.[7] In the first screening, various bacterial strains maintained in our laboratory were inoculated onto agar media containing $^{125}I^-$. After incubation, bacterial colonies were transferred to a membrane filter by toothpicks, and radioactivity in the colonies was visualized with a Molecular Imager FX system (Bio-Rad). This transfer technique enabled us to obtain a good autoradiograph with a relatively low background, while direct transfer of colonies without toothpicks resulted in a very high background. Bacterial strains showing dense and black spots were chosen. In the second screening, the strains were cultured in liquid media containing $^{125}I^-$. After incubation, the cultures were put on a silicone oil layer (35:65 mixture of SH556 and SH550, Toray Dow Corning Silicone) that was overlaid on distilled water in a microcentrifuge tube. After centrifugation, the tube contents were frozen, and the distilled water layer containing the cell pellet was cut off for determining radioactivity.

For phylogenetic analysis of bacterial strains, the 16S ribosomal RNA gene (16S rDNA) was sequenced as described previously.[8] The sequences were aligned using the CLUSTAL W program, version 1.6.[9] A phylogenetic tree was constructed by the neighbor-joining method.[10]

3. Results and Discussion

Volatilization of Iodide from Environmental Samples. When diluted soil slurries were incubated with 1 mM of stable iodide for 20 days, CH_3I production in the bottle headspace was detected by GC-ECD.[8] Production of other organic iodine compounds (e.g. CH_3CH_2I and CH_2I_2) was not observed. The addition of glucose sometimes enhanced CH_3I production, but little CH_3I was produced under anaerobic incubation conditions. The soil slurries were then incubated with iodide tracer ($^{125}I^-$), and iodide volatilization under various incubation conditions was determined. This method allowed us to study iodide volatilization under a natural concentration of stable iodide (without any addition of excess stable iodide). Autoclaving of soil slurries strongly inhibited the volatilization, while yeast extract sometimes enhanced the volatilization. Interestingly, the volatilization of iodide was almost completely inhibited by prokaryotic antibiotics (streptomycin and tetracycline), but not by the eukaryotic antibiotic cycloheximide. These results suggest that iodide in soil is methylated and volatilized into the atmosphere mainly as a result of aerobic soil bacteria.

A similar experiment was carried out using fresh seawater.[11] The volatilization of iodide did not occur in autoclaved or 0.22 μm-filtered seawater. In addition, both prokaryotic and eukaryotic antibiotics inhibited the volatilization significantly. Thus, it was considered that iodide in seawater is volatilized as a result of microbial activities, and that marine bacteria, at least partially, participate in the process.

In soil slurries and seawater, iodide was volatilized mainly as an organic iodine compound (probably CH_3I), and volatilization of inorganic iodine (i.e. molecular iodine: I_2) was not observed. However, when we incubated natural gas brine water with $^{125}I^-$, not only organic iodine but also I_2 was produced. Table 1 shows new experimental data related to the production of I_2 in brine water. I_2 production was much higher than that of organic iodine (see "untreated" samples in Table 1), and it was enhanced by the addition of 0.1% yeast extract. The volatilization of I_2 was strongly inhibited by autoclaving, filtration, and prokaryotic antibiotics. These results suggest that I_2 production in brine water is also mediated by bacteria. From the viewpoint of global iodine cycling, natural gas brine water is of great interest because it is highly enriched with iodide. Brine water in Japan, especially that in Chiba prefecture, often has iodide concentrations of approximately 1 mM, which is 2,000 times higher than that in natural seawater.[12] Natural gas, mainly methane, is associated with brine water in this area. The annual production of iodine in this area accounts for 6,000 tons, which constitutes approximately one third of the world iodine production. Our results indicate that bacteria with capacities for producing I_2 and organic iodine compounds inhabit brine water. Isolation and characterization of such bacteria is described below (see *Iodide-oxidizing Bacteria* (*IOB*)).

TABLE 1: Organic and Inorganic Iodine Volatilization from Natural Gas Brine Waters[a]

		Untreated	Autoclaved	Filtered[b]	YE[c]	Stp+Tc[d]	Ch[e]
Brine 1	Org-I	100	0	0	943	0	86
	Inorg-I	266	0	9	10,059	0	49
Brine 2	Org-I	100	2	2	211	33	71
	Inorg-I	600	0	0	2,781	N.D.[f]	N.D.

[a]All values are expressed as relative % of volatilization, and volatilization of organic iodine in untreated samples is expressed as 100%. [b]Samples were filtered through a 0.22-μm membrane filter. [c]Yeast extract was added. [d]Streptomycin and tetracycline were added. [e]Cycloheximide was added. [f]Not determined.

Iodide-volatilizing Bacteria (IVB). Of 100 bacterial strains tested, approximately 40% showed percentages of volatilization of more than 0.01%.[8, 11] Based on 16S rDNA sequences, we concluded that they were affiliated with *Proteobacteria* (α, β, and γ subdivisions), the *Cytophaga-Flexibacter-Bacteroides* (CFB) group, and High G+C Gram positive bacteria, indicating that IVB were not confined to any particular group of bacteria and were found throughout the bacterial domain. GC-ECD and GC-MS analyses showed that the gaseous iodine compound emitted by bacteria was CH_3I. We also measured the iodide-methylating activity by using cell free extracts of *Rhizobium* sp. MRCD 19.[13] We found that *S*-adenosyl-L-methionine (SAM) could serve as a methyl donor for methylation of iodide. The apparent affinity constants (K_m) for substrates (i.e. iodide and SAM), calculated from Lineweaver-Burk plots, were 0.26 and 0.024 mM, respectively.

Iodide-oxidizing Bacteria (IOB). A large number of IOB strains were isolated from natural gas brine water, seawater, and marine sediment.[6] Interestingly, an enrichment of the environmental samples with 1 mM of iodide (7 to 180 days) was essential for isolation of IOB from seawater and sediment, while IOB could be isolated without enrichment from brine water. IOB were phylogenetically divided into two groups within the α-subclass of the *Proteobacteria*. One of the groups was most closely related to *Roseovarius tolerans* with sequence similarities between 94 and 98%. The other group was most closely related to *Rhodothalassium salexigens*, although the sequence similarities were relatively low (89 to 91%). The iodide-oxidizing reaction was mediated by an extracellular enzyme, and oxygen was indispensable for the reaction. This inferred that certain oxidases were involved in the oxidation of iodide.

Radiotracer experiments and GC-MS analyses revealed that IOB produced not only I_2 but also organic iodine compounds such as CH_2I_2 and CH_2ClI.[6] Table 2 shows our new experimental data on gaseous iodine production by IVB (strain C-19) and IOB (strains A6 and C3). IVB did not produce I_2, and the production amounts of organic iodine compound (CH_3I) under 0.1 μM and 0.1 mM of iodide were 21 and 730 pmol/20 mL of culture, respectively. Since the organic iodine production increased only 35-fold in the presence of 0.1 mM iodide, CH_3I production from iodide was considered to be saturated. In IOB

TABLE 2: Production of Organic and Inorganic Iodine Compounds by IVB and IOB[a]

Strain	Most-related organism		Iodide concentration added to the medium		
			0.1 M	0.1 mM	1 μM
C-19 (IVB)	*Algoriphagus antarcticus*	Org-I	21	730	N.D.[b]
		I_2	<0.04	<40	N.D.
A-6 (IOB)	*Roseovarius tolerans*	Org-I	0.11	17,000	N.D.
		I_2	<0.04	840	N.D.
C-3 (IOB)	*Rhodothalassium salexigens*	Org-I	<0.04	64,000	5,200,000
		I_2	<0.04	1,200	160,000

[a]All values are expressed as pmol of iodine/20 mL of culture. The strains were cultured with $^{125}I^-$ and appropriate concentrations of stable iodide (KI) in Marine Broth 2216 liquid medium (Difco). After 3 days of incubation, volatile organic iodine and I_2 were collected with activated charcoal and silver wool traps, respectively. [b]Not determined.

strains, however, both organic (CH_2I_2 and CH_2ClI) and inorganic (I_2) iodine compounds were produced, and the production was strongly enhanced under high levels of iodide (Table 2). This may imply that the expression of enzyme(s) that produced I_2, CH_2I_2, and CH_2ClI was induced or enhanced under high iodide concentrations. However, in our previous study,[6] the levels of iodide-oxidizing activity were constant regardless of iodide concentrations in the media. Thus, it might be possible that the activity of the enzyme(s) involved in the production of I_2, CH_2I_2, and CH_2ClI is induced (or enhanced) by I_2 but not by iodide. Further study is needed to understand the biochemical mechanism of organic and inorganic iodine production by IOB.

Iodide-accumulating Bacteria (IAB). A screening of bacteria with capacities for accumulating iodide was carried out.[7] Combining autoradiography with radiotracer experiment was very efficient for selection of IAB. Of 91 bacterial strains tested, 6 strains were chosen as IABs. They removed 80 to 90% of iodide from the liquid media within 24 h, and a corresponding amount of iodide was detected in their cells. All of these strains originated from marine sediment, and they were phylogenetically most closely related to *Arenibacter troitsensis* and *Flexibacter aggregans* NBRC15975, members of the family *Flavobacteriaceae*. Figure 1 is a phylogenetic tree of strain C-21, which was chosen for further investigation. When strain C-21 was grown in a liquid medium containing 0.1 μM of iodide, iodide concentration in the medium decreased to 0.02 μM within 24 h, and the maximum iodide content of the strain was 220 pmol/mg dry cells. The iodide concentration factor was calculated based on the ratio of the iodide concentration in cells (on a dry weight basis) to that in the medium. In strain C-21, the maximum value of the iodide concentration factor was 5,500 at 24 h. The kinetics of iodide uptake by IAB were then determined using a washed cell suspension of strain C-21. A cumulative uptake of iodide was observed, but it occurred only in the presence of glucose. Iodide transport by this strain showed Michaelis-Menten kinetics with an apparent affinity constant (K_t) and a maximum velocity (V_{max}) of 0.073 μM and 0.55 pmol/min/mg dry cells, respectively (Figure 2). From these results, we considered that the uptake and accumulation of iodide by IAB was mediated by an active transport system which requires metabolic energy, but not by simple diffusion or physicochemical adsorption onto the bacterial cell surface. Determinations of the energy source (ATP or ion gradient) and the substrate specificity of the iodide uptake system are underway in our laboratory.

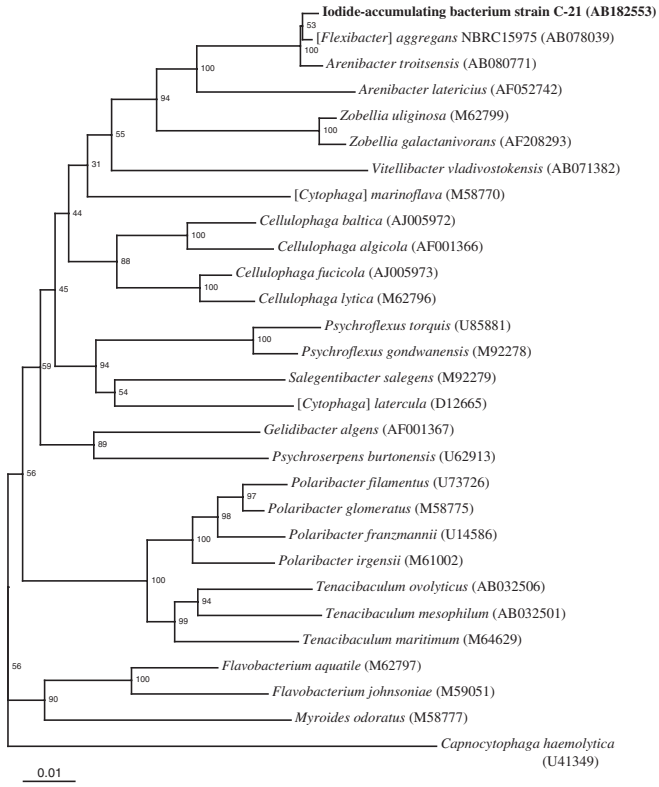

Figure 1. Phylogenetic position of strain C-21 among the family *Flavobacteriaceae* on the basis of 16S rDNA sequences. Numbers at nodes show bootstrap values obtained from 100 resamplings. The GenBank accession number for each reference strain is shown in parentheses. The bar shows 1 nucleotide substitution per 100 nucleotides. *Capnocytophaga haemolytica* was used as the outgroup.

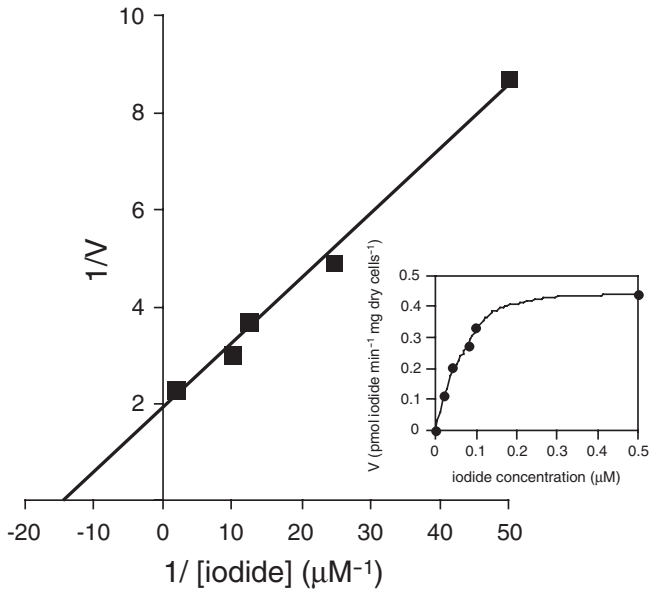

Figure 2. A double-reciprocal plot of iodide uptake by strain C-21. The inset shows the initial uptake of iodide as a function of iodide concentrations from 0.02 to 0.5 μM.

4. Conclusion

Microorganisms play an important role in the biogeochemical cycling of many trace elements by changing their chemical behavior in the environment. Our results demonstrated that microorganisms, especially bacteria, have a potential to influence the chemical behavior of iodine through such processes as volatilization, oxidation, and accumulation. Another important process in iodine cycling is the reduction of iodate (IO_3^-) to iodide, and a sulfate-reducing bacterium (*Desulfovibrio desul-*

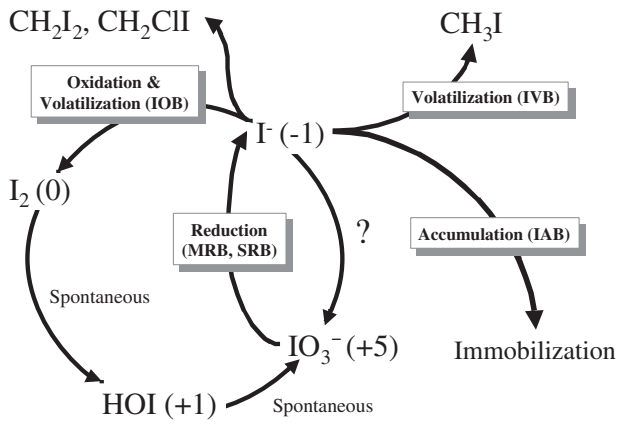

Figure 3. The biogeochemical cycling of iodine. Pathways that can be mediated by microorganisms are also shown.

furicans) and a metal-reducing bacterium (*Shewanella putrefaciens*) have been reported to possess iodate-reducing abilities.[14, 15] In Figure 3, we illustrate the biogeochemical cycling of iodine, and also show several pathways that can be mediated by microbial activities. Since bacteria comprise a great biomass and are distributed widely in the environment, it is not surprising if they contribute significantly to global iodine cycling. Therefore, bacterial influence on the mobility and the transformation of iodine should be considered in the assessment of ^{129}I released from nuclear facilities. Furthermore, such information may be useful in predicting the environmental risk of ^{129}I possibly released from ground storage of nuclear waste.

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