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Department of Marine Sciences, Texas A&M University, Galveston, Texas 77553, and Savannah River National Laboratory, Aiken, South Carolina 29808

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129I is a major by-product of nuclear fission that is of concern because of its mobility in the environment, excessive inventory, toxicity (it accumulates in the thyroid), and long half-life (~16 million years). The aim of this study was to determine if bacteria from a 129I-contaminated oxic aquifer at the F area of the U.S. Department of Energy’s Savannah River Site, SC, could accumulate iodide at environmentally relevant concentrations (0.1 μM I⁻). Iodide accumulation capability was found in 3 out of 136 aerobic bacterial strains isolated from the F area that were closely related to Streptomyces/Kitasatospora spp., Bacillus mycoides, and Ralstonia/Cupriavidus spp. Two previously described iodide-accumulating marine strains, a Flexibacter aggregans strain and an Arenibacter trottsensis strain, accumulated 2 to 50% total iodide (0.1 μM), whereas the F-area strains accumulated just 0.2 to 2.0%. Iodide accumulation by FA-30 was stimulated by the addition of H₂O₂, was not inhibited by chloride ions (27 mM), did not exhibit substrate saturation kinetics with regard to I⁻ concentration (up to 10 μM I⁻), and increased at pH values of <6. Overall, the data indicate that I⁻ accumulation likely results from electrophilic substitution of cellular organic molecules. This study demonstrates that readily culturable, aerobic bacteria of the F-area aquifer do not accumulate significant amounts of iodide; however, this mechanism may contribute to the long-term fate and transport of 129I and to the biogeochemical cycling of iodine over geologic time.

129I is major by-product of nuclear fission that is of concern because of its mobility in the environment, excessive inventory, long half-life (~16 million years), and potential toxicity due to bioaccumulation through the food chain and bioconcentration in the thyroid gland (16, 17, 22). Currently, 146 Ci of 129I is inventoried in soils at two U.S. Department of Energy (DOE) sites, the Hanford Site and the Savannah River Site (SRS), where it has been identified as a key risk driver in contaminated soils and groundwater (21, 24). Furthermore, the global inventory of 129I will increase significantly if just a fraction of the expected “Nuclear Renaissance” is realized (e.g., between China and India alone, 50 to 60 new nuclear reactors are expected to come online by the year 2020) (16, 50). Thus, it is critical to understand the environmental behavior of 129I in order to rigorously assess storage and disposal options for current and future stockpiles of 129I.

In general, little information is available about the chemical properties and mobility of iodine in subsurface aquifers, particularly its tendency to form organo-iodine or its mobility as organo-iodine. However, our own measurements of groundwater from several of the F-area aquifer bore holes found that organo-iodine could account for up to 25% of total iodine (43, 52). The various isotopes of iodine can be strongly bound to macromolecular organic matter, which can significantly decrease or increase its transport, bioavailability, and transfer to humans, depending on the molecular weight and physicochemical properties of the resulting iodine-organic matter species (15, 19, 41–43, 49).

Microbial activity has been linked to the production of organo-iodine and sorption of iodine to soil, and a small but growing body of literature has implicated microbial oxidases, perhydroxylases, and particularly peroxidases in the halogenation of soil organic matter (4, 10, 20, 23, 27, 32–34, 37, 38). Yet, details concerning the mechanisms and bacterial species or groups involved are lacking. The most-recent advances in research concerning microbial-iodine interactions have been contributed by Amachi et al. (1–8, 18). In a series of papers, Amachi’s research group has isolated (i) iodide-accumulating bacteria (IAB) from marine sediments that concentrate iodide by a factor of 6 × 10³ (6), (ii) iodide-oxidizing bacteria from seawater and natural gas brine water that transform iodide into I₂ and volatile organo-iodine species (8), and (iii) iodide-methylating bacteria from a variety of soil and seawater samples (4, 5). For the marine IAB, Amachi et al. (2) proposed a model whereby extracellular H₂O₂, generated by glucose oxidase, oxidizes iodide to I₂ or hypiodous acid (HIO) via an unidentified haloperoxidase. HIO is then transported across the cell membrane via a facilitated diffusion-type mechanism. Once inside the cell, HIO either is reduced to iodide or forms organo-iodine.

Notably, iodide-oxidizing and iodide-accumulating strains were readily obtained from marine or brine waters but not from surface soils (22 samples from a rice paddy, upland field, and forest soils) (6, 7). The only study to date examining the potential for microorganisms from subsurface aquifers to associate with iodine was performed using enrichments from anoxic natural gas formation waters that contain exceedingly...
high concentrations of iodine (∼120 mg liter⁻¹) and a set of 16 anaerobic bacterial strains obtained from culture collections that included sulfate and iron reducers, denitrifiers, and methanogens (3). The results indicated very limited adsorption or accumulation of iodine by anaerobic microorganisms. Overall, the research conducted thus far demonstrates that microbial activity is an important factor in iodine biogeochemical cycling (particularly in enhancing iodine binding to high-molecular-weight organic matter) and leads us to hypothesize that select soil and sediment bacteria could be capable of influencing the chemical behavior of iodide (the most common form of ¹²⁹I found in groundwater) via accumulation, volatilization, and oxidation under aerobic conditions. In particular, we wish to develop an understanding of the role that microorganisms play in the formation of organo-iodine in the subsurface and how they influence iodine mobility. As an initial step toward this overarching goal, the aim of this study was to determine if naturally occurring bacteria from a ¹²⁹I-contaminated oxic aquifer at the Savannah River Site, SC, could accumulate iodide at environmentally relevant concentrations.

MATERIALS AND METHODS

Isolation of bacteria. Sediment samples were collected from three locations and multiple depths within the F-area plume zone (10 to 100 pCi/liter ¹²⁹I) at the Savannah River Site, SC (Fig. 1). Sediments from the first two locations, FAW-4 and FAW-1, were collected from a sandy/clay aquifer 13.1 to 20.7 m and 24.1 to 25.9 m below the surface, respectively. The other sampling location, FSP-07, was an organic-rich wetland zone (0.15 to 1.2 m below the surface). Two approaches, sonication or pyrophosphate based, were used to isolate microbes directly from each of the subsurface soils. For the sonication method, soil samples (1 g) were suspended in 100 ml sterile distilled water (dH₂O) and stirred for 15 min. A portion (1 ml) of this soil slurry was sonicated for 15 s to generate a 10⁻² dilution of the original soil slurry. For the pyrophosphate method, soil samples (1 g) were suspended in 100 ml sterile phosphate-buffered saline (pH 7.0) containing 25 mM pyrophosphate and stirred for 30 min and then allowed to settle for 30 min to generate a 10⁻² dilution of the original soil slurry.

Subsequent dilutions (10⁻³ to 10⁻⁶) were prepared from the sonication- and pyrophosphate-generated soil slurries in both R2A (1/10 strength; Difco) and DNB (Oxoid) medium. A portion (100 μl) of each dilution (10⁻² to 10⁻⁶) series prepared in R2A was spread onto R2A (1/10 strength) plates solidified with either noble agar (Difco) or gellum gum (Sigma Aldrich), with or without cycloheximide (100 μg ml⁻¹; Sigma Aldrich). A portion (100 μl) of each dilution (10⁻² to 10⁻⁶) series prepared in DNB was spread onto DNB agar plates, with or without cycloheximide (100 μg ml⁻¹). Plates were incubated at room temperature in the dark and monitored for growth over a period of 1 week to 3 months. As colonies with unique morphologies appeared on the plates, they were transferred to fresh R2A (1/10 strength) or DNB plates. In this manner, 325 distinct morphotypes were isolated and stored (agar plates at 4°C and glycerol stocks at −80°C) for further analysis.

Enrichment cultures containing 1 mM iodide were also prepared with sediments collected from the aquifer and wetland zones as means to isolate IAB. Enrichments were generated by suspending sediments (0.05 g) in 50-ml conical tubes containing (i) 20 ml dH₂O, (ii) 20 ml 10% nutrient broth (Oxoid), or (iii) 20 ml 10% nutrient broth plus 50 μg ml⁻¹ cycloheximide. Iodide was added (as KI; Sigma) to give a final concentration of 1 mM. This resulted in a total of 24 enrichment cultures (8 sediment samples, with 3 treatments for each sediment sample). The enrichments were incubated in the dark at room temperature and agitated by tube inversion once each day. At 2, 9, and 22 weeks, 50 μl from each tube was transferred to sterile tubes containing 5 ml R2A (1/10 strength) to
generate a $10^{-2}$ dilution. Dilutions of $10^{-4}$ and $10^{-6}$ were then prepared in R2A (1/10 strength), and 25 µl of each dilution was spread on both 25% DNB-IS (IS concentrate of 1.2 g liter$^{-1}$ KI and 1 g liter$^{-1}$ soluble starch) and 25% R2A-IS agar plates. In this manner, 29 distinct morphotypes were isolated and stored (agar plates at 4°C and glycerol stocks at -80°C) for further analysis.

16S rRNA gene sequencing and phylogenetic analysis. To determine the taxonomic identity of the isolates, a portion of the 16S rRNA gene was amplified and sequenced. Crude bacterial lysate was collected by placing a small amount of isolated colonies into 10 µl Tris-EDTA (TE) buffer (pH 7.5) with sterile pipette tips. Amplification was performed using 1 µl of the crude lysate as a template in a 50-µl reaction mixture containing 5 µl 10× buffer, MgCl$_2$ (2.5 mM), the 27f primer (5′-GAGTTGATCATGCGTCGACT-3′) (10 pmol), the 1492r primer (5′-GGTACCTTGTTACGACTT-3′) (10 pmol), deoxynucleoside triphosphates (200 µM each), and 1.25 U of HotStar Taq DNA polymerase (Qiagen). Reactions were amplified in a PTC-100 thermocycler (MJ Research, Inc.) as follows: 95°C for 10 min and 32 cycles of 95°C for 1 min, 45°C for 45 s, and 72°C for 1 min, followed by a 7-min elongation step. PCRs were visualized on a 1.2% agarose gel to ensure amplification of a single product of the expected size. The 16S rRNA gene amplicon was purified with a MinElute PCR purification kit (Qiagen), and the concentration of the purified product was adjusted to -15 ng µl$^{-1}$ in dH$_2$O using a Nanodrop 1000 spectrophotometer (Thermo Scientific). Sequencing of the 16S rRNA PCR products was performed by the Georgia Sequencing Facility at the University of Georgia, Athens, GA.

Phylogenetic similarity of 16S rRNA sequences was first determined with BLAST searches of the GenBank (http://www.ncbi.nlm.nih.gov/genbank/index.html) and the RDP classifier (13), followed by maximum-likelihood reconstruction of phylogenetic trees with ARB (48). Culture conditions. Marine iodide-accumulating bacterial (IAB) strains, Flexibacter aggregans NBRC15975 and A. trotsenensis JCM11736, were purchased from the NITE Biological Research Center, Chibin, Japan, and used as reference strains for the iodide accumulation assay. The two reference strains and all microbial isolates from the F area were grown on marine agar 2216 (Difco) and 1/4-strength R2A agar medium (EMD), respectively.

Iodide accumulation screening. Two methods using $^{125}$I$^{-}$ as a tracer were combined to determine the iodide-accumulating ability from F-area microbes. The first used autoradiography, similar to the procedure of Amachi et al. (7). A small portion of microbial colonies, applied evenly to R2A agar plates. Microbial isolates from the F area were vortexed for 1 min, the radioactivity of accumulated$^{125}$I was determined by a scintillation vial. Radioactivity of $^{125}$I was determined by liquid scintillation counting for 10 min (count errors were consistently below 20%). The iodide-accumulating ability (percent accumulation) of microbial cells was calculated as the ratio of “cell-associated$^{125}$I” versus “$^{125}$I activity in the microbial culture.” Cell densities in aliquots were measured at 600 nm with a spectrophotometer (Turner SP8001). Correlations of bacterial dry weight with optical density at 600 nm (OD$_{600}$) for each target bacterium were obtained during different periods of exponential growth through linear regression, resulting in an $r^2$ value higher than 0.98 for each strain.

Effect of chloride on iodide-accumulating abilities. Bacteria were grown in two types of aqueous minimal media, M9 and Cl$^{-}$-deficient M (M9X). M9 contained 47.75 mM Na$_2$HPO$_4$, 22.04 mM KH$_2$PO$_4$, 8.56 mM NaCl, 18.70 mM NH$_4$Cl, 2 mM MgSO$_4$, 0.1 mM CaCl$_2$, 22.22 mM glucose, and 0.5% yeast extract. M9X was prepared by replacing NaCl, NH$_4$Cl, and CaCl$_2$, with the same concentrations of Na$_2$SO$_4$, (NH$_4$)$_2$SO$_4$, and Ca(OH)$_2$, respectively. Cells grown in M9 and M9X media were exposed to 2 different concentrations of stable I$^{-}$ (0.1 and 10 µM) while maintaining the same concentration of $^{125}$I$^{-}$ (1.33 kBq liter$^{-1}$). Incubations were performed in the dark at room temperature with continuous shaking at 150 rpm for 24 h. H$_2$O$_2$ (final concentration, 5 mM) was then added to the microbial culture. After an additional 24-h incubation, aliquots were collected for the determination of cellular $^{125}$I activity and cell density as described above.

Effect of pH and sodium azide on iodide-accumulating abilities. To evaluate iodide accumulation as a function of pH, IAB from the F area were grown in the M9 medium containing 0.5% yeast extract at room temperature with (shaking, 150 rpm). When the optical density (600 nm) of the cultures reached ~0.8, cells were harvested by centrifugation (3,500 × g at 20°C for 15 min) and washed twice with medium with pH 4, 6, 8, and to 9, respectively. To avoid bursting or shrinking of cells by transferring them from M9 to pH-adj usted minimal medium, the iodine concentration of pH-adjusted medium was maintained at 177 mM, which was the same ionic strength as the M9 medium. Cell pellets were suspended in pH-adjusted minimal medium containing stable I$^{-}$ (0.1 µM), $^{125}$I$^{-}$ (1.33 kBq liter$^{-1}$) and H$_2$O$_2$ (5 mM). In order to investigate the role of heme-containing enzymes or other active cell processes on iodide accumulation, a parallel assay was performed with the addition of sodium azide (Na$_3$N$_3$, Na$_3$N$_5$ (10 mM) was added to the cell suspension to inhibit enzymatic activities before addition of I$^{-}$ and H$_2$O$_2$. Cell suspensions were then incubated at room temperature in the dark for 24 h, after which aliquots were collected for the determination of $^{125}$I activity and cell density as described above.

Iodide desorption. A procedure similar to that of MacLean et al. (30), with slight modifications, was performed to determine if iodide desorption could occur after accumulation. After iodide accumulation at pH 4 to 9 (described above), cells were collected via centrifugation (16,000 × g for 20 min at 4°C), suspended in M9 medium containing 10% Na$_2$O$_2$ and incubated with continuous shaking (150 rpm) for 2 h. Aliquots were then collected for the determination of $^{125}$I$^{-}$ activity and cell density as described above.

Statistical analyses. Analyses of variance (ANOVA) were performed to evaluate the significant differences in iodide-accumulating abilities as a function of iodide concentrations, as well as the presence versus absence of chloride ions. Tukey’s post hoc tests were used to compare means of values for iodide-accumulating abilities from significant (P < 0.05) ANOVA test results. The statistical analyses were performed using SPSS 16.0 (SPSS Institute, Inc.).

RESULTS

Isolation and phylogenetic analysis of F-area soil bacteria. A total of 325 aerobic microbes were directly isolated from subsurface sediments of the F area at the Savannah River Site (Fig. 1). Of these isolates, 32% were cultured from sandy/clay sediments of an aquifer (sites FAW-1 and FAW-4, 13.1 to 25.9 m below the surface) and 29% and 39% were cultured from the dark, organic rich sediments of the seep zone (site FSP-07), 0.15 to 1 m and 1 to 1.2 m below the surface, respectively. Analysis of the 16S rRNA gene revealed that the isolates were members of four phyla within the domain Bacteria. The majority of isolates (55%) were classified as Proteobacteria.
followed by Firmicutes (20%), Actinobacteria (17%), and Bacteroidetes (8%). No obvious trends relating the phylogeny of the isolates to their environmental source (seep zone versus sand/clay aquifer or depth of the sediment) or the isolation method employed (pyrophosphate versus sonication or gellan gum versus noble agar) were observed (data not shown).

A yellow coloration was noted on the tubes of 2/24 enrichment cultures (8 sediment sources, with 3 enrichment conditions for each culture; see Materials and Methods) after 22 weeks of incubation in the dark. A strong I₂ smell was also detected after the caps of the discolored conical tubes were opened, indicating the transformation of I⁻ to I₂ (8). The two enrichment cultures that exhibited yellow coloration were derived from the seep zone (FSP-07) sediments collected 0.15 to 1 and 1 to 1.2 m below the surface, and both were incubated in dH₂O with 1 mM I⁻. Isolation efforts from the 24 enrichment cultures yielded 29 distinct morphotypes. Analysis of 16S rRNA gene sequences revealed that these 29 strains were members of the same 4 phyla, as the isolates recovered directly from sediments. The majority of isolates from the enrichment cultures (76%) were classified as Proteobacteria, followed by Actinobacteria (14%), Bacteroidetes (7%), and Firmicutes (3%).

Screening for iodide-accumulating strains. Forty-two strains, representing the phylogenetic diversity of isolates from F-area soils and enrichment cultures, were screened for their ability to represent the phylogenetic diversity of isolates from F-area A. troitsensis. A yellow coloration was noted on the tubes of 2/24 enrichment cultures; see Materials and Methods) after 22 incubations.

Table 1. Iodide accumulation by F-area bacterial isolates and two known IAB isolates.

<table>
<thead>
<tr>
<th>Bacterial isolate(s)</th>
<th>Without H₂O₂</th>
<th>With H₂O₂ added at 4 h</th>
<th>With H₂O₂ added at 24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cell activity (cpm)</td>
<td>Accumulation (%)</td>
<td>Cell activity (Bq/ml)</td>
</tr>
<tr>
<td>F. aggregans NBRC15975&lt;sup&gt;a&lt;/sup&gt;</td>
<td>726</td>
<td>2,410</td>
<td>637</td>
</tr>
<tr>
<td>A. troitsensis JCM11736&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10,170</td>
<td>25,482</td>
<td>10,365</td>
</tr>
<tr>
<td>FA-30</td>
<td>105</td>
<td>0.383</td>
<td>120</td>
</tr>
<tr>
<td>FA-2C-B*</td>
<td>87</td>
<td>0.357</td>
<td>101</td>
</tr>
<tr>
<td>FA-191</td>
<td>27</td>
<td>0.099</td>
<td>40</td>
</tr>
<tr>
<td>Other strains&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31 (±18)</td>
<td>0.143 (±0.087)</td>
<td>26 (±13)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Activity associated with cells washed 3 times following incubation of cells in the presence of 125I⁻ for 24, 28, or 56 h. H₂O₂ was added after 24 h for the 28- and 56-h incubations.

<sup>b</sup> (Number of cpmp 125I in cell pellet after incubation/number of cpmp 125I in supernatant after incubation) × 100.

<sup>c</sup> Marine strains previously identified as IAB (6).

<sup>d</sup> Average values ± SD for the 133 strains that did not consistently accumulate iodide.

A second method, in which bacteria were grown in liquid medium containing 125I⁻, was used to assess iodide accumulation among 139 of the F-area isolates (the 42 strains initially screened by autoradiographic analysis of colony material and 97 additional strains) as well as the two I⁻-accumulating marine bacterial strains, the F. aggregans strain and the A. troitsensis strain. With the use of this method, nine of the F-area strains exhibited the potential to accumulate iodide. However, upon reexamination using the same procedure with 3 additional washes, only one F-area strain (FA-30) and the marine strains consistently exhibited appreciable 125I⁻-accumulating abilities (Table 1).

It has been reported that H₂O₂ plays a role in iodide accumulation and oxidation by marine bacteria (6, 18); thus, iodide accumulation was assessed for the same 139 strains and 2 marine IAB strains in liquid cultures as described above, except that H₂O₂ was added to the cultures either 4 or 24 h prior to harvesting of the cells. H₂O₂ addition resulted in greater iodide accumulation for FA-30 and the two marine strains and enabled the identification of two additional iodide-accumulating strains, FA-2C-B* and FA-191 (Table 1). In each of these strains, iodide accumulation was greater in cells that had been exposed to H₂O₂ for a longer period of time (24 versus 4 h). However, the three IAB strains isolated from the F area, FA-30, FA-2C-B*, and FA-191, exhibited an iodide-specific accumulation that was 2 orders of magnitude less than that observed for the marine IAB strains, the F. aggregans and A. troitsensis strains (Table 1). Among the F-area isolates, the only strain that exhibited an iodide accumulation phenotype...
with both the plate and the liquid culture assays was FA-30 (among the F-area isolates, FA-30 also exhibited the strongest $^{125}\text{I}$ accumulation phenotype with the use of both assays).

Phylogenic analysis of the partial 16S rRNA genes (750 to 900 bp) from FA-30, FA-2C-B*, and FA-191 revealed that these strains were members of the Actinobacteria, Firmicutes, and Proteobacteria phyla, respectively. FA-30 was most closely related to various *Streptomyces* and *Kitasatospora* species (16S rRNA gene similarity, 94%), FA-2C-B* was closely related to *Bacillus mycoides* L2S8 (EU221418; 98% similarity), and FA-191 was most closely related to various *Ralstonia* and *Cupriavidus* species (97 to 98% similarity). FA-191 and FA-30 were cultured from seep zone (FSP-07) sediments, 0.15 to 1 and 1 to 1.2 m below the surface, respectively, whereas strain FA-2C-B* was isolated from one of the enrichment cultures. No IAB were found among the isolates obtained from the deeper, oligotrophic sandy/clay aquifer material.

**Impact of chloride on iodide accumulation.** To evaluate whether Cl$^-$ present in M9 medium could inhibit iodide accumulation by the F-area IAB, cells of FA-30, FA-2C-B*, and FA-191 were incubated in medium with and without added chloride (27 mM total Cl$^-$ concentration, added as 8.56 mM NaCl, 18.70 mM NH$_4$Cl, and 0.1 mM CaCl$_2$). Strains FA-30 and FA-2C-B* accumulated more iodide in the presence of chloride ($P < 0.05$), whereas chloride did not have a significant effect on I$^-$ accumulation in strain FA-191 (Fig. 3).

**Impact of pH and sodium azide on iodide accumulation.** The iodine-accumulating ability of strains FA-30, FA-2C-B*, and FA-191 in liquid cultures grown in pH-adjusted medium (pH 4 to 9) containing iodide (0.1 μM) was evaluated. Between pH 4 and pH 6, there were significant decreases in cellular iodine content in strains FA-30 and FA-191 as the pH increased (Fig. 4). Above pH 6, the cellular iodine content was low, <0.1 μg/g dry cell weight, and either gradually decreased with increasing pH (FA-30) or remained at a constant low level with increasing pH (FA-191). A relationship between pH and iodide accumulation was not observed for FA-2C-B* (data not shown).

Strains FA-30 and FA-191 were tested in a parallel pH assay where sodium azide (NaN$_3$; 10 mM) was included. Both strains exhibited lower I$^-$ accumulation when incubated with NaN$_3$, particularly below pH 6.0 (Fig. 4). At pH 4.0, cells of FA-191 and FA-30 accumulated 40% and 90% less I$^-$, respectively, in the presence of NaN$_3$. At pH levels of >6.0, I$^-$ accumulation was essentially unaffected by NaN$_3$ (it should be noted, however, that the cellular iodine content of cells incubated in medium with a pH value of >6.0 was barely above background levels).

**Desorption of iodine from IAB.** Desorption experiments were conducted with strains FA-30 and FA-191 to evaluate the nature of the interaction between iodide and the cells. Desorption of iodine as a function of pH was not observed in cells of FA-30 or FA-191 (only ±10% variation in the cellular iodine content of the cells before and after the desorption step) (data not shown).

**DISCUSSION**

Mobility of radioactive iodine in the subsurface environment is affected by the iodine’s chemical speciation and interactions with soil constituents, including minerals, organic matter, and microorganisms. Based on thermodynamic principles, the main iodine species in SRS F-area groundwater and sediments...
should be iodide, which is thought to have the highest subsurface mobility, i.e., to be least sorbed or taken up by sediments, compared to iodate and organo-iodine (33, 40, 43). However, organo-iodine has been found to contribute a significant fraction (up to 25%) of total iodine in groundwater from the F area (43, 52). The extent to which iodide binds to or is incorporated within bacterial cells in oxic subsurface aquifers has not previously been investigated; thus, we tested the iodide-acculumating ability of 139 phylogenetically distinct bacterial isolates from F-area sediments.

Three aerobic bacterial strains from the F area, FA-30, FA-2C-B*, and FA-191, were shown to accumulate iodide. Iodide accumulation by these three strains was significantly different from the background value observed for 136 other strains evaluated (consistently 2 to 10 times higher), and in the case of FA-30, this result was validated by two different approaches, (i) autoradiography of cell material grown on 125I-agar plates and (ii) liquid scintillation analysis of washed cell material that had been grown in liquid medium containing 125I -. Compared to that of IAB isolated from marine sources, however, the iodide-accumulating capacity of the F-area strains was quite small. Amachi et al. (6) reported accumulation of 80 to 90% of total iodide by various marine strains, including F. aggregans NBRC15975 and A. troitsensis JCM11736, when these strains were incubated in the presence of 0.1 μM I-. Under our experimental conditions (i.e., much more rigorous cell washing and higher pH [7.0 versus 6.0] than those used by Amachi et al.), F. aggregans and A. troitsensis accumulated 2 to 50% total iodide (0.1 μM), whereas F-area strains FA-30, FA-2C-B*, and FA-191 accumulated 0.2 to 1.5% of the total iodide (0.1 μM).

Another difference between the IAB isolated from F-area sediments and the IAB previously identified from marine sediments (6) is that the latter microorganisms were classified exclusively within the Flavobacteriaceae family of the Bacteroidetes, whereas the F-area IAB represented three phyla, Acetinobacteria (FA-30, most closely related to Streptomyces and Kirtasatospora spp.), Firmicutes (FA-2C-B*, a putative Bacillus sp.), and Betaproteobacteria (FA-191, closely related to Ralstonia/Cupriavidus spp.). As these three phyla are often dominant members of terrestrial soil microbial communities (they comprise >90% of the isolates obtained from F-area sediments) whereas Bacteroidetes spp. are more common in marine environments, it appears that iodine accumulation is not restricted to a distinct phylogenetic lineage(s) but rather is manifested in select bacterial taxa adapted to their respective environments.

There are two mechanisms that provide the most parsimonious explanation for iodide accumulation by bacteria. One is electrostatic adsorption of I- by positively charged functional groups, such as amines from proteins and peptides, present on the surface of the cell. For example, MacLean et al. (30) used Bacillus subtilis to model iodide-bacterium adsorption through electrostatic interaction and found that readily adsorbed iodide at pH values of <4 and that iodide could then be quickly desorbed (~2 h) by raising the pH to 7.0. In that study, an aqueous solution of diluted HNO3 was used as the experimental wash solution, precluding other halide ions from competing with iodide for electrostatic interaction with bacterial cells. In the present study, the three terrestrial IAB strains were incubated and maintained in a M9 minimal medium solution that contained ~27 mM chloride ions, which is ~2.7 × 105 times higher than the iodide concentration (0.1 μM) present in the experimental assays. Iodide accumulation by FA-30, FA-2C-B*, and FA-191 was not inhibited by chloride ions (27 mM) (Fig. 3). Indeed, at low iodide concentrations (0.1 μM), I- accumulation by FA-30 and FA-2C-B* was greater in M9 medium than in chloride-deficient M9 medium (we note that there was a difference in ionic strength between the standard M9 medium and chloride-deficient M9 medium used in this experiment). Furthermore, pH-dependent desorption tests failed to reveal reversibility of the interaction between iodide and cells of FA-30 and FA-191. These results indicate that I- accumulation by the F-area terrestrial strains was not due to electrostatic surface adsorption when pH levels were ≥4.0.

The second possible mechanism for adsorption between iodide and cellular constituents is electrophilic substitution resulting in iodination of organic molecules. Strong electrophiles such as HOI/I2/I3- produced by biotic or abiotic processes from I- could attack aromatic rings or other organic moieties of the cell and replace -H with -I to form a stable organo-iodine bond (12, 31, 44). Given the high concentration of organic matter in a bacterial culture, it is reasonable to expect that the oxidized I species, i.e., HOI/I2/I3-, could react with it. Several observations signify that the iodide accumulation phenotype exhibited by F-area strains FA-30, FA-2C-B*, and FA-191 likely proceeds via this mechanism. First, the iodide-accumulating abilities from FA-30, FA-2C-B*, FA-191 were all facilitated by the addition of H2O2. Since H2O2 is a strong oxidant, I- is readily oxidized to I2/I3-, without enzymatic catalysis, especially under acidic conditions. However, at the near-neutral pH values (pH 6 to 7) that were used during the screening portion of our study, H2O2 consistently stimulated iodide accumulation in just 5 of 141 strains tested (including the two marine strains). Thus, it is unlikely that H2O2, itself acting as an oxidizing agent, transformed I- into a highly reactive species capable of binding nonspecifically to cell material (though at lower pH values [<4 to 5], this mechanism could be more relevant). Alternatively, haloperoxidases, which are found in animals, plants (including algae), fungi, and bacteria, utilize H2O2 as a cosubstrate and are considered the primary enzyme responsible for nonspecific halogenation of organic substrates in nature (11, 45, 46). NaN3 significantly inhibited I- accumulation in the F-area strains, implying the involvement of an enzymatic driven process, such as an active transport system or heme haloperoxidases, which are NaN3 sensitive (45). The F-area strains also exhibited increased I- accumulation with decreasing pH, characteristic of many haloperoxidases, which exhibit optimal activity under acidic conditions (14, 36, 38). Although our data support electrophilic substitution or internalization rather than electrostatic adsorption to the cell surface, the precise nature and location of bacterially bound I- in these terrestrial strains and the accumulation mechanism remain to be determined.

Background concentrations of stable I in F-area groundwater range from 10 to 100 nM, and plume concentrations of 129I are typically ~60 pCi/liter (2.4 nM) at “hot spots” but can reach levels of >900 pCi/liter in the organic-rich seep zone. Our results demonstrate that the majority (98%) of aerobic bacteria isolated from F-area sediments do not accumulate iodide (<0.2% accumulation) at ambient I- concentrations (0.1 μM), and the three IAB strains that were identified accu-
We are currently examining that possibility through a microbial species yet to be discovered from the F area or fungi may contribute appreciably to the organo-iodine pool over centuries to decades (10). Carefully controlled, long-term cultivation of IAB, such as Roseovarius sp., could contribute appreciably to the organo-iodine pool (25), whose biomass typically accounts for 1% or less of sedimentary organic matter (e.g., humic or fulvic acids). Even when bacteria, whose biomass typically accounts for 1% or less of sedimentary organic matter (such as the F-area seep zone sediments) (25), incorporate less than 2% of iodine into their cells, this process could contribute appreciably to the organo-iodine pool over the long term. Similar mechanisms have been proposed to explain chloride retention in forest soils and peat bogs over decades to centuries (10). Carefully controlled, long-term column studies are needed to examine the extent that IAB, such as those identified in this study, affect the speciation and mobility of I in F-area seep zone sediments. Finally, uncultivated bacterial species yet to be discovered from the F area or fungi may be capable of much higher levels of iodide accumulation (9). We are currently examining that possibility through a microradiography-fluorescence in situ hybridization (MAR-FISH) approach (29).

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