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Verrucomicrobia Are Candidates for Polysaccharide-Degrading Bacterioplankton in an Arctic Fjord of Svalbard

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In Arctic marine bacterial communities, members of the phylum *Verrucomicrobia* are consistently detected, although not typically abundant, in 16S rRNA gene clone libraries and pyrotag surveys of the marine water column and in sediments. In an Arctic fjord (Smeerenburgfjord) of Svalbard, members of the *Verrucomicrobia*, together with *Flavobacteria* and smaller proportions of *Alpha-* and *Gammaproteobacteria*, constituted the most frequently detected bacterioplankton community members in 16S rRNA gene-based clone library analyses of the water column. Parallel measurements in the water column of the activities of six endo-acting polysaccharide hydrolases showed that chondroitin sulfate, laminarin, and xylan hydrolysis accounted for most of the activity. Several *Verrucomicrobia* water column phylotypes were affiliated with previously sequenced, glycoside hydrolase-rich genomes of individual *Verrucomicrobia* cells that bound fluorescently labeled laminarin and xylan and therefore constituted candidates for laminarin and xylan hydrolysis. In sediments, the bacterial community was dominated by different lineages of *Verrucomicrobia*, *Bacteroidetes*, and *Proteobacteria* but also included members of multiple phylum-level lineages not observed in the water column. This community hydrolyzed laminarin, xylan, chondroitin sulfate, and three additional polysaccharide substrates at high rates. Comparisons with data from the same fjord in the previous summer showed that the bacterial community in Smeerenburgfjord changed in composition, most conspicuously in the changing detection frequency of *Verrucomicrobia* in the water column. Nonetheless, in both years the community hydrolyzed the same polysaccharide substrates.

A major fraction of heterotrophic activity in the ocean is carried out by marine microbial communities (1). These communities use substrates such as high-molecular-weight carbohydrates (polysaccharides), which constitute a large percentage of phytoplankton biomass, particulate organic matter, and dissolved organic matter (DOM) in the ocean (2–5) and therefore fuel a considerable proportion of heterotrophic activity. To initiate the degradation of complex organic matter, bacteria must initially hydrolyze high-molecular-weight substrates by using extracellular enzymes in order to yield substrates sufficiently small to be taken into the microbial cell for further processing (6).

The substrate spectrum and the rates of polysaccharide-hydrolyzing extracellular enzymes produced by microbial communities vary by location and depth in the ocean (7–9, 83) and can change through processes such as aggregate formation (10). Bacterial groups differ in their enzymatic spectra, as shown through field studies (11, 12), as well as genomic and microbiological investigation (13–16). Microbial communities involved in polysaccharide degradation in the water column include heterotrophic *Gammaproteobacteria*, fast-growing opportunists that can adapt quickly to changing substrate availability (17) and that include isolates that grow on rich standard media (18), as well as members of the *Bacteroidetes* phylum, which are consistently abundant in phytoplankton-amended incubations and enrichment experiments, as well as field studies of natural phytoplankton blooms (14, 19–22). Among the *Alphaproteobacteria*, members of the metabolically versatile *Roseobacter* cluster are particularly abundant in nearshore waters and often occur in association with algae, phytoplankton, and particles (23, 24).

In organic-rich marine sediments, the phylogenetic composition and the physiological capabilities of benthic bacteria shift toward a differently structured community that uses a wide range of anaerobic carbon degradation pathways, working in concert to degrade complex organic substrates. Sediment-dwelling bacterial populations include sulfate-, sulfur-, and metal-reducing *Deltaproteobacteria* specializing in the oxidation of low-molecular-weight organic compounds (25), bacteria of the phylum *Chloroflexi*, whose relatively few cultured isolates perform halorespiration or fermentation (26), and members of the phylum *Planctomycetes*, whose cultured representatives include heterotrophic aerobes, anaerobic fermenters, and anaerobic ammonia oxidizers (27). Interestingly, some *Chloroflexi* and *Planctomycetes* are specialists in glycoside hydrolysis (13, 28).

Among the bacterial phyla that appear consistently in molecular surveys of Arctic and temperate marine environments, the *Verrucomicrobia* are a widespread minority component in clone libraries from the marine water column (29–31) and sediments (32–37). The free-living, epibiotic, and symbiotic lifestyles of *Verrucomicrobia*, their predominantly heterotrophic, carbohydrate-degrading metabolism, and their wide-ranging occurrence in different freshwater and marine habitats suggest that these bacteria play significant ecophysiological and biogeochemical roles that require closer examination. The *Verrucomicrobia* (38) include the family *Verrucomicrobiaceae*—congruent with intraphylum subdivision 1 (39)—which harbors the carbohydrate-degrading genus *Verrucomicrobium sensu stricto*, defined by its possession of cytoplasm-containing cell wall extensions, or prosthecae (40), the

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prosthecate carbohydrate degraders within the genus *Prosthecobacter*, isolated from freshwater habitats (41), and several marine heterotrophic genera and species that have been isolated from marine sediment, sponges, algae, and seawater (42, 43). The class *Spartobacteria* (subdivision 2) contains the brackish water taxon "*Candidatus* Spartobacteria baltica," represented by a genome assembled from an environmental metagenome in Baltic seawater (44), and the heterotrophic soil isolate *Chthoniobacter flavus* and multiple related isolates from the same pasture soil (45); soil isolates from the same source also constitute the so-far unnamed subdivision 3 (46). Subdivision 4 includes the obligate anaerobe *Opitutus terrae* and multiple phylotypes from paddy soil (47) and the aerobic seawater and coral-associated species *Coraliomargarita akajimensis*, as well as the facultative anaerobe *Alterococcus agarolyticus* (48) and the highly unusual epixenosomes; these bacterial epibionts of the marine ciliate *Euplotidium* undergo a complex life cycle, possess microtubulins, and form an extrusive apparatus that ejects portions of the bacterial cytoplasm and genome tethered to a 40-μm-long tube (49). Subdivision 5 is so far represented by sediment phylotypes and remains uncultured (39). The *Verrucomicrobia* also contain extremophiles: a monophyletic group of aerobic, thermophilic, and acidophilic methanotrophs termed *Verrucomicrobia* thermoacidophilic methanotrophs (VTAM) has been isolated by three different groups independently and simultaneously from acidic hot springs (50–52).

Given this unusual diversity in habitats and lifestyles, the contribution and function of *Verrucomicrobia* within marine bacterioplankton communities emerges as an open research question. In this study, a 16S rRNA gene-based bacterial community survey of the water column and sediments in an Arctic fjord (Smeerenburgfjord) of Svalbard yielded *Verrucomicrobia* as the dominant clone group in the deep water column and provided the opportunity for a parallel survey of hydrolytic enzyme activities in water column and sediment to investigate whether the presence of *Verrucomicrobia* changed the polysaccharide hydrolysis patterns in the marine environment.

MATERIALS AND METHODS

Study site and sample collection. Seawater and sediment samples were collected at Station J, Smeerenburgfjord, 79.42°N, 11.05°W, where the water column depth was 211 m (53) on 15 August 2008. The surface and bottom water temperatures were 3.3°C and 1.6°C, respectively, and the sediment temperature was 1.2°C. The upper 2 cm of these sediments is soft and brown, grading to darker brown and black at deeper depths. Brittle stars and worm tubes are frequently found at this site (52). Surface water, collected at a depth of ca. 2 m, and bottom water, collected at a depth of 205 m, were transferred from a 10-liter Niskin bottle to 5-liter carboys immediately after sampling. Water was maintained at ca. 4°C in the 5-liter carboys until the ship reached the field station approximately 12 h post-sample collection. The water samples for clone library analysis (750 ml) were filtered with a hand pump through a Millipore Durapore 0.22-μm-pore-size filter approximately 3 h after arrival at the field station; the water was maintained at 3.5°C at the field station. The filters for DNA extraction and sequencing were transferred to sterile plastic centrifuge tubes and frozen immediately at −80°C. Measurements of enzyme activities were initiated once the control seawater had been autoclaved using equipment in the shore lab, approximately 18 h after sampling.

Sediment was collected via a Haps core. Sediments for enzyme activity measurements were transferred to gas-tight bags, which were maintained at ca. 4°C until experiments were initiated at the shore lab. (The subsurface sediment bag was closed without headspace; the surficial sediment bag was closed with generous headspace.) For clone library analysis, surface sediments (25 ml, from the upper 0 to 2 cm; light brown oxidized sediment) were transferred by clean spatula into a sterile 50-ml centrifuge tube. Brown/black sediments (25 ml) with a faint smell of H_2S from the sulfate-reducing zone at 3 to 9 cm of depth (53) were also transferred into a 50-ml centrifuge tube, and both tubes were frozen immediately at −20°C. After the ship reached the shore lab (ca. 12 h), sediment samples for clone library analysis were transferred to a −80°C freezer. All samples for clone library analysis were kept frozen (dry ice or −80°C freezer) until processing.

Measurements of enzyme activities. Extracellular enzyme activities were measured using fluorescently labeled polysaccharides as the substrates. Six substrates were selected that span a range of structural complexities for a focused study of enzyme activities within the polysaccharides, a broad class of biomolecules quantitatively significant for marine carbon metabolism. These polysaccharides were pullulan [α(1,6)-linked maltotriose (glucose)], laminarin [β(1,3 glucose)], xylan (xylose), fucoidan (sulfated fucose), arabinogalactan (arabinose and galactose), and chondroitin sulfate (sulfated *n*-acetyl galactosamine and glucuronic acid), which were all purchased from Sigma or Fluka. The polysaccharides were labeled with fluoresceinamine (isomer II; Sigma) and characterized as described previously (54, 55). These polysaccharides are constituents of marine algae (2), and the activities of enzymes hydrolyzing these substrates have been demonstrated in marine bacteria (16, 56–58). Genes encoding enzymes that hydrolyze these polysaccharides have also been identified in the genomes of fully sequenced marine bacteria (13–16).

Enzymatic activities were measured in seawater and sediments in experiments initiated at the shore lab in Ny Ålesund, Svalbard, ca. 18 h after sample collection. All experiments (surface and bottom water, as well as surface and subsurface sediments) were incubated at 3.5°C (the temperature of the cold room available to us) in the dark. Each 60-ml portion of seawater received a single substrate at a concentration of 3.5 μmol liter^{-1} monomer-equivalent polysaccharide. This approach ensures the same carbon concentration in every incubation, irrespective of differences in molecular weight of polysaccharides. The 60-ml portion was then divided into three 20-ml replicates. Killed controls consisted of autoclaved seawater, with substrate added after seawater had cooled. Since the equipment necessary to monitor substrate hydrolysis was not available in Ny Ålesund, sampling time points were selected based on previous work in surface waters at this site (59). Incubations were sampled after 0, 3, 7, 10, and 15 days. At each time point, ca. 1.5 ml of sample was removed from each vial, filtered through a 0.2-μm-pore-size surfactant-free cellulose acetate filter, and then stored frozen. Water column hydrolysis rates have been reported previously (60).

Measurements of enzyme activities in sediments were made by homogenizing sediments (0- to 2-cm surface layer; 3- to 9-cm subsurface layer) thoroughly and then transferring replicate 20-ml portions of sediments to 50-ml centrifuge tubes for individual incubations. The deep sediments were gassed with N_2 during processing and stored in N_2-filled gastight bags. Since analytical equipment necessary to measure substrate signal and monitor substrate transformations was not available in Ny Ålesund, substrate addition levels and the time course of incubation were based on previous experience with sediments from this fjord (7, 59). Each substrate was added to triplicate tubes. For surface sediments, the substrate addition levels were 3.5 μmol per tube (equivalent to 175 μmol liter^{-1} of sediment) with an additional set of tubes at 7.0 μm per tube for fucoidan. Substrate addition levels for deep sediments were 7.0 μmol per tube, with additional sets at 14 μmol per tube for pullulan, fucoidan, and arabinogalactan. Controls consisted of a 1:1 slurry of seawater and sediment that was autoclaved and cooled prior to substrate addition. At each time point (0, 24, 48, 72, and 120 h), tubes were centrifuged in a refrigerated centrifuge to obtain a small amount of pore water, which was filtered through a 0.2-μm-pore-size filter and frozen until analysis. Samples were rehomogenized, and deep sediment samples were gassed again with N_2 and incubated at 3.5°C in the dark.

Seawater and pore water samples were shipped frozen to the home lab, where they were thawed and injected via autosampler on a gel permeation chromatography–high-pressure liquid chromatography (HPLC) system with fluorescent detection as described previously (54, 55). Hydrolysis rates were calculated from the molecular weight distribution of substrates as they were progressively hydrolyzed (54, 55). The rates reported here are the maximum rates observed over the time course of incubation.

The rates we measured are potential rates, since they were measured with an externally added substrate that competes with naturally occurring substrates of unknown concentration for enzyme active sites. The concentration of carbohydrates added to seawater likely doubles the concentration of total dissolved carbohydrates, assuming a dissolved organic carbon (DOC) concentration of ca. 80 μM and a carbohydrate concentration of 25% of DOC (3); data from other fjords of Svalbard suggest that our assumptions about DOC concentrations are reasonable (61, 62). Sedimentary carbohydrate additions represent approximately a 1- to 2-fold increase in pore water total dissolved carbohydrates (53); particulate carbohydrates, which fuel the dissolved carbohydrate pool, are not included in this calculation. These carbohydrate additions likely represent saturation conditions, which would make the hydrolysis rates zero order (independent of substrate concentration) with respect to enzyme kinetics.

DNA extraction, PCR amplification, cloning, and sequencing of 16S rRNA genes. DNA from filter samples and sediments was extracted according to standard protocols (see the supplemental material). Bacterial 16S rRNA genes were amplified by PCR using the bacterial primers 8f and 1492r (63). Each 25-μl PCR mixture contained 1 μl DNA template, 0.3 μl primer solution (100 pmol μl^{-1}), 1 μl bovine serum albumin (10 mg ml^{-1}), 2.5 μl 10× PCR buffer (Fast buffer 1; Promega, Madison, WI), 4 μl deoxynucleoside triphosphate (dNTP) (2.5 mM each), 16.075 μl sterile RNA-free water (Qiagen, Valencia, CA), and 0.125 μl (0.625 U) Speed Star *Taq* polymerase (Promega). PCR amplification was performed in a Bio-Rad i-Cycler. The conditions were as follows: an initial denaturation at 94°C for 2 min, followed by 25 cycles, each consisting of 10 s of denaturation at 98°C, 15 s at a primer annealing temperature of 58°C, and 20 s of elongation at 72°C, plus a final cycle of 72°C for 10 min. The PCR products were visualized on a 1.5% agarose gel using an ethidium bromide stain. Blank extractions, serving as controls for contamination during DNA extraction, yielded no visible products. All PCR products were purified using an MoBio PCR cleanup kit and cloned using the TOPO TA PCR cloning kit before being transformed into *Escherichia coli* according to the manufacturer's protocols (Invitrogen, San Diego, CA). Near-complete 16S rRNA gene sequences were obtained from Genewiz, Inc., which directly amplified clone colony DNA by using a nonspecific amplification procedure, followed by a single-primer sequencing reaction using either 8f/1492r or M13 vector primers.

Sequence analysis. Sequence reads were checked and assembled into contigs using Sequencher (Gene Codes Corp.). Assembled contigs were initially screened for chimeras using Bellerophon 3 (64) using the default settings. Three percent operational taxonomic unit (OUT) groupings were then defined using DOTUR and provided the basis for rarefaction curves (65). Representative sequences for each 3% OTU were then aligned in ARB, with final adjustments by eye, to the closest relatives in the SILVA v.95 REF database (66). After the addition of representative phylotypes, all sequences that appeared in the final tree were checked for chimeras by running them through Bellerophon 3 (64), using a window size of 200 bp and a divergence ratio cutoff of 1.08. Selected sequences flagged as chimeras were then double checked using Pintail and excluded from the data set. The alignment used for phylogeny consisted of sequence regions (ca. 1,200 nucleotides) that passed a 40% conservation filter using the filter tool in ARB. The bacterial phylogenies were estimated using the neighbor-joining algorithm (67), a maximum likelihood-estimated model of evolution, and a gamma-corrected rate distribution, in MEGA 4.0 (68). Branch support was estimated with a 2,000-replicate interior branch (IB) test (68), which uses a hypothesis-testing approach to determine the probability that a particular interior branch has a length greater than 0 (i.e., that the node in question should not be collapsed into a polytomy). Values greater than 95% should be considered strong support for a particular node (69). In phylogenetic trees of this study, bacterial clades were defined using the most-basal marker sequences from published phylogenies and named and referenced in accordance with published phylogenies. Bacterial clades showed long stem branches relative to internal branch lengths and were confirmed by high interior branch support.

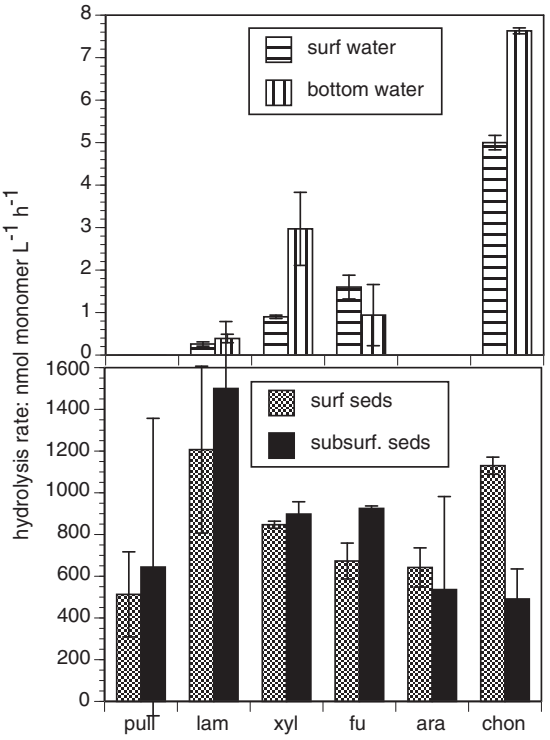

FIG 1 Hydrolysis rates of polysaccharides in surface (surf) water (horizontal-striped bars) and bottom water (vertical-striped bars) samples (A) and in homogenized sediments (seds) from depths of 0 to 2 cm (gray bars) and 3 to 9 cm (black bars) (B). The substrates are pullulan (pull), laminarin (lam), xylan (xyl), fucoidan (fu), arabinogalactan (ara), and chondroitin sulfate (chon). Error bars show the standard deviations of triplicate incubations. Water column hydrolysis rates were replotted from reference 60.

Nucleotide sequence accession numbers. The nucleotide sequences discussed in this study can be found under GenBank accession no. KJ566220 to KJ566304 (see Table S1 in the supplemental material).

RESULTS AND DISCUSSION

Enzymatic hydrolysis rates. In surface as well as bottom waters, chondroitin, fucoidan, xylan, and laminarin were hydrolyzed over a 15-day time course; arabinogalactan and pullulan were not (Fig. 1). Hydrolysis rates ranged from 0.26 to 7.6 nmol monomer $liter^{-1}$ h^{-1} and decreased in the order chondroitin > fucoidan > xylan > laminarin in surface waters and chondroitin > xylan > fucoidan > laminarin in bottom waters; in both cases, chondroitin accounted for over half of the measured activities. In surface and subsurface sediments, in contrast, all substrates were hydrolyzed at mostly overlapping rates ranging from 419 to 1,500 nmol monomer $liter^{-1}$ h^{-1}; neither chondroitin nor another substrate dominated the overall hydrolytic activities exclusively.

Bacterial community composition. The 16S rRNA gene clone library for the water column bacterial community was dominated by *Verrucomicrobia*, *Bacteroidetes*, and *Gammaproteobacteria*; *Al-*

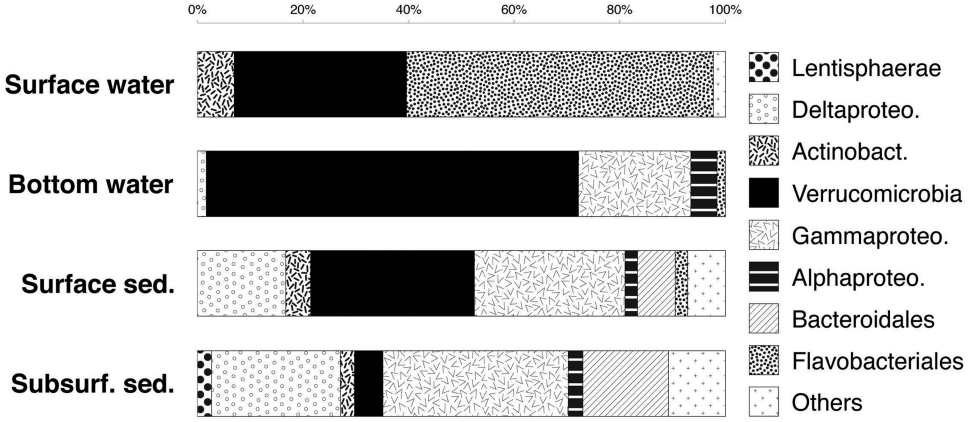

FIG 2 Percent representation of bacterial phylum- and subphylum-level groups in 16S rRNA gene clone libraries from water column and sediment samples in Smeerenburg Fjord, Svalbard, obtained in summer 2008. The total clone numbers are 43 in surface water, 62 in deep water, 44 in surface sediment, and 44 in subsurface sediment (see Table S1 in the supplemental material).

phaproteobacteria were predominantly found in the bottom water. *Deltaproteobacteria* appeared as a dominant clone group in the sediment samples, and several additional phyla were represented in the sediments by a few clones each (Fig. 2). The proteobacterial results and most phylum-level bacterial groups are discussed in detail in the supplemental material.

In the surface and bottom water samples, *Verrucomicrobia* accounted for nearly 33% and 68% of the clone library, respectively—far more than previously obtained in water column clone libraries obtained from samples at the same location in summer 2007 (ca. 3%). *Verrucomicrobia* were also highly abundant in the surface sediment, where they constitute 30% of the bacterial clone library; their clone library representation dropped to near 8% in the subsurface sediment (Fig. 2). The *Verrucomicrobium* phylotypes in water column and sediment were phylogenetically distinct (Fig. 3). The water column clones belonged to the family *Verrucomicrobiaceae* and were related to the marine genera *Luteolibacter*, *Roseibacillus*, *Persicirhabdus* (42), and *Haloferula* (43). These marine, heterotrophic and oxygen- or nitrate-respiring *Verrucomicrobia* form a sister group to the freshwater genera *Prosthecobacter* and *Verrucomicrobium* (41) within subdivision I of the *Verrucomicrobia*, following the classification of Hugenholtz and colleagues (39). Sediment clones belonged to the not-yet-cultured subdivision V of the *Verrucomicrobia* (39). This group contained diverse marine benthic clones (Fig. 3), encompassing the range from hydrothermal sediments to previously analyzed Svalbard sediments (32).

Fluctuating detection of *Verrucomicrobia*. Does the conspicuous appearance of *Verrucomicrobia* in the 16S rRNA gene clone libraries from Smeerenburgfjord constitute a major anomaly? Published 16S rRNA gene clone libraries, Fluorescent *in situ* hybridization (FISH) surveys and denaturing gradient gel electrophoresis (DGGE) surveys from the Arctic marine water column contain *Verrucomicrobia*, if at all, as a minority population (30, 32, 35, 36, 70). A comparison of multiple 16S rRNA gene clone libraries from polar environments showed that *Verrucomicrobia* were detectable in marine sediments and soils and in Arctic seawater but were not detectable in Antarctic seawater or sea ice (35). *Verrucomicrobia* were not reported in high-throughput sequencing surveys of the polar oceans, where *Alphaproteobacteria*, *Gammaproteobacteria*, and *Flavobacteria* dominated (71). In a comparative analysis of abundant versus rare bacterial V6-tagged 16S rRNA gene phylotypes from the Arctic Ocean, *Verrucomicrobia* accounted for approximately 3% of the tags in deep and surface water, and were assigned to the rare bacterial biosphere (72, 73). A single-cell sequencing survey of the Atlantic and Pacific water column recovered *Verrucomicrobia* in substantial proportions (6 to 8% in the mesopelagic and 20% in the surface waters of North Pacific Station ALOHA) (74). Within a 3-year survey of coastal seawater from offshore Delaware, *Verrucomicrobia* phylotypes belonged to the rarely detected phylotypes that were found at less than half of all time points; if detected, they accounted for approximately 1% of all detected phylotypes (75).

Viewed in context, these marine bacterioplankton surveys show that *Verrucomicrobia* are generally less abundant than the dominant *Proteobacteria* or *Bacteroidetes* and that specific *Verrucomicrobia* phylotypes may cycle through periods when they are detectable and others when they are not. Such a cycle may be related to the presence of specific complex substrates or phytoplankton blooms; *Veruccomicrobia* were among the organisms that responded strongly to addition of comparatively low concentrations of diatom-derived dissolved organic matter (DOM) to continuous cultures (76). From this perspective, the 2008 clone library results from Svalbard may represent a conspicuous population spike of naturally variable *Verrucomicrobia*; these fluctuations suggest the ability to exploit energy and carbon sources opportunistically.

***Verrucomicrobia* as potential glycoside hydrolysis generalists.** We propose that the Svalbard *Verrucomicrobia* phylotypes in the water column and sediment are involved in polysaccharide hydrolysis. In a previous Svalbard study, phylotypes of *Bacteroidetes* and *Gammaproteobacteria* dominated the clone library composition of the water column and sediment in summer 2007 and were considered the bacteria that are mostly responsible for complex carbohydrate degradation (36). Since then, an innovative combination of isolation and sequencing of single cells that were tagged with fluorescently labeled laminarin and xylan has called attention to the *Verrucomicrobia*. When fluorescently labeled polysaccharides were added to seawater and freshwater bacterioplankton, subsequent fluorescence-activated cell sorting and

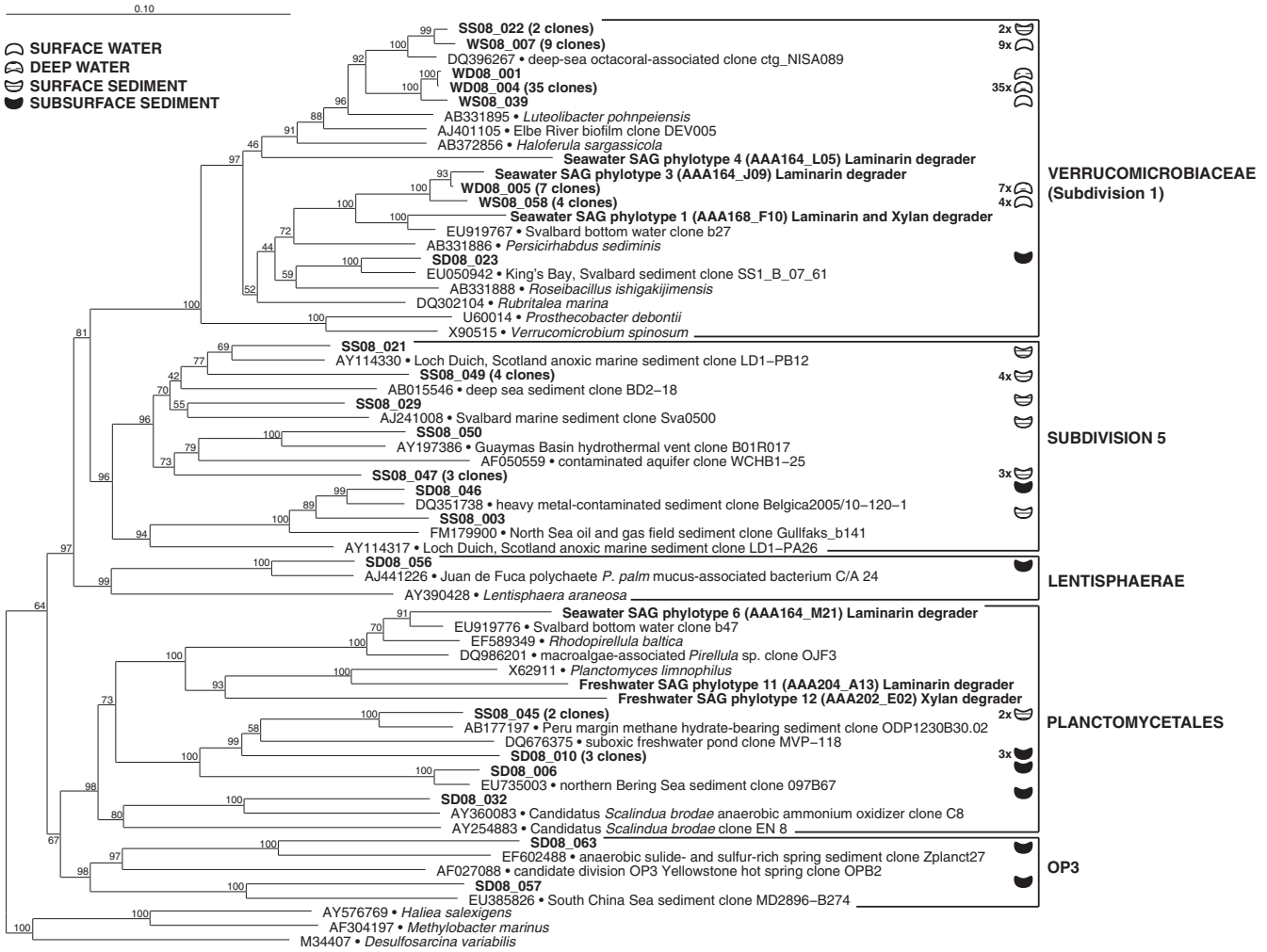

FIG 3 Neighbor-joining phylogeny of Smeerenburg Fjord *Verrucomicrobia* and related phyla *Lentisphaerae*, *Planctomycetes*, and OP3, based on an ~1,200-bp alignment of bacterial 16S rRNA gene sequences. The Svalbard phylotypes are labeled with the habitat indicator (water_surface [WS], water_deep [WD], sediment_surface [SS], and sediment_deep [SD]) followed by the clone designation and are highlighted in boldface.

sequencing of cells that were labeled with polysaccharides revealed that laminarin was almost exclusively and xylan was preferentially associated with *Verrucomicrobia*; cells identified as *Gammaproteobacteria* and *Bacteroidetes* also were labeled with xylan (77). The seawater phylotypes were mostly associated with *Verrucomicrobiaceae* (subdivision 1) and with the *Opitutaceae* (subdivision 4), whereas the freshwater phylotypes were members of subdivision 3. The specific attachment of fluorescently labeled substrates to *Verrucomicrobia* cells would precede uptake into the cell and is consistent with genome content. The amplified genome of one of these substrate-labeled cells (AAA168-F10, in subphylum I) and the published genomes of other *Verrucomicrobia* were particularly rich in glycoside hydrolase genes (77), on a par or even above the glycoside hydrolase gene frequency in genomes of *Bacteroidetes*, acknowledged specialists for the degradation of complex carbohydrates (21). Several Svalbard *Verrucomicrobiaceae* phylotypes formed a monophyletic group with single-cell amplified genomes AAA168-F10 and AAA164-J09 (Fig. 3); most Svalbard *Verrucomicrobiaceae* clones formed a lineage with substrate-labeled cell genome AAA164_L05, with *Luteolibacter* and *Haloferula* (Fig. 3). Although phylogenetic affinity *per se* should not be overinterpreted, it calls attention to the *Verrucomicrobiaceae* in Smeerenburgfjord as a potential contributor to the observed activities and spectrum of glycoside hydrolases. Interestingly, a genome rich in glycoside hydrolases was also reported for the taxon "*Candidatus* Spartobacteria baltica" within the *Spartobacteria* (44), suggesting that polysaccharide hydrolysis is widely distributed across the *Verrucomicrobia*. The polysaccharides laminarin and xylan, which attached to *Verrucomicrobiaceae* cells in single-cell sorting experiments (77) and constituted some of the inferred substrates for the hydrolases of "*Candidatus* Spartobacteria baltica" (44), accounted for 15 to 28% of the integrated hydrolysis rates for all substrates measured in Smeerenburgfjord seawater and 41 to 48% of the integrated rates for sediment samples, respectively (Fig. 1).

The question of functional redundancy. The hydrolysis rates and enzymatic capabilities of the water column and sediment bacterial communities in 2008 were similar to the rates and substrate spectrum measured at the same location 1 year previously, in summer 2007. In both cases, chondroitin sulfate was the dominant substrate hydrolyzed in the water column, followed by xylan,

laminarin, and fucoidan in changing proportions; pullulan and arabinogalactan were not hydrolyzed in the water column in either year. In the sediment, the full range of substrates, including pullulan and arabinogalactan, were hydrolyzed in 2007, as well as in 2008, although in somewhat changing proportions; pullulan and laminarin hydrolysis rates dominated the hydrolytic spectrum more strongly in 2007 than in 2008 (Fig. 1; see Fig. S4 in the supplemental material). This continuity of hydrolytic capabilities contrasted with changes in bacterial community composition. In contrast to 2007, the SAR11 lineage within the *Alphaproteobacteria* was not detected in the 2008 clone libraries, the *Bacteroidetes* in 2008 were more strongly skewed toward the surface water layer and depleted in bottom water, and the proportion of the *Verrucomicrobia* greatly increased from ca. 5% of both water column clone libraries in 2007 toward 25% to 70% in the water column clone libraries of 2008 (36).

The extent to which microbial communities that differ in composition diverge or converge in their function and the relationship of function to metabolic potential are a major focal point of investigation in microbial ecology (78–80). Detailed case studies that focus on a genetic comparison of composition and function, where function is deduced from an analysis of genetic potential of the microbial communities, show that microbial communities that differ in community composition remain capable of performing the same functions. For example, gene-based functional redundancy studies have focused on associations of bacterial communities with marine green macroalgae (81) and bacterial and archaeal symbionts of marine sponges (82). In this study, the difficulty of establishing taxon-specific hydrolytic capabilities and the possibility of unrecorded hydrolytic activities (for example, bacterial preferences for substrates other than those that were used here) preclude taxon-specific claims about functional redundancy. Establishing such taxon-specific substitutions—that the functional repertoire and gene expression pattern of a particular microbial group enable it to take the place of another group in carbohydrate hydrolysis—requires a distinct molecular toolbox, including, for example, metatranscriptomics, microautoradioactivity combined with FISH (MAR-FISH), or single-cell sequencing directly from Smeerenburgfjord samples. The Smeerenburgfjord water column would provide a suitable model system to address this issue, since the changing bacterial community as a whole maintains its observed hydrolytic capabilities.

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