Novel strains isolated from a coastal aquifer suggest a predatory role for flavobacteria
Erin C. Banning 1, Karen L. Casciotti 2 & Elizabeth B. Kujawinski 2

1 MIT/WHOI Joint Program in Oceanography/Applied Ocean Science and Engineering, Woods Hole, MA, USA; and 2 Department of Marine Chemistry and Geochemistry, Woods Hole Oceanographic Institution, Woods Hole, MA, USA

Correspondence: Erin C. Banning, 266 Woods Hole Road, MS #52, Woods Hole, MA 02543, USA. Tel.: +1 508 289 3067; fax: +1 508 457 2076; e-mail: ebanning@whoi.edu


DOI: 10.1111/j.1574-6941.2010.00897.x

Editor: Michael Wagner

Keywords
predatory bacteria; top-down regulation; Olleya; Tenacibaculum; lytic bacteria.

Abstract
Three newly isolated strains of flavobacteria from coastal aquifer sediments have been found to be predatory, lysing a range of live and pasteurized microbial prey. The three strains have been classified on the basis of 16S rRNA gene phylogeny as belonging to the recently described Olleya (strains VCSA23 and VCSM12) and Tenacibaculum (strain VCSA14A) genera. Two of the closest cultured relatives to the strain VCSA14A, Tenacibaculum discolor and Tenacibaculum gallaecium, were also found to be bacteriolytic. These five predatory strains exhibit gliding motility and have been observed to lyse prey cells after surrounding them with social swarms, similar to known predatory bacteria such as myxobacteria and members of the genus Lysobacter. Flavobacteria are often numerically significant in a wide variety of freshwater and marine environments, particularly in association with particles, and are thought to be involved in the degradation of biopolymeric substances. If predatory capability is widespread among flavobacteria, they may be a previously unrecognized source of ‘top-down’ bacterial mortality with an influence on the composition and activity of surrounding microbial communities.

Introduction
Microbial communities can be structured by top-down mortality resulting from the grazing of predators, which include protists, viruses and bacteria. The action of microbial predators also causes the release of dissolved organic matter (DOM), much of which is accessible to heterotrophic microorganisms, and can support secondary production in the microbial loop (Azam et al., 1983). Many experimental studies have shown that protistan grazing can strongly influence the morphological, physiological and phylogenetic composition (reviewed by Hahn & Höfe, 2001; Pernthaler, 2005) and metabolic activity (Cutler & Bal, 1926; Griffiths, 1989; Tso & Taghon, 2006) of prey communities. Because of high host specificity and rapid production rates, viruses are hypothesized to affect the most abundant populations in a community, promoting high overall community diversity (Fuhrman, 1999). However, this assumption ignores the trophic diversity known to exist within the bacterial domain.

Predatory bacteria, capable of growing with live bacterial prey as a sole substrate, have been detected in and cultured from a wide variety of environments and phylogenetic groups (Martin, 2002; Jurkevitch, 2007). The longest-studied predatory bacteria, the myxobacteria and the genus Bdellovibrio and its close relatives, belong to the Deltaproteobacteria (Shimkets & Woese, 1992; Davidov & Jurkevitch, 2004), but predatory species have also been characterized from the Alpha-, Beta- and Gammaproteobacteria (Ensign & Wolfe, 1965; Daft et al., 1975; Casida, 1982; Lambina et al., 1983; Makkar & Casida, 1987; Davidov et al., 2006a, b) and the phyla Chloroflexi (Quinn & Skerman, 1980), Bacteroidetes (Lewin, 1997) and Actinobacteria (Casida, 1983). These well-characterized predatory species have been cultured from soils, estuaries, rivers, lakes, bogs and marine and freshwater sediments, and have been detected using PCR-based techniques in an even wider range of environments.
including groundwater, human skin and hydrothermal vents. In addition to these relatively well-studied predatory bacteria, a growing number of less well-characterized lytic bacteria have been reported in culture, microscopy and stable-isotope probing studies (Guererro et al., 1986; Mitsutani et al., 1992; Nogales et al., 1997; Manage et al., 2000; Sacchi et al., 2004; Amaro et al., 2005; Lueders et al., 2006). Many of these potentially predatory bacteria belong to genera such as Cytophaga, Pseudoalteromonas and Alcaligenes, which, while actively studied, had not been previously thought to include predatory species (Mitsutani et al., 1992; Manage et al., 2000; Amaro et al., 2005).

Predatory bacteria use a wide array of predatory mechanisms, ranging from the invasion of Gram-negative periplasms carried out by Bdellovibrio and its relatives (Stolp & Starr, 1963) to the production of lytic exoenzymes by myxobacteria (Hart & Zahler, 1966; Harcke et al., 1972) and some members of the genus Lysobacter (Ensign & Wolfé, 1965). Other predatory mechanisms include attachment to prey cells coupled with the production of a diffusible lytic factor (Casida, 1980, 1982) and the capture of prey cells by their flagella (Lewin, 1997). Predatory bacteria can be considered obligate or nonobligate predators, depending on whether they can assimilate exogenous DOM as a growth substrate in addition to utilizing live prey. Most described predatory bacteria are nonobligate predators, the exceptions being members of the genera Bdellovibrio, Bacteriovorax and Peridibacter in the Deltaproteobacteria and Micavibrio in the Alphaproteobacteria, which require live prey as their growth substrate.

The huge diversity in the phylogeny and physiology of known predatory bacteria makes it difficult to study them as a group using culture-independent techniques. For example, some predatory bacteria, such as Ensifer adhaerens, are very closely related to nonpredatory bacteria (Willems et al., 2003), which renders 16S rRNA gene-based phylogenetic probes incapable of distinguishing predatory from nonpredatory organisms. In addition, the culture-independent study of nonobligate predatory bacteria is complicated by the current inability to assess whether an organism was actively lysing prey or merely assimilating exogenous DOM at the time of its detection. One recent study (Lueders et al., 2006), designed to detect predatory bacteria using $^{13}$C-labeled prey bacteria, successfully detected known nonobligate predatory bacteria such as myxobacteria and a member of the genus Lysobacter, as well as many gene sequences belonging to groups of bacteria containing no well-characterized predatory species. However, this approach is complicated by the fact that predatory bacteria are unlikely to be the only assimilators of lysed prey biomass, resulting in the possibility of uptake of $^{13}$C-labeled biomolecules by nonpredatory heterotrophs.

In order to assess the efficacy of culture-independent approaches for the detection of predatory bacteria and to examine the environmental and ecological significance of the functional guild as a whole, a wider range of predatory bacteria needs to be isolated and characterized. With this goal in mind, we conducted a study to identify the presence and diversity of culturable predatory bacteria from a coastal aquifer on Cape Cod, MA. This site contains a variety of chemical niches (Testa et al., 2002; Talbot et al., 2003; Charette et al., 2005) in a surface-rich environment that could provide a substrate for microbial communities supporting a variety of predatory bacteria. Aquifers have been found to harbor significant protist communities, as reviewed by Novarino et al. (1997), but other than the detection of Bdellovibrio in a groundwater-fed cave (Hutchens et al., 2004), other sources of top-down bacterial mortality have not been studied extensively in groundwater systems.

**Materials and methods**

**Field collection**

A vibracorer was used to sample aquifer sediments beneath the intertidal zone at the Waquoit Bay National Estuarine Research Reserve, Cape Cod, MA, in November 2007. The Cape Cod aquifer is primarily composed of fine to coarse sand and gravel (Cambraeri & Eichner, 1998). A mixing zone between fresh groundwater and sea water intruding into the aquifer from the head of the bay results in a steep, tidally and seasonally influenced salinity gradient (Testa et al., 2002; Talbot et al., 2003; Charette & Sholkovitz, 2006), providing a variety of chemical environments. Before coring, a piezometer profile was taken, sampled every 6 in., to locate the salinity gradient. A vibracoring rig and 4-in.-diameter aluminum barrels, rinsed with sea water from the intertidal zone, were used for coring within the intertidal zone at the head of Waquoit Bay. The core barrel was split lengthwise with an electric saw and sediment was collected at 4-in. depth intervals from the center of the core using flame-sterilized spatulas. The sediment samples were transported to the laboratory in autoclaved glass jars on ice and kept refrigerated for < 24 h before being sampled for culturing.

**Reference and prey bacterial cultures**

Tenacibaculum discolor DSM 18842, Tenacibaculum galli- cum DSM 18841, Kocuria kristinae DSM 20032, Pseudomo- nas putida DSM 50906 and Planctomycyes maris DSM 8797 were obtained from the German Collection of Microorgan- isms and Cell Cultures (DSMZ). Bacillus subtilis PY79 was kindly provided by Tonja Bosak, Saccharomyces cerevisiae by Lynn Miller, Flavobacterium johnsoniae by Mark McBride, Shewanella oneidensis MR-1 by Dianne Newman, Pseudo- monas corrugata by Edouard Jurkevitch and Escherichia coli...
JM109 by Daniel Rogers. Olleya marilimosa strain CAM030T was kindly provided by Carol Mancuso Nichols. Nitrosomonas sp. C-113a (Red Sea isolate) is maintained in the lab of Karen Casciotti. Halomonas halodurans is maintained in the lab of Elizabeth Kujawinski (originally isolated by G. Jones, University of New Hampshire). Unless otherwise noted, all reagents were obtained from Thermo Fisher Scientific Inc. (Waltham, MA). The details of the culture media used are described in the Supporting Information, Appendix S1.

Predatory culture isolation and purification

We prepared bacterial prey for initial enrichment and isolation from cultures of Serratia marcescens, S. oneidensis MR-1 and Nitrosomonas sp. C-113a, grown as described in the Supporting Information, Appendix S1. Cultures of S. oneidensis and S. marcescens were harvested by centrifugation and washed three times in HEPES buffer at a salinity of 20. The final cell suspension represented a 20-fold concentration of the original culture. As a result of relatively low cell densities, Nitrosomonas sp. C-113a was handled differently - 300–500 mL of a stationary-phase culture was filtered onto a 0.2-μm filter and resuspended in its own sterile medium, with a final concentration factor of at least a 100-fold. The bacterial prey smears were prepared by spreading 30 μL of the concentrated prey suspension over an area about 1 cm wide and several centimeters in length on non-nutrient water agar containing cycloheximide (WCX agar) (Shimkets et al., 2006). Cycloheximide was included in the initial enrichment agar to prevent fungal growth, but was excluded after the first two to three transfers. The smears were allowed to dry before inoculation with sediment aliquots.

Small (approximately thimble sized) aliquots of sediment, taken from different depths in the sediment core, were placed directly at one end of bacterial prey smears. After inoculation, plates were observed every 1–3 days using a dissecting microscope at magnifications of × 6 and × 12. Swarms associated with unambiguous clearing of the prey smear were transferred to fresh prey smears by cutting out a small block of agar from the leading edge of the swarm using a sterile syringe needle. Purification was accomplished by transfer of the freshest, leading edges of expanding swarms to new prey smears at least 10 times and then to dilute nutrient water agar containing cycloheximide (WAT) once fungal contaminants had been eliminated. Two salinities were used for the initial enrichments to represent the salinity range of the sampling site: one using deionized water and the other substituting artificial sea water (ASW, see Supporting Information, Appendix S1) for 71.5% of the final volume to reach a final salinity of about 25.

Culture experiments

The production of lytic exoenzymes by VCSA23 was tested by resuspending a live S. oneidensis cell concentrate in the cell-free supernatant from a VCSA23 stationary-phase culture, grown non-predatorily in Marine broth. The cell-free supernatant was obtained by pelleting the VCSA23 cells by centrifugation and then filtering the collected fluid through a 0.2-μm syringe filter. The S. oneidensis culture was divided into two equal volumes and harvested by centrifugation. One of the resulting pellets was resuspended in spent Marine broth from VCSA23 (10 mL) and the other was resuspended in the spent Marine broth from the S. oneidensis culture (10 mL). The absorbance of the two suspensions was monitored by spectrophotometry over the course of 2 weeks relative to sterile Marine broth.

Unless specified otherwise, DN broth with a salinity of 25 was used as a basal medium for nonpredatory growth tests, and WAT with a salinity of 25 (WAT25) was used as a basal medium for predatory growth tests with S. oneidensis MR-1 as the live prey organism. Pasteurized prey smears were also used as a predatory growth substrate, prepared by exposing the S. oneidensis prey to 70 °C for at least 15 min, followed by normal washing procedures as described above.

Both predatory and nonpredatory growth of VCSA23, VCSM12 and VCSA14A were tested at five different temperatures (4, 15, 23, 30 and 37 °C) and salinities (0, 10, 20, 25 and 30). The response of the three strains to increasing concentrations of organic matter was also assessed by growing them on WAT25 amended with 0.01, 0.1 or 1 g yeast extract L⁻¹ with pasteurized S. oneidensis MR-1 prey washed and concentrated to about 10 times the stationary culture cell density (final concentration about 10¹⁰ cells mL⁻¹). Degradation of casein and starch by the three isolates and O. marilimosa, T. discolor and T. gallowaecum was tested by inoculation onto FMM agar (Suzuki et al., 2001; Piñeiro-Vidal et al., 2008), prepared in the same seawater matrix as WAT25 and amended with 0.4% w/v casein or starch.

Prey specificity was tested by inoculating each test strain onto live smears of each prey organism, prepared by repeated centrifugation and resuspension as described above, except for Nitrosomonas sp. C-113a and P. maris, which were concentrated by filtration. Two different prey densities were tested for many of the other prey
organisms – about 10 times the prey’s stationary-phase cell density and about one tenth the prey’s stationary-phase cell density. Clearing presence and progress was monitored every 2–3 days via a dissecting microscope. A strain was scored as predatory on a particular prey organism if macroscopically visible clearing expanded progressively in either of the replicate smears for the test prey.

To facilitate observation of the predatory behavior and spatial relationships between predators and prey, cultures were grown on autoclaved polycarbonate membrane filters (Millipore, Billerica, MA; 0.2-μm pore size) placed onto WAT25 and analyzed by FISH and confocal microscopy. One hundred and eighty microliters of an S. oneidensis live cell suspension was spread evenly onto the filters using a pipette tip. In parallel, prey was applied in the same manner directly onto the agar to facilitate the monitoring of clearing progress during the experiment. Excess fluid from the suspensions was allowed to absorb overnight. For each predatory strain tested (strains VCSA23, VCSM12, VCSA14 and T. gallaecum A37.1T), a small agar block covered with the strain in question was inoculated onto the center of the prey-covered membrane filters, as well as onto prey spots on the agar. Each experiment included three replicate plates per predatory strain, with each plate containing two prey-covered filters inoculated with predator, one filter inoculated with predator only and two filterless prey spots, one inoculated with predator and one not. In addition, a separate plate contained prey-covered filters that were not inoculated with predator. The experiment was monitored daily by checking the filterless predator + prey spots using a dissecting microscope.

Once clearing zones of approximately 1 cm diameter had developed (approximately 3 days after predator inoculation), one predator + prey filter from each plate and three prey-only filters were fixed and frozen. Filters were fixed in Petri dishes by successive immersion in each dish for at least 5 min at room temperature. After washing, each filter was dipped into a 1 : 1 mixture of PBS and ethanol and dried in a Petri dish before freezing at −20 °C.

16S rRNA gene sequencing

Cells from strains VCSA23, VCSM12 and VCSA14A were suspended in HEPES buffer at a salinity of 20 and frozen at −20 °C. Thawed suspensions (1 μL per reaction) were used as the template for PCR amplification. General bacterial 16S rRNA gene primers (10 μM; 27F: 5′-AGA GTT TGA TCC TGG CTC AG-3′, 1492R) and 2 × GoTaq Green Master Mix (Promega Corp., Madison, WI) were used in the reaction mixes. Thermal cycling was performed under the following conditions: denaturation at 94 °C for 2 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 47 °C for 30 s and extension at 72 °C for 30 s, followed by a final extension of 10 min at 72 °C. PCR products were checked for single, coherent bands of the appropriate size (about 1500 base pairs) by agarose gel electrophoresis and ethidium bromide staining.

Full-length 16S rRNA gene sequences (about 1500 base pairs) were cloned from VCSA23, VCSM12 and VCSA14A using the pGEM®-T Easy cloning kit (Promega Corp.) according to the manufacturer’s instructions. For each strain, 16 colonies were picked and grown overnight in Luria–Bertani broth at 37 °C with shaking at 180 r.p.m. No colonies were recoverable from the VCSM12 clone library, which was not further pursued due to the high similarity between the partial 16S rRNA gene sequences of VCSM12 and VCSA23. Cells were collected by centrifugation and plasmids were extracted and purified using a Beckman-Coulter BiomekFX alkaline-lysis plasmid preparation machine and used as a template in two parallel sequencing reactions with the M13F (5′-GTA AAA CGA CGG CCA G-3′) and M13R primers (5′-CAG GAA ACA GCT ATG AC-3′), respectively. Partial sequences (600–800 base pairs) were acquired for strain VCSM12 using the full-length PCR product as the template for sequencing with the 1492R primer after purification using the Wizard SV PCR Cleanup Kit (Promega Corp.). Sequencing reactions were carried out in 6 μL volumes using Applied Biosystems BigDye 3.1 chemistry. All sequencing was performed using an Applied Biosystems 3730XL capillary sequencer at the Josephine Bay Paul Center at the Marine Biological Laboratory in Woods Hole, MA.

Phylogenetic analysis

Bases were called and vector sequences were trimmed from the 16S rRNA gene clone sequences using the Ribosomal Database Project pipeline (Cole et al., 2007, 2009). The trimmed sequences were imported into ARB (Ludwig et al., 2004, version December 2007) and aligned to closely related sequences in the Greengenes ARB database (DeSantis et al., 2006) using the ARB aligner. The ARB editor was used to construct consensus sequences from cloned 16S rRNA gene sequences for each half of the full 16S rRNA gene, which were then exported to FASTA files and manually merged into a single consensus full-length contig and imported into the Silva Reference database (Pruesse et al., 2007, SSURef 97 release). The full-length consensus sequences from VCSA14A and VCSA23 and a partial sequence from VCSM12 have been deposited in GenBank under the accession numbers GQ996383, GQ996384 and GQ996385, respectively. The percent similarity between the full-length consensus 16S rRNA
gene sequences of VCSA23 and VCSA14A, the approximately 750 base pair partial sequence of VCSM12 and their closest relatives was determined using the ‘Align two sequences’ function of the BASIC LOCAL ALIGNMENT SEARCH TOOL (BLAST) (Zhang et al., 2000).

The full-length consensus sequences for strains VCSA23 and VCSA14A were aligned with the ARB aligner and manually checked against the 16S rRNA gene sequences of 204 species of marine flavobacteria in the Living Tree Project database release 100 (Yarza et al., 2008). The 16S rRNA gene sequence of O. marilimosa CAM030T was obtained from GenBank and aligned in the Living Tree Project database as well. A positional conservancy filter was calculated using the filter by base frequency method in ARB at 30% minimal similarity, then manually checked against the 207 aligned sequences and ultimately included 1266 positions present and aligned in all sequences. Trees were constructed and compared in ARB using the neighbor-joining (ARB), maximum-likelihood (RAXML) and maximum-parsimony methods in PHYML. Trees were built using the neighbor-joining, maximum-likelihood and maximum-parsimony methods in PHYML (Felsenstein, 2005). A smaller set of 76 species was exported using the conservancy filter for tree-building in PHYML.

Probes were determined to be specific to the single mismatch at a formamide concentration of 35%, although the VCSA14A probe did exhibit weak hybridization to T. gallaecium cells at formamide concentrations up to 50%. In all cases, FISH was carried out on whole membrane filters by placing them on a microscope slide and pipetting 300 μL of hybridization buffer (35% formamide) and 9 μL of each probe’s working stock (GAM42A conjugated with fluorescein and a predator probe conjugated with Cy3 on each filter as appropriate; 100 ng μL⁻¹) onto each filter. Hybridization and wash buffers and probe stock solutions were prepared according to previously published protocols (Pernthaler et al., 2001). The slides were hybridized in 50-mL centrifuge tubes prewarmed to 46 °C with hybridization-buffer-soaked Kim-wipes inside. Hybridization was conducted at 46 °C for 2–3 h before removing the filters from the humidification chambers and gently placing them in Petri dishes filled with wash buffer and treating them as described elsewhere (Pernthaler et al., 2001). To hybridize filters inoculated with T. gallaecium, which has a single mismatch with the VCSA14A probe, a hybridization buffer containing 0% formamide was used. After hybridization, each filter was mounted with a small drop of 4',6-diamidino-2-phenylindole (DAPI) mountant mix (Pernthaler et al., 2002) on a pre-cleaned microscope slide with a large coverslip (24 by 50 mm) and kept at 4 °C in the dark until imaging.

Confocal microscopy

While probe optimization slides were examined using a Zeiss Axioplan 2 microscope, experimental filters were imaged on a Zeiss LSM 510 META NLO confocal microscope using 488 nm Argon gas and 543 nm Helium–Neon gas lasers and a Zeiss Plan-Apochromat × 63 oil-immersion objective with a numerical aperture of 1.4. In most cases, the filter was initially explored in the fluorescence mode using a mercury lamp to visualize DAPI, Cy3 and fluorescein isothiocyanate (FITC) labels and to mark locations of interest in the LSM software for later imaging. Detector gain and amplifier offset settings were manually optimized on each filter in response to varying signal-to-noise ratios between filters. Cy3 and FITC image z-stacks were collected in the frame scanning mode using the averaging method over four scans at either 1024 × 1024 or 2048 × 2048 pixel resolution. For each stack, slices were captured at 0.5-μm intervals and pinhole settings were optimized for one airy unit. Images were collected primarily in transsects crossing the predatory–prey interaction zones.

Image analysis

z-Stacks were exported as TIFF files using the ZEISS LSM IMAGE BROWSER version 4.2.0.121 and imported into the DAIME digital image analysis tool, version 1.2 (Daims et al., 2006).
The histogram stretch and noise reduction tools of DAIME were used to improve the contrast between cells and filter backgrounds. The same settings were used for all images collected in a given channel from a particular filter. Two-dimensional image projections were then calculated using the maximum intensity projection tool of DAIME and exported to the TIFF file format for display. Scale bars were added manually using ADOBE PHOTOSHOP.

For labeled cell volume measurements, subtransects of 128 × 128 pixel stacks were cropped from selected full image stacks (1024 × 1024 pixels) in transects using ADOBE PHOTOSHOP and individually processed in DAIME using the same settings for histogram stretch and noise reduction as for the larger image stacks. The cropped image stacks were segmented using the three-dimensional segmentation tool in DAIME with the edge detection algorithm, ignoring any putative objects of 5 voxels or smaller. Volumes for each channel in each cropped image stack were measured using DAIME’s measurement tool.

Results

Predatory activity

Live bacterial prey were used as the sole carbon source to isolate three strains of predatory bacteria, designated VCSA23, VCSM12 and VCSA14A, from a Cape Cod aquifer. These isolates generated macroscopic clearing zones in a variety of live and pasteurized bacterial prey smears and swarmed beyond the prey smears on water agar. In addition, two already-described species closely related to strain VCSA14A, T. discolor and T. gallaicum, also cleared prey smears. Figure 1 shows the images of typical macroscopic clearings caused by T. gallaicum and strains VCSA23, VCSM12 and VCSA14A on live prey lawns of S. oneidensis. Olleya marilimosa strain CAM030T, which is closely related to strains VCSA23 and VCSM12, did not generate any clearings on the same prey bacteria. We cannot currently determine whether O. marilimosa CAM030T was never predatory or whether it has lost its predatory capability in culture.

The predatory specificity of the five strains was tested on 11 prey organisms selected on the basis of cell wall structure and taxonomic diversity (Table 1). Strains VCSA23 and VCSM12 had the broadest prey specificity of the strains tested, showing unambiguous and expanding clearing on smears of all except three of the prey organisms onto which they were inoculated. With the Gram-positive K. kristinae and the yeast S. cerevisiae, clearing zones developed around the inoculum site and expanded to varying degrees before ceasing growth well short of full prey consumption. Neither strain cleared H. halodurans. The three Tenacibaculum strains (VCSA14A, T. discolor LL04 11.1.1T and T. gallaicum A37.1T) displayed narrower specificity than strains VCSA23 and VCSM12. Strain VCSA14A had the narrowest prey specificity tested, unambiguously clearing only S. oneidensis
and *F. johnsoniae*. *Tenacibaculum discolor* and *T. gallaicum* each cleared both of those prey as well as *B. subtilis*, *E. coli* and, in the case of *T. gallaicum*, *Nitrosomonas* sp. C-113a. None of the three *Tenacibaculum* strains visibly cleared *K. kristinae*, *P. maris*, *S. cerevisiae* or any of the pseudomonads tested. Like the *Olleya* strains, none of the *Tenacibaculum* strains affected *H. halodurans*. All five strains were also found to be nonobligate predators, capable of growing heterotrophically on complex organic media.

Because predatory bacteria are known to use a variety of lytic mechanisms (Martin, 2002; Jurkevitch, 2007), the predatory flavobacteria were further investigated to constrain the mechanism of prey lysis. We investigated the possibility that lytic exoenzymes are released into the culture fluid by the predator, as has been observed for a *Lysobacter* sp. (Ensign & Wolfe, 1965). In our study, live *S. oneidensis* cells were resuspended into a cell-free culture supernatant collected from a stationary-phase broth culture of VCSA23. No decrease in cell density was observed relative to a control probe were observed (Fig. S3). Such shortened, often spherical cells have been reported from older, late

Table 1. Prey specificity of predatory species tested

<table>
<thead>
<tr>
<th>Predator species:</th>
<th>VCSA23</th>
<th>VCSM12</th>
<th>VCSA14A</th>
<th><em>T. discolor</em> LL04 11.1.1T</th>
<th><em>T. gallaicum</em> A37.1T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prey species</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Shewanella oneidensis</em> MR-1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Escherichia coli</em> JM109</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Pseudomonas putida</em> DSM 50906</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>Pseudomonas corrugata</em></td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>Halomonas halodurans</em></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>Nitrosomonas</em> sp. C113a</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td><em>Flavobacterium johnsoniae</em></td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Planctomyces maris</em> DSM 8797</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>Kocuria kristinae</em> DSM 20032</td>
<td>+/−</td>
<td>+/−</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em> PY79</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Saccharomyces cerevisiae</em></td>
<td>+/−</td>
<td>+/−</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

+, progressively spreading clearing of prey smear observed coincident with swarm emergence; −, no clearing observed on prey smear; +/-, some clearing observed, but ceases expanding shortly after leaving the original inoculum area.

In order to facilitate imaging of predator–prey spatial relationships with a minimum of physical disturbance, membrane filters were used as a growth surface for 3- to 5-day incubations and visualized using FISH. The two sets of predatory flavobacteria (*Olleya* sp. VCSA23 and VCSM12 and *Tenacibaculum* sp. VCSA14A and *T. gallaicum* A37.1T) exhibited different patterns of prey clearing when visualized at a high magnification. On prey-coated filters inoculated with strain VCSA23, a decrease in prey abundance was observed coincident with the position of a dense, expanding front of VCSA23 cells (Fig. 2). Measurements of labeled prey and predator biovolume show that *S. oneidensis* cell volume decreased by approximately two orders of magnitude within about 150 μm crossing into the predator swarm (Fig. 2). At least 1 mm inward from the main interaction zone, both VCSA23 and VCSM12 formed very dense round aggregates with small central hollows. These aggregates appeared to expand over time to become a dense mass of predator cells (Figs S1 and S2). However, despite the extremely high densities of predator cell in the aggregates, a small number of prey cells were still present.

On filters inoculated with strain VCSA14A, prey cell density declined quickly to very low levels coincident with the preparation of a wet mount slide.

We also explored the possibility that direct cell-to-cell contact was required for prey lysis. Live cell microscopy was used in an attempt to visualize lytic events caused by cell contact between individual cells of all three isolated strains and the *S. oneidensis* prey. The predators frequently exhibited gliding motility on slides in wet mount preparations, but no direct lysis of prey cells by single predator cells was observed. However, it is possible that the wet mount environment is not conducive to predatory activity (or its observation) due to a number of factors, such as low predator cell density or disturbance associated with the preparation of a wet mount slide.
Novel strains suggest a predatory role for flavobacteria

Fig. 2. (a) Two-dimensional confocal projections of VCSA23 cells (labeled with the VCSA23-Cy3 probe, top, in red) and Shewanella oneidensis prey (labeled with the GAM42a-FITC probe, bottom, in green). From left to right, the most recently cleared zone, the interface between the expanding VCSA23 cell front and the prey lawn, and the prey lawn relatively undisturbed by VCSA23 cells are shown. Scale bars = 10 µm. In the lower graph (b), labeled cell volume data are plotted against the transect coordinate using a section of the collected images, delineated by white boxes on the two-dimensional projections in (a). Each point was calculated from a 321 µm² square. The volumes of GAM42a-FITC-labeled cells (S. oneidensis) are plotted with green squares, while the VCSA23-Cy3-labeled cell volumes (strain VCSA23) are represented by red circles. Gray shading in the graph indicates the spatial orientation of the two-dimensional projections shown in (a).
exponential- or stationary-phase cultures in several *Tenacibaculum* sp. (Wakabayashi *et al.*, 1986; Hansen *et al.*, 1992; Suzuki *et al.*, 2001; Frette *et al.*, 2004), and may represent a dormant life stage. *Tenacibaculum gallaicum* A37.1*T* was observed to have a similar pattern of predation on *S. oneidensis* cells (Fig. S4).

For all strains tested, at least a few cells hybridizing with the prey-specific probe could be found in cleared areas, suggesting that not all susceptible prey cells are lysed. In addition, small numbers of scattered predatory cells were observed well in advance of the main density fronts for all four strains. Despite this, the areas ahead of the predator density fronts appeared essentially identical to prey lawns on control filters that never received predatory bacteria. A general tendency of higher prey cell volumes in close proximity to predatory cell fronts was observed on most filters. We attribute this to the regrowth of prey cells on DOM released from neighboring prey cell lysis at and behind the predatory cell front.

Because VCSA23, VCSM12 and VCSA14A are nonobligate predators, we examined whether their lytic behavior was affected by the availability of exogenous DOM using yeast extract as a complex DOM source. All three strains cleared the pasteurized prey smears at all of the DOM concentrations tested. However, the growth habits of strains VCSA23 and VCSM12 changed from a swarming growth habit with filamentous margins to a thick, slimy growth habit with smooth edged, entire margins at the highest DOM concentration tested (0.1% w/v yeast extract). In contrast, strain VCSA14A retained a swarming growth habit with initially entire edges when grown on marine agar, which has a concentration of complex DOM of about

![Two-dimensional confocal projections of strain VCSA14A cells (labeled with the VCSA14A-Cy3 probe, top, in red) and *Shewanella oneidensis* prey (labeled with the GAM42a-FITC probe, bottom, in green) show, from left to right, the most recently cleared zone, the interface between the expanding VCSA14A cell front and the prey lawn, and the prey lawn relatively undisturbed by VCSA14A cells. The three sets of two-dimensional projections were acquired adjacent to each other along a transect crossing the VCSA14A cell front. The graph at bottom (b) was constructed identical to that in Fig. 2b.](image-url)
0.6%. These results showed that although all three of the tested strains continued to track and lyse pasteurized prey cells in the presence of high concentrations of exogenous DOM, they changed their social and motility behavior in response to increasing DOM concentrations.

**Phylogenetic analysis**

The 16S rRNA gene sequences of VCSA23 (1483 base pairs) and VCSM12 (three partial sequences between 708 and 756 base pairs) are nearly identical, with 99% identity between the overlapping regions of the full-length consensus sequence for VCSA23 and the partial sequences for VCSM12, suggesting that they may be two strains belonging to a single species. Phylogenetic analysis based on 1299 well-aligned base pairs of the 16S rRNA gene sequences indicates that VCSA23 belongs to the genus *Olleya* (Fig. 4). The 16S rRNA gene sequence of VCSA23 is 97% identical to *O. marilimosa* CAM030®, which is the only described species in the genus (*Mancuso Nichols et al.*, 2005). These levels of 16S rRNA gene sequence identity suggest that VCSA23 and VCSM12 could represent a second species in the *Olleya* genus, given the often-used threshold of 97% identity in this marker gene for species-level differentiation. The next closest cultured relative on the basis of the 16S rRNA gene sequence is *Lacinutrix algicola*, which is 95% identical to VCSA23.

The 16S rRNA gene sequence (1486 base pairs) of the third isolate, VCSA14A, is 89–91% identical to those of VCSA23 (full length) and VCSM12 (partial), respectively. It branches tightly within the genus *Tenacibaculum*, and is most closely related to the recently described species *T. discolor*, *T. gallaicum* (*Piñeiro-Vidal et al.*, 2008), and *T. litoreum* (*Choi et al.*, 2006), which are 99%, 97% and 99% identical to VCSA14A in their 16S rRNA gene sequences, respectively (Fig. 4).

**Physiology**

Physiological tests used to compare the three newly isolated strains with other closely related cultured strains are shown in Table 2. With respect to the physiological characteristics tested, strains VCSA23 and VCSM12 are both very similar to their closest cultured relative, *O. marilimosa* strain CAM030®. These three strains have gliding motility, are mesophiles incapable of growth at 37 °C and require at least some salt for growth. In fact, the only major characteristic assessed in which the two predatory strains differ from strain CAM030® is the ability to grow by lysing prey bacteria and a requirement for sea salts. Their next closest cultured relative, *L. algicola* strain AKS29®, cannot glide, is incapable of growing at 30 °C and does not require salt (*Nedashkovskaya et al.*, 2008). Strain VCSA14A was found to be very similar to its closest relatives, *T. discolor* strain LL04 11.1.1®, *T. litoreum* strain CL-TF13® and *T. gallaicum* strain A37.1®. All four strains possess gliding motility, are capable of growth at 37 °C and are incapable of utilizing either (+)-d-glucose or citrate as the sole carbon sources with ammonia and nitrate as nitrogen sources. All six of the strains tested in this study were unable to degrade starch with ammonia and nitrate as nitrogen sources, but were able to degrade casein, exhibiting clearing halos indicative of diffusible proteases on casein FMM agar plates.

**Discussion**

Surface-associated predatory flavobacteria such as those described in this study could influence the biogeochemistry of a wide variety of environments. In a manner similar to that already known for predatory protozoa (Hahn & Höfe, 2001; Pernthaler, 2005), they could increase the mobility and cycling of organic matter and suppress or stimulate particular members of microbial communities. The strength and persistence of such an influence is dependent on a variety of factors, including the accessibility of susceptible prey and details of the predatory biology of particular predatory flavobacteria present, such as the breadth of their prey specificity and environmental cues that either stimulate or discourage predatory activity. The isolates described in this study are undergoing further characterization with respect to their predatory biology. In addition, the discovery that two type strains of the genus *Tenacibaculum* are predatory raises the possibility that existing culture collections may contain clues as to the phylogenetic distribution of additional predatory bacteria.

This study provides the first definitive evidence for predatory activity within the class *Flavobacteria*. Flavobacteria are found in a wide variety of habitats, including seawater (*DeLong et al.*, 2006), lakes (*Eiler & Bertilsson*, 2007; *Michael et al.*, 2009), marine sediments (*Llobet-Brossa et al.*, 1998; *Musat et al.*, 2006), polar sea ice (*Brinkmeyer et al.*, 2003) and hydrothermal settings (*Kormas et al.*, 2006). This group has been reported as being important in the degradation of polymeric substances in aquatic environments (*Pinhassi et al.*, 1999; *Cottrell & Kirchman*, 2000; *Janse et al.*, 2000; *Covert & Moran*, 2001). They are also frequently associated with particulate matter in the oceans (*DeLong et al.*, 1993; *Rath et al.*, 1998), as well as with phytoplankton and bacterial blooms (*Abell & Bowman*, 2005; *Eiler & Bertilsson*, 2007). If some fraction of the flavobacteria in these habitats is accessing living biomass as a growth substrate, our observations have broad implications for the role of flavobacteria in microbial ecology and biogeochemistry.

Our results suggest that the predatory flavobacteria described in this study might use a lytic mechanism requiring at least close proximity, if not direct contact, to prey cells. Many myxobacteria and some members of the genus...
Lysobacter release constitutively produced lytic exoenzymes (Ensign & Wolfe, 1965, 1966; Hart & Zahler, 1966; Harcke et al., 1972; Kobayashi et al., 2005). Although all of the strains produced diffusible proteases (Table 2), the culture supernatant of strain VCSA23 showed no lytic activity (data not shown). This suggests either that the production of lytic exoenzymes is contingent on the presence of prey cells or that they are not released extracellularly. If lytic enzymes are not released extracellularly, they may be membrane-associated similar to those commonly found in genomic and biochemical studies of marine flavobacteria (Tsugawa et al., 1996; Nedashkovskaya et al., 2005; Bauer et al., 2006; González et al., 2008). Further study is needed to fully constrain the lytic mechanism of the predatory flavobacteria.

The diversity in prey specificity displayed by the strains tested in this study suggests varying degrees of specialization within the predatory flavobacteria, as has been found for other predatory bacteria (Mathew & Dudani, 1955; Stolp & Starr, 1963; Jurkevitch et al., 2000; Bull et al., 2002; Davidov et al., 2006a,b). At least with respect to the prey species and growth conditions assayed here, strains VCSA23 and VCSM12 appear to be more cosmopolitan in terms of susceptible prey than the Tenacibaculum strains. Their range

**Fig. 4.** Maximum-likelihood tree showing the phylogenetic relationship between strains VCSA23, VCSA14A and other members of the family Flavobacteriaceae based on full-length 16S rRNA gene sequences. The tree was built from an alignment containing the 76 species shown here using 1266 positions present and aligned in all 76 sequences. GenBank nucleotide accession numbers are provided for each sequence in parentheses. Numbers at nodes indicate the percent bootstrap support for that node in a 100 replicate bootstrap analysis of the maximum-likelihood tree. Open black circles and filled gray circles indicate the nodes recovered in neighbor-joining and maximum-parsimony trees, respectively, built from the same alignment. The scale bar indicates the branch length corresponding to 0.1 changes per mean nucleotide position.
of susceptible prey was very broad, with only *H. halodurans* proving completely impervious (Table 1) under the conditions tested. Even among the *Tenacibaculum* strains, all of which are very closely related to each other according to 16S rRNA gene analysis, there was considerable diversity in prey specificity. In fact, no two *Tenacibaculum* strains had the same prey specificity profile. Prey specificity may be related to the predators’ mechanism of lysis and to the physiological or the chemical characteristics of the prey under the tested conditions. It is clear from the prey specificity profiles that the cell wall structure of the prey is not a dominant factor in prey susceptibility to the predatory flavobacteria. Four out of the five predatory strains tested were found to lyse both Gram-negative and Gram-positive prey. Despite the susceptibility of *S. oneidensis* to all the predatory strains tested, at least one other member of the class *Gammaproteobacteria* proved to be resistant to predation by each predator. Because the lytic mechanism of these predatory flavobacteria appears to require direct contact (or at least close proximity) with prey, other prey characteristics may be important, including the production of extracellular polymeric substances, the presence or absence of specific proteins or compounds on the prey cell surface or the production and release of compounds antagonistic to predator cells.

Interestingly, the images collected from cleared zones of prey lawns on membrane filters indicate that *S. oneidensis* prey is not completely eliminated by any of the predators tested (Figs 2 and 3, Figs S1–S4). Although incomplete elimination of prey populations has been commonly observed for obligate planktonic predators, such as *Bdellovibrio* (Keya & Alexander, 1975; Jurkevitch et al., 2000; Shemesh & Jurkevitch, 2004), it has often been assumed that gliding predators such as myxobacteria completely lyse their local prey populations (Mathew & Dudani, 1955). However, attempts to assess prey survival after myxobacterial predation have shown that the complete elimination of prey cells is rare (Singh, 1947; Hillesland et al., 2007) and that a small number of viable prey cells can be recovered after the lysis of > 99% of the original prey population. The basis for predatory resistance of these surviving cells is currently unknown, whether it be from genotypic or phenotypic differences from lysed prey cells. A minority of prey cells under *Bdellovibrio* predation have been shown to develop a temporarily increased resistance to predatory attack, termed plastic phenotypic resistance (Shemesh & Jurkevitch, 2004). Prey populations under attack by gliding nonobligate predators, such as myxobacteria and the flavobacteria in this study, may undergo a similar physiological change in which a small subset becomes temporarily resistant to predation. Another, less studied, possibility is that the predatory flavobacteria may stop active predation in response to external factors, such as nutrient concentration or prey cell density. Either possibility could serve as a mechanism that allows prey populations to escape complete elimination by bacterial predation.

In addition to differences in prey specificity, the tested predatory strains displayed contrasting predatory growth efficiencies. As represented in Figs 2 and 3, the same density of prey cells presented to and lysed by the different predator strains resulted in an order of magnitude more cells (as measured by the total volume) of *Olleya* sp. VCSA23 (Fig. 2) and VCSM12 (Fig. S2) than those of *Tenacibaculum* sp. VCSA14A (Fig. 3) and *T. galliacum* A37.1T (Fig. S4). Although both sets of predators were responsible for approximately the same amount of prey lysis, the lower

### Table 2. Physiological comparison between predatory strains and close relatives

<table>
<thead>
<tr>
<th>Species:</th>
<th>Olleya marilimosa</th>
<th>Lacinutrix algicola</th>
<th>Tenacibaculum discolor</th>
<th>T. litoreum</th>
<th>T. galliacum</th>
<th>T. mesophilum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strains:</td>
<td>VCSA23 VCSM12 CAM030</td>
<td>AKS293</td>
<td>VCSA14A</td>
<td>LL04 11.1.1</td>
<td>CL-TF13</td>
<td>A37.1T MBIC 1140</td>
</tr>
<tr>
<td>References:</td>
<td>This study</td>
<td>This study</td>
<td>Nedashkovskaya et al. (2008)</td>
<td>This study</td>
<td>Choi et al. (2006)</td>
<td>Piñeiro-Vidal et al. (2008)</td>
</tr>
<tr>
<td>Characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gliding motility</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Growth at 0 salinity</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Growth at 25 salinity w/only NaCl</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>W</td>
<td>W</td>
</tr>
<tr>
<td>Growth at 30°C</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Growth at 37°C</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Bacteriolysis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Starch degradation</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Casein degradation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* *Mancuso Nichols et al. (2005) reports no casein hydrolysis, but Olleya marilimosa strain CAM030 was observed to hydrolyze casein in this study.*

**W. weak growth without characteristic swarming at colony edges observed.**

**ND. no data.**

Published by Blackwell Publishing Ltd. All rights reserved.

predatory growth efficiency of the *Tenacibaculum* strains suggests that they may be unable to assimilate as much prey biomass as the *Olleya* strains. Both *T. discolor* and *T. gallaecium*, as well as other members of the genus *Tenacibaculum*, have been reported to be unable to assimilate a variety of sugars (Suzuki et al., 2001; Yoon et al., 2005; Choi et al., 2006; Piñeiro-Vidal et al., 2008), while the closest relative to strains VCSA23 and VCSM12, *O. marilimosa CAM030*¹, has been described as utilizing several sugars (Mancuso Nichols et al., 2005). This suggests that the predatory *Olleya* strains could owe their higher predation efficiency to a broader capacity for carbohydrate assimilation. However, carbohydrates generally compose a minority of bacterial cell dry weight – < 10% in *E. coli*, as reviewed by Neidhardt et al. (1990) – and so seem unlikely to be solely responsible for the large differences in the predatory cell density between the strains. The *Tenacibaculum* and *Olleya* strains may also differ in their hydrolytic capabilities, with *Tenacibaculum* strains able to access fewer of the prey macromolecules.

Determining the prevalence of predation among flavo-
bacterial species remains a significant challenge. As men-
tioned earlier, predatory capability is not a monophyletic
trait and predatory bacteria can be very closely related to
nonpredatory species. Nonobligate predatory lifestyles may
have evolved repeatedly and independently from saprophy-
tic gliding bacteria (Jurkevitch, 2007), both within the class
*Flavobacteria* and in other groups. In addition, the utiliza-
tion of functional gene markers, which has been successful
in assessing the presence and diversity of other functional
guilds, is not possible for detecting predatory bacteria at this
time. Many of the hydrolytic enzymes presumably used by
predatory flavobacteria to break down prey macromolecules
after cell lysis could also be used to break down nonliving
particulate and DOM, and so would not be diagnostic of
predatory capability. Until an unambiguous functional
marker for predatory flavobacteria can be identified,
culture-based approaches may be the most reliable way
of establishing whether a particular species is capable of
predation.

Nonetheless, in addition to this study, other reports have
evoked reported evidence that supports the possibility that preda-
tion may not be a rare lifestyle for flavobacteria. For
example, *Tenacibaculum maritimum* was described to lyse
dead cells of *E. coli*, *Aeromonas hydrophila* and *Edwardsiella
tarda* (Wakabayashi et al., 1986; Suzuki et al., 2001),
though it is unclear whether it was tested for growth on
live prey cells. *Kordia algicida* has been described to have a
strongly algidical effect on some diatoms and other algae in
liquid (Sohn et al., 2004). At least four other types of marine
flavobacteria have also shown lytic activity towards eukar-
yotic phytoplankton, although they have not been charac-
terized taxonomically (Maeda et al., 1998; Amaro et al.,
2005). In addition, a stable-isotope probing study using
¹²C-labeled prey cells added to soil microcosms detected a
flavobacterial 16S rRNA gene sequence along with sequences
belonging to known predatory bacteria such as myxobacter-
ia and members of the genus *Lysobacter* within the ¹³C-
labeled RNA fraction (Luéders et al., 2006). This result
suggests that the labeled *Flavobacterium* sp., which took up
stable-isotope-labeled carbon from the added prey cells, is a
predatory bacterium. However, this type of labeling study is
ambiguous by nature, because it is possible that nonpreda-
tory bacteria could have scavenged labeled carbon from the
prey after it was lysed.

The results of this study and mounting circumstantial
vidence in the recent literature indicate that predation
should be considered as a potential ecological role for
flavobacteria and other members of the *Bacteroidetes*. If
predatory flavobacteria are significant sources of microbial
mortality, then flavobacteria as a group cannot be accurately
treated as equivalent to other, more ‘passive,’ heterotrophic
bacteria in biogeochemical models as some may represent a
higher trophic level. In other words, these results argue that
the bacterial size fraction in aquatic environments can
possess significant trophic complexity, with potential con-
sequences for the efficiency of biogeochemical cycling.

**Acknowledgements**

We would like to thank Ed Leadbetter, Dan Rogers, Mark
Martin and James Saenz for helpful discussions during the
course of this study and four anonymous reviewers whose
useful comments improved this manuscript. We are grateful
for field support and assistance from Dan Rogers, Paul
Henderson, Matt McIlvin, James Saenz, Sandy Baldwin,
Laura Erban, Matt Charette and Kevin Kroeger. We thank
Jeff Donnelly and Maya Gomes for the loan of a vibracorer
and advice, and Cornelia Wuchter and Stefan Sievert for
assistance with FISH. Louis Kerr is acknowledged for his
technical assistance with confocal microscopy. This work
was supported by NSF grant EAR-0525166 to E.B.K., a
WHOI Ocean Ventures Fund award and a WHOI Coastal
Ocean Institute fellowship, both awarded to E.C.B.

**References**

dynamics of class *Flavobacteria* on diatom detritus in
experimental mesocosms based on Southern Ocean seawater.

Amaro AM, Fuentes MS, Ogalde SR, Venegas JA & Suarez-Isla BA
(2005) Identification and characterization of potentially
algal-lytic marine bacteria strongly associated with the toxic
dinoflagellate *Alexandrium catenella*. *J Eukaryot Microbiol* 52:
191–200.
Novel strains suggest a predatory role for flavobacteria


Felsenstein J (2005) *PHYLIP (Phylogeny Inference Package)* Version 3.67. Distributed by the author, Department of Genome Sciences, University of Washington, Seattle, WA.


Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Detailed culture media.

Fig. S1. Two-dimensional projections of z-stacks of VCSCA23 and *Shewanella oneidensis* prey in a membrane filter culture captured by confocal microscopy.

Fig. S2. Two-dimensional projections of z-stacks of strain VCSM12 and *Shewanella oneidensis* in a membrane filter culture, acquired by confocal microscopy.

Fig. S3. Two-dimensional projections of z-stacks of VCSCA14A and *Shewanella oneidensis* prey in a membrane filter culture captured by confocal microscopy.
Fig. S4. Two-dimensional projections of *Tenacibaculum gallaecum* A37.1<sup>T</sup> cells (labeled with the VCSA14A-Cy3 probe hybridized at 0% formamide, top, in red) and *Shewanella oneidensis* prey (labeled with the GAM42a-FITC probe, top, in green).

Please note: Wiley-Blackwell is not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.