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Shewanella and *Photobacterium* spp. in Oysters and Seawater from the Delaware Bay▽

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***Shewanella algae*, *S. putrefaciens*, and *Photobacterium damselae* subsp. *damselae* are indigenous marine bacteria and human pathogens causing cellulitis, necrotizing fasciitis, abscesses, septicemia, and death. Infections are rare and are most often associated with the immunocompromised host. A study was performed on the microbiological flora of oysters and seawater from commercial oyster harvesting sites in the Delaware Bay, New Jersey. From 276 water and shellfish samples tested, 1,421 bacterial isolates were picked for biochemical identification and 170 (12.0%) of the isolates were presumptively identified as *S. putrefaciens*, 26 (1.8%) were presumptively identified as *P. damselae* subsp. *damselae*, and 665 (46.8%) could not be identified using the API 20E identification database. Sequencing of the 16S rRNA genes of 22 *S. putrefaciens*-like isolates identified them as *S. abalonesis*, *S. algae*, *S. baltica*, *S. hafniensis*, *S. marisflavi*, *S. putrefaciens*, *Listonella anguillarum*, and *P. damselae*. Beta-hemolysis was produced by some *S. algae* and *P. damselae* isolates, while isolates of *S. baltica* and *L. anguillarum*, species perceived as nonpathogenic, also exhibited β-hemolysis and growth at 37°C. To our knowledge, this is the first time these beta-hemolytic strains were reported from shellfish or seawater from the Delaware Bay. Pathogenic *Shewanella* and *Photobacterium* species could pose a health threat through the ingestion of contaminated seafood, by cuts or abrasions acquired in the marine environment, or by swimming and other recreational activities.**

The genus *Shewanella* has been studied for decades and, over the years, has been classified taxonomically as *Achromobacter*, *Pseudomonas*, *Alteromonas*, and *Shewanella* (34). Because of a genetic similarity to *Vibrionaceae*, MacDonell and Colwell (21) recommended that *Shewanella* and a related genus, *Listonella*, be placed in the *Vibrionaceae* family. *Shewanella* species are gram-negative long, short, or filamentous rods that are generally oxidase positive, indole negative, and nonfermentative to most sugars and usually produce H_2S on Kligler or triple sugar iron agar (TSI) (34). The human pathogenic species *Shewanella putrefaciens* and *Shewanella algae* (formerly *S. alga*) have been associated with septicemia (5, 7, 26); cellulitis, skin, and soft tissue infections (7–9, 26), including wound infections (6); ear infections (15, 32); cerebellar abscesses (32); leg ulcers (9); osteomyelitis (4); and arthritis (20). *Shewanella algae* is believed to be the more dominant human pathogen (14, 18, 27) of marine and clinical origin (35, 36), whereas *S. putrefaciens* is often isolated from food and environmental samples (12, 37). Other *Shewanella* spp., especially *S. baltica*, are commonly associated with food spoilage (12, 37). The incidence of shewanellosis is rare; however, a recent report from Japan indicates that *Shewanella* infections are increasing, that raw fish have been associated with illness in patients with liver disease, and that at least one case was misdiagnosed as *Vibrio vulnificus*, based on the presence of necrotizing fasciitis, liver cirrhosis, and a history of raw-fish consumption (25). Immunocompromised individuals appear to be at higher risk for *Shewanella* infections (8, 16, 17, 19).

Another marine bacterium, *Photobacterium damselae* (formerly known as *Vibrio damselae*), has been associated with morbidity and deaths due to necrotizing fasciitis from wound infections (2, 10, 33, 38, 39). *Photobacterium damselae* is also a significant pathogen in wild and farm-raised fish (13), and strains are biochemically similar to *Shewanella* spp. in that they are gram negative, oxidase positive, indole negative, and nonfermentative to most sugars and produce H_2S. *Photobacterium* is currently a genus within the *Vibrionaceae* family.

In this paper, we report the isolation of *Shewanella* spp. and *P. damselae* subsp. *damselae* from oysters and seawater as part of a 2-year survey for *Vibrionaceae* in the Delaware Bay. There are little data on the presence or distribution of *Shewanella* spp. in the Delaware Bay, and this bay contains commercial shellfish harvesting areas. Because presumptive *Shewanella* species were being isolated and some species are human pathogens, we sought to evaluate the efficacy of conventional biochemical methods for their detection and differentiation and to confirm, by biochemical and sequence analyses, the identity of some of the isolates. Isolates of *P. damselae* subsp. *damselae* were also evaluated since they were occasionally identified by biochemical testing as presumptive *S. putrefaciens* isolates.

MATERIALS AND METHODS

Stock cultures. *Shewanella* stock cultures consisted of *S. algae* (ATCC 51192; American Type Culture Collection, Manassas, VA), *S. algidipiscicola* (31), *S. baltica* (NCTC 10735; National Collection of Type Cultures, London, England), *S. frigidimarina* (ACAM 591; Australian Collection of Antarctic Microorganisms, Hobart, Tasmania, Australia), *S. morhuae* (ATCC U1417), and *S. putrefaciens* (ATCC BAA-1097). The authenticity of the stock cultures was confirmed by sequence analysis of the 16S rRNA genes by the methods described below.

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Oysters and seawater. Oysters and overlying seawater samples were collected during monthly sampling cruises from May 2004 to June 2006 to commercial oyster seedbeds along the New Jersey coast as part of a survey for total *Vibrionaceae* in the Delaware Bay. Oysters were harvested by dredge while seawater was obtained with a 2.2-liter-capacity Niskin-type sampler (Wildlife Supply Co., Buffalo, NY). Samples were transported at ambient temperatures to the Haskins Shellfish Research Laboratory, Port Norris, NJ, where testing was initiated the same day (generally within 6 h of collection). Each month for approximately 2 years, oysters and seawater were collected from six sites, and samples from each site were analyzed for a total of 138 oyster samples and 138 seawater samples.

Cultural and preliminary biochemical analyses. Each oyster sample was a composite of 50 g of shucked meats from four to six oysters and was homogenized in 450 ml of 0.1% peptone (Becton Dickinson and Co., Sparks, MD) water. One hundred microliters of seawater, oyster homogenates, and serial 10-fold dilutions (in 0.1% peptone water) of the water and homogenates were separately spread plated on tryptic soy agar (Becton Dickinson and Co.) containing 0.5% additional NaCl (1% total) (TSA-N) and incubated at 37°C for 24 h. Up to 120 colonies/month were randomly picked for biochemical identifications from among the countable dilution plates.

Biochemical testing was performed using an API 20E system (bioMérieux Industries, Hazelwood, MO) and a BactiDrop oxidase test kit (Remel, Lenexa, KS). The nonselective nature of TSA-N allowed the rapid growth of many marine bacteria and potential human pathogens, including *Shewanella* spp., from both seawater and shellfish. *Shewanella* and *Photobacterium* spp. could be identified only if they were among the predominant organisms, since biochemical testing was performed only on colonies picked from the countable plates; lower-dilution plates were typically overgrown.

Biochemical and nucleotide sequence analyses of selected isolates. Ten oyster and seawater isolates identified by API 20E as *S. putrefaciens*, 15 isolates that were nonfermentative but could not be identified by the API system, and the six stock *Shewanella* spp. were subjected to further biochemical analyses. The analyses were conducted at 37°C, unless otherwise noted, and included the suite of API-20E tests: growth on tryptic soy agar containing 1% and 6% total NaCl; the production of H_2S on TSI (Becton Dickinson and Co.); hemolysis on plates of tryptic soy agar containing 5% sheep's blood (bioMérieux); growth on TSA-N at 25, 37, and 42°C; and the presence of phosphoglucose isomerase with a lysyl aminopeptidase (PGI-LysAP) activity. Lower-temperature incubations (25°C) were needed for four stock cultures that would not grow at 37°C, specifically, *S. algidipiscicola*, *S. baltica*, *S. frigidimarina*, and *S. morhuae*. The PGI-LysAP activity is characteristic of bacteria in the *Vibrionaceae* family and was determined by a colony overlay procedure for peptidases (29, 30). *Shewanella algae* is reported to grow at elevated NaCl concentrations (up to 6% NaCl) and at high temperatures (up to 42°C), but *S. putrefaciens* is not (14, 18).

Nucleotide sequence analyses were performed for confirmation of species identity. For sequencing, genomic DNA was purified from overnight cultures of the isolates after growth in LB or marine broth 2216 media (Becton Dickinson and Co.) by using a DNeasy tissue kit (Qiagen, Valencia, CA). A fragment of the 16S rRNA gene was PCR amplified from each genomic preparation using forward primer 63F (5′-CAG GCC TAA CAC ATG CAA GTC-3′) and reverse primer 1387R (5′-GGG CGG WGT GTA CAA GGC-3′), as described by Marchesi et al. (22). PCRs were performed in a total volume of 50 μl containing 1 U of Eppendorf HotMaster *Taq* DNA polymerase (Brinkmann Instruments Inc., Westbury, NY), 0.25 μg of each primer, approximately 100 ng of template DNA, 200 μM deoxynucleoside triphosphates, and the buffer supplied with the HotMaster *Taq* polymerase. Reaction mixtures were incubated in an Eppendorf Mastercycler gradient thermocycler with an initial denaturation for 2.0 min at 94°C, followed by 25 cycles of 20 s at 94°C, 20 s at 50°C, and 80 s at 65°C. The PCR mixtures were checked for amplification of the 16S rRNA fragment by visualization of the reaction mixture on a 1% agarose gel, and the remaining PCR solution was prepared for sequencing by purification using a QIAquick PCR purification kit (Qiagen). The products were sequenced in the forward and reverse directions using the 63F and 1387R primers, respectively, by SeqXcel Inc., San Diego, CA. Approximately 750 bp of unambiguous sequence was obtained in both directions, and identification was made by comparing the sequence to the GenBank database using the BLASTn program (1). All matches were at 100% identity unless otherwise stated.

RESULTS AND DISCUSSION

A total of 1,421 bacterial colonies isolated on TSA-N plates from oysters and seawater were subjected to biochemical identification using the API 20E system, and 170 isolates (12% of all picked colonies) were identified as presumptive *S. putrefaciens* isolates. Of these, eight were listed in the API database as excellent identifications (ID), 97 were very good ID, two were good ID, and 63 were acceptable ID. Examples of biochemical profiles for these IDs are shown in footnote *a* in Table 1, and the biochemical tests corresponding with these codes are listed in Table 2. A lack of biochemical reactivity is evident in most of the reactions (Table 2) and leads to poor discrimination of the isolates. Twenty-six isolates (1.8% of all colonies picked) were identified as *P. damselae* isolates, while 665 of the isolates (46.8%) could not be identified (did not have codes in the API database). Many of these were generally nonfermentative bacteria. Twenty-four-hour colonies of presumptive *S. putrefaciens* and other unidentified nonfermentative bacteria were small (≤3 mm), convex, circular, smooth, and pale orange, pink, or reddish. Presumptive *S. putrefaciens* isolates did not ferment D-glucose, D-mannitol, inositol, D-sorbitol, L-rhamnose, D-sucrose, D-melibiose, amygdalin, or L-arabinose (Table 2). It should be noted that the API database was originally designed to identify clinical pathogens but has evolved to include some environmental bacteria as well. *Shewanella putrefaciens* is the only species of *Shewanella* listed in the database, even though *S. algae* may be a more common pathogen (14, 18, 27).

Semiquantification of presumptive *S. putrefaciens* isolates was determined by API biochemical testing of isolates picked from dilution plates. Over the 2-year study, the highest counts were obtained for oysters in August 2004 ($\geq 3 \times 10^4$/g) and the second-highest counts were obtained in August 2005 ($\geq 2 \times 10^4$/g). Counts are listed as greater than or equal to a value because only a portion of the isolates on the TSA-N plates were picked for identification. The highest counts for seawater occurred in August 2004, with $\geq 2 \times 10^3$ presumptive *S. putrefaciens* counts/ml. As total *Vibrionaceae* counts diminished during the winter and early spring, *Shewanella* identifications were made more readily in lower-dilution plates from both oyster and seawater samples. Presumptive *S. putrefaciens* counts ranged from nondetectable to $\geq 4 \times 10^2$/g of oyster samples and $\geq 2 \times 10^1$/ml of seawater samples during late winter and early spring (March to June).

Oyster and seawater isolates identified by API 20E as either *S. putrefaciens* or nonfermentative bacteria that were not listed in the API database but had biochemical profiles similar to that of *S. putrefaciens* were sequenced and identified as *S. abalonesis*, *S. algae*, *S. baltica*, *S. hafniensis*, and *S. marisflavi* with 100% sequence identity (Table 1). To our knowledge, this is the first time these species have been isolated from shellfish or seawater from the Delaware Bay. One nonfermentative isolate that was unidentifiable by API was *S. putrefaciens* with >99% sequence identity; however, none of nine presumptive *S. putrefaciens* isolates, as determined by API 20E, were identified by sequencing as *S. putrefaciens* (Table 1). Of these nine presumptive *S. putrefaciens* isolates, two were sequenced as *P. damselae* subsp. *damselae*; five were *S. abalonesis*, *S. baltica*, *S. marisflavi* (two isolates), and the human pathogen *S. algae*; and the remaining two were *Listonella anguillarum*, a significant fish pathogen. The inability to differentiate *Shewanella* spp. using the API system was reported previously when *S. algae* was misidentified as *S. putrefaciens* (9, 14). Concerning the stock cultures, five of the six were incorrectly identified by API as *S. putrefaciens*, while the *S. putrefaciens* stock culture was uniden-

TABLE 1. Biochemical profiles and sequence identification of nonfermentative bacterial isolates from oysters and seawater from the Delaware Bay

Stock or source	API code[a]	Sequence identification[b]	Growth on 6% NaCl[c]	H_2S on TSI[d]	Hemolysis[e]	Growth at[f]:		
						25°C	37°C	42°C
Stock cultures								
Shewanella algae	**0402004**	*Shewanella algae*	+	+	α	+	+	+
Shewanella algidipiscicola	**0400004**	*Shewanella algidipiscicola*	– (25°C)	+	– (25°C)	+	–	–
Shewanella baltica	**0502004**	*Shewanella baltica*	– (25°C)	+	– (25°C)	+	–	–
Shewanella frigidamarina	**0400004**	*Shewanella frigidimarina*	– (25°C)	–	– (25°C)	+	–	–
Shewanella morhuae	**0402004**	*Shewanella morhuae*	– (25°C)	–	– (25°C)	+	–	–
Shewanella putrefaciens	0000004	*Shewanella putrefaciens*	–	–	α	+	+	–
Environmental samples								
Oysters	1402004	*Shewanella abalonesis* (99%)	+	+	α	+	+	–
Seawater	**0502004**	*Shewanella abalonesis* (>98%)	+	+	α	+	+	+
Oysters	**0402004**	*Shewanella algae*	–	+	β	+	+	+
Oysters	**0402004**	*Shewanella baltica* (>99%)	–	+	α	+	+	–
Seawater	0002004	*Shewanella baltica*	–	–	β	+	+	–
Oysters	0002004	*Shewanella baltica*	–	+	α	+	+	–
Oysters	0000004	*Shewanella hafniensis* (99%)	–	–	–	+	+	–
Oysters	**0400004**	*Shewanella marisflavi* (>99%)	–	+	α	+	+	–
Oysters	**0400004**	*Shewanella marisflavi* (>99%)	–	+	α	+	+	–
Oysters	0002004	*Shewanella putrefaciens* (>99%)	–	+	α	+	+	–
Oysters	**0400004**	*Photobacterium damselae* subsp. *damselae*	–	+	α	+	+	–
Oysters	**0402004**	*Photobacterium damselae* subsp. *damselae*	–	+	β	+	+	+
Oysters	1040004	*Photobacterium damselae* subsp. *damselae*	+	–	α	+	+	–
Oysters	0000004	*Listonella anguillarum*	–	+	α	+	+	–
Oysters	**0402004**	*Listonella anguillarum*	–	+	α	+	+	–
Oysters	0000004	*Listonella anguillarum*	–	+	α	+	+	–
Oysters	0002004	*Listonella anguillarum*	–	+	α	+	+	–
Oysters	0000004	*Listonella anguillarum*	–	–	α	+	+	–
Seawater	0002004	*Listonella anguillarum*	–	–	β	+	+	–
Seawater	**0402004**	*Listonella anguillarum*	–	+	α	+	+	–
Oysters	1002004	*Listonella anguillarum*	+	–	–	+	+	–
Seawater	1000004	*Listonella anguillarum*	+	–	α	+	+	–
Oysters	1040004	ND	+	–	α	+	+	–
Oysters	**0402004**	ND	–	+	α	+	+	–
Oysters	0002004	ND	–	–	α	+	+	–

[a] Bold type signifies API 20E identification of *Shewanella putrefaciens* (often in error), with API codes of 0502004 indicating an excellent ID, 0402004 indicating a very good ID, and 0400004 indicating an acceptable ID, according to the API database. Specific biochemical reactions for these codes are shown in Table 2.
[b] Identification based on 16S rRNA gene at 100% sequence identity (or other percentage indicated in parentheses). ND, sequence not determined.
[c] Growth on tryptic soy agar containing 6% total NaCl in 24 h at 37°C or, if noted in parentheses, at 25°C.
[d] H_2S produced from TSI in 24 h at 25°C.
[e] Alpha or beta hemolysis on blood agar plates in 24 h at 37°C or, if noted in parentheses, at 25°C. A minus (−) indicates no hemolysis.
[f] Growth within 24 h on tryptic soy agar containing 1% total NaCl at various incubation temperatures.

tifiable by API (Table 1). Seven other nonfermentative isolates that were not listed in the API database were sequenced and were identified as *L. anguillarum* (Table 1).

All isolates and stock cultures grew readily on TSA-N; however, only *S. abalonesis*, one of three biotypes of *P. damselae* subsp. *damselae*, one biotype of *L. anguillarum*, and the stock culture of *S. algae* were capable of growing in the presence of 6% NaCl (Table 1). Literature indicates that *S. algae* can be differentiated from *S. putrefaciens* by its ability to grow in 6% NaCl (14, 18); however, an oyster-associated *S. algae* isolate with 100% sequence identity to the 16S rRNA gene of *S. algae* could not be grown on agar containing 6% NaCl. This isolate did produce H_2S on TSI slants and exhibited β-hemolysis, both of which are reportedly characteristics of *S. algae* (14) (Table 1). Although oyster-associated *S. algae* produced β-hemolysis, the stock culture produced only α-hemolysis. Enzymes that are responsible for β-hemolysis are well established as virulence factors in a variety of bacterial pathogens, including the enterococci (23), *Escherichia coli* (3), *Listeria monocytogenes* (28), and *Vibrio parahaemolyticus* (24), and may well serve as an indicator of virulence in *Shewanella* spp. One of three species of *P. damselae* subsp. *damselae* was also beta-hemolytic (Table 1). *Listonella anguillarum* and *S. baltica*, species perceived as nonhuman pathogens, also exhibited β-hemolysis and growth at 37°C (Table 1).

Oyster and seawater isolates all grew at 25 and 37°C; however, the stock cultures, with the exception of *S. algae* and *S. putrefaciens*, grew only at 25°C (Table 1). It is generally reported that *S. baltica*, a cold-water species, will not grow at 37°C (40); however, three strains from the Delaware Bay (two of which had sequence identity to the 16S rRNA gene of *S. baltica*) grew readily at 37°C, suggesting the adaptation of *S. baltica* to warmer climates. Alternatively, stock cultures could behave atypically due to repeated laboratory passage. The fact that one seawater isolate with sequence identity to *S. baltica* was also beta-hemolytic at 37°C suggests a potential human pathogenic strain. In addition to the anticipated growth of *S. algae* at 42°C, one of the two biotypes of *S. abalonesis* and one

TABLE 2. Biochemical characteristics of bacterial isolates by API codes shown in Table 1

Biochemical reaction	Reactivity for isolate with API code[a]:								
	0000004	0002004	1000004	1002004	1040004	**0400004**[b]	**0402004**[c]	**0502004**[d]	1402004
β-Galactosidase	−	−	+	+	+	−	−	−	+
Arginine dihydrolase	−	−	−	−	−	−	−	−	−
Lysine decarboxylase	−	−	−	−	−	−	−	−	−
Ornithine decarboxylase	−	−	−	−	−	−	−	+	−
Citrate utilization	−	−	−	−	−	−	−	−	−
H_2S production	−	−	−	−	−	+	+	+	+
Urease	−	−	−	−	−	−	−	−	−
Tryptophane deaminase	−	−	−	−	−	−	−	−	−
Indole production	−	−	−	−	+	−	−	−	−
Voges Proskauer (acetoin production)	−	−	−	−	−	−	−	−	−
Gelatinase	−	+	−	+	−	−	+	+	+
D-Glucose fermentation/oxidation	−	−	−	−	−	−	−	−	−
D-Mannitol fermentation/oxidation	−	−	−	−	−	−	−	−	−
Inositol fermentation/oxidation	−	−	−	−	−	−	−	−	−
D-Sorbitol fermentation/oxidation	−	−	−	−	−	−	−	−	−
L-Rhamnose fermentation/oxidation	−	−	−	−	−	−	−	−	−
D-Sucrose fermentation/oxidation	−	−	−	−	−	−	−	−	−
D-Melobiose fermentation/oxidation	−	−	−	−	−	−	−	−	−
Amygdalin fermentation/oxidation	−	−	−	−	−	−	−	−	−
L-Arabinose fermentation/oxidation	−	−	−	−	−	−	−	−	−
Cytochrome oxidase	+	+	+	+	+	+	+	+	+

[a] Codes represented in bold type indicate the only codes for *Shewanella* spp. in the API database.
[b] API code indicating acceptable ID of *S. putrefaciens*.
[c] API code indicating very good ID of *S. putrefaciens*.
[d] API code indicating excellent ID of *S. putrefaciens*.

of the three biotypes of *P. damselae* subsp. *damselae* were capable of growth at this temperature (Table 1).

The seawater and oyster isolates and the stock cultures listed in Table 1 produced brightly fluorescent foci when colonies were overlaid with membranes during the colony overlay procedure for peptidases. This indicates that these cultures contain PGI-LysAP activity, which has been observed for all *Vibrionaceae* and *Aeromonidaceae* family members tested to date (29). Such activity was not previously detected in pathogens or nonpathogens from other families of bacteria (29), further suggesting the relatedness of *Shewanella* spp. to members of the *Vibrionaceae* family (21). *Photobacterium damselae* also produced bright fluorescent foci, as anticipated, since it is classified as *Vibrionaceae*.

Although API 20E misidentified isolates as *S. putrefaciens*, the isolates were related to each other biochemically as oxidase-positive, nonfermentative bacteria within the genera *Photobacterium* and *Listonella* (both *Vibrionaceae*) and *Shewanella* (also related by sequence to the *Vibrionaceae*). Unfortunately, there are no simple assays to detect *S. algae* or *S. putrefaciens* from among the many nonfermentative isolates present in the marine environment, and routine sequence confirmation of isolates is impractical. The lack of discrimination by the API-20E system for *Shewanella*-like bacteria is understandable since the isolates show little reactivity in biochemical testing, due in part to their nonfermentative nature (Table 2). The inability to differentiate *Shewanella* spp. from each other, the misidentification of species as *S. putrefaciens*, and the inability to identify even a stock culture of *S. putrefaciens* as *S. putrefaciens* highlight a need for improved identification schemes to identify pathogenic and nonpathogenic strains of *S. algae*, *S. putrefaciens*, and *P. damselae* subsp. *damselae*.

Quantification and analysis of the *Shewanella* and *Photobacterium* isolates were not an objective of our initial *Vibrio* study, and our data are very limited; however, the identification of beta-hemolytic *S. algae*, *S. baltica*, and *P. damselae* subsp. *damselae* isolates is of interest because of their potential pathogenicity. In fact, there is a general lack of knowledge about the presence and proliferation of these species in the Delaware Bay and in bays and tributaries worldwide. The infectious doses for these species are unknown, and summertime levels may be sufficiently high to elicit infection, particularly in immunocompromised individuals. The inability to properly identify *Shewanella* and *Photobacterium* species complicates disease diagnosis and may mask the true incidence of infection. When our isolates were subjected to the API-20E test, most were misidentified as *S. putrefaciens* or they remained unidentified because they were not listed in the API database, further demonstrating the need for improved differentiation methods. Our supplemental biochemical and growth temperature studies showed variability in reactions, depending on individual strain characteristics.

Previously, *Shewanella algae* was found at higher levels in seawater and shellfish during the warmer summer months (11, 14) and was associated with a higher incidence of illness during the summer (14). The beta-hemolytic strains of *S. algae* and *P. damselae* subsp. *damselae* showed both tolerance to and growth at temperatures as high as 42°C, thus demonstrating their permissiveness to high temperatures. It is not known whether *Shewanella* or *Photobacterium* species from the Delaware Bay have caused illnesses through ingestion of contaminated oysters, clams, or other seafood; by cuts or abrasions acquired in the marine environment; or by swimming or other recreational activities. However, their detection suggests the need for closer scrutiny of their levels and of any association they may have with disease.

In summary, this paper identified potentially pathogenic species of *Shewanella* and *Photobacterium* for the first time in the Delaware Bay. Species were identified by sequencing of their 16S rRNA genes, and this, at the present time, is the method of choice for their identification, since conventional biochemical methods failed to reveal their identity. We further identified hemolytic activity in some isolates, suggesting a pathogenic potential for these bacteria. High numbers of *Shewanella* and *Photobacterium* isolates in oysters and seawater during the summer months suggest that monitoring the levels of pathogenic species and strains should be continued in the Delaware Bay and expanded to other geographic locations.

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