Characterization and Identification of Marine *Alteromonas nigrifaciens* Strains and Emendation of the Description

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Nine nonpigmented strains of gram-negative, aerobic, marine bacteria with polar flagella were isolated from the mussels *Crenomytilus grayanus* and *Patinopecten jessoensis*. These organisms were conspecific and exhibited relatively high levels of genetic relatedness (61 to 100%). The G+C contents of the DNAs of these strains were 38.5 to 40.2 mol%. The strains isolated from mussels were phenotypically distinct from previously described *Alteromonas* species that have similar DNA G+C contents (*Alteromonas haloplanktis*, *Alteromonas tetradonitis*, *Alteromonas atlantica*, and *Alteromonas carrageenovora*), and their DNAs exhibited only 12 to 41% similarity with the DNAs of the type strains of these species. DNA-DNA hybridization data revealed that the levels relatedness between the strains which we studied and the type strain of *Alteromonas nigrifaciens* were significant (66 to 70%). Production of a melanin-like pigment, which is characteristic of *A. nigrifaciens*, was observed only in tyrosine-containing media. The strains isolated from mussels were identified as *A. nigrifaciens*. We present an emended description of *A. nigrifaciens* that includes several phenotypic and chemotaxonomic characteristics.

The genus *Alteromonas* Baumann et al. 1972 includes both nonpigmented and pigmented gram-negative, aerobic, polarly flagellated, marine bacterial species (4, 5, 7, 11), and there have been numerous proposals of new *Alteromonas* species recently (3, 10, 12, 16, 22). However, phenotypic discrimination of the species of this genus is problematic because of significant variations in their phenotypic traits. Phenotypic differences are frequently observed among even genetically closely related strains (2). Tests to determine utilization of specific carbon sources, which have been used successfully in *Pseudomonas* systematics (9), are less informative when they are used for differentiation of *Alteromonas* species. Some characteristics, such as requirements for growth factors and the production of pigments and some secondary metabolites, which were described as specific characteristics of certain species, have been shown to be strain-specific characteristics in recent studies. For example, one strain of *Alteromonas tetradonitis* produces tetrodotoxin, and recent investigations of the DNA relatedness of *Alteromonas* species have shown that *A. tetradonitis* is closely related genetically to *Alteromonas haloplanktis*, which never produces tetrodotoxin (10). Thus, genetic and chemotaxonomic methods appear to provide more reliable information than differential phenotypic characteristics in *Alteromonas* systematics.

We previously isolated several strains belonging to the genus *Alteromonas* from the Far Eastern mussels *Crenomytilus grayanus* and *Patinopecten jessoensis* (13). The purpose of this study was to characterize and identify these strains by using phenotypic, genetic, and chemotaxonomic methods in order to provide additional taxonomic data for *Alteromonas* species.

MATERIALS AND METHODS

Bacterial strains and isolation. The strains which we used are listed in Table 1. Mussels (*C. grayanus* and *P. jessoensis*) were collected in 1989 and 1990 at the Pacific Institute Bio-Organic Chemistry Marine Experimental Station, Troitz Bay, Gulf of Peter the Great, Sea of Japan. The mussels were collected at a depth of 8 m (salinity, 33‰; temperature, 12°C) and were prepared aseptically. Each sample of tissue (3 to 5 g) was homogenized in 10 to 15 ml of sterile seawater in a sterile glass homogenizer. Then 0.1 to 0.2 ml of the homogenate was spread onto plates containing marine agar 2216 (Difco) (1) and plates containing medium B, which contained 0.2% (wt/vol) Bacto Peptone (Difco), 0.2% (wt/vol) casein hydrolysate (Merck), 0.2% (wt/vol) yeast extract (Difco), 0.1% (wt/vol) glucose, 0.02% (wt/vol) KH_2PO_4 , 0.005% (wt/vol) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1.5% (wt/vol) Bacto Agar (Difco), 50% (vol/vol) natural seawater, and 50% distilled water. The pH of the medium was adjusted to 7.8 with 1 M NaOH. All of the plates were incubated at 25 to 28°C. The strains were isolated from single colonies after 7 days of incubation, purified (14), and stored on the same semisolid medium in tubes under mineral oil at 4°C.

Phenotypic analysis. The phenotypic characteristics of each strain were determined by the methods described by Baumann et al. (4, 5) and Smibert and Krieg (23). The following physiological and biochemical properties were examined: oxidation or fermentation of glucose (18); arginine dihydrolase, lysine decarboxylase, and ornithine decarboxylase activities; accumulation of poly- β -hydroxybutyrate; cell pigmentation; cell morphology; Gram staining; motility; sodium requirement; oxidase (17), catalase, urease, indole, and H_2S production; and the ability to hydrolyze gelatin, starch, Tween 80, and chitin. The requirement for Na^+ ions was determined on medium that contained 0.25% (wt/vol) yeast extract, 0.1% (wt/vol) glucose, 0.02% (wt/vol) KH_2PO_4 , and 0.005% (wt/vol) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (pH 7.8). The tests for utilization of various organic substrates as sole carbon sources (at a concentration of 0.1% [wt/vol]) were performed on synthetic solid BM medium (4). In some cases liquid BM medium (10 ml per tube) was used; the bacteria were grown with shaking on a rotary shaker at 160 rpm for 72 h at 24 to 26°C.

Susceptibility to antibiotics was determined by using the routine diffusion plate technique, plates containing solid medium B, and disks impregnated with following antibiotics: rifampin (15 μg), ristomycin (150 μg), kanamycin (10 μg), ampicillin (10 μg), benzylpenicillin (10 μg), streptomycin (15 μg), neomycin (15 μg), erythromycin (15 μg), gentamicin (10 μg), oxacillin (20 μg), cephalixin (10 μg), polymyxin (50 μg), lincomycin (10 μg), and ofloxacin (10 μg).

Genetic analysis. DNA was isolated by the method of Marmur (20). The G+C content of each DNA was determined by the thermal denaturation method of Marmur and Doty (21). Levels of DNA-DNA hybridization were determined spectrophotometrically, and initial renaturation rates were determined as described by De Ley et al. (8) and Levanova et al. (19).

Production and characterization of soluble pigment. Production of a soluble pigment was studied by using medium B and medium BT (medium B supple-

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TABLE 1. Strains studied

Species	Strain ^a	Other designation(s) ^a	Source ^a
<i>A. nigrifaciens</i>	KMM 160	2MC41	Mussel (<i>C. grayanus</i>), Troitza Bay, Sea of Japan
	KMM 159	2MC45	Mussel (<i>C. grayanus</i>), Troitza Bay, Sea of Japan
	KMM 155	2MC12	Mussel (<i>C. grayanus</i>), Troitza Bay, Sea of Japan
	KMM 154	2MC19	Mussel (<i>C. grayanus</i>), Troitza Bay, Sea of Japan
	KMM 153	2MC32	Mussel (<i>C. grayanus</i>), Troitza Bay, Sea of Japan
	KMM 156	2ML26	Mussel (<i>C. grayanus</i>), Troitza Bay, Sea of Japan
	KMM 298	4ML18	Mussel (<i>C. grayanus</i>), Troitza Bay, Sea of Japan
	KMM 158	2MM6	Mussel (<i>C. grayanus</i>), Troitza Bay, Sea of Japan
	KMM 161	2GM 32	Mussel (<i>P. jessoensis</i>), Troitza Bay, Sea of Japan
	KMM 661 ^{Tb}	LMG 2227 ^T , NCTC 10691 ^T , ATCC 19375 ^T , ATCC 23327 ^T	LMG
<i>A. macleodii</i>	KMM 661 ^T	IAM 13010 ^T , ATCC 19375 ^T , NCIMB 8614 ^T , NCTC 10691 ^T	U. Simidu
<i>A. haloplanktis</i>	KMM 568 ^T	ATCC 27126 ^T , IAM 12920 ^T , NCIMB 1963 ^T , Baumann 107 ^T	ATCC
	KMM 460 ^T	IAM 12915 ^T , ATCC 14393 ^T , NCIMB 2084 ^T , Zobell & Upham 545 ^T	U. Simidu
<i>A. tetraodonis</i> (<i>A. haloplanktis</i>)	KMM 458	IAM 14160	U. Simidu
<i>A. carrageenovora</i>	KMM 656 ^T	IAM 12662 ^T , ATCC 43555 ^T , NCIMB 302 ^T	M. Akagawa-Matsushita
<i>A. atlantica</i>	KMM 655 ^T	IAM 12376 ^T , IAM 12927 ^T , NCIMB 301 ^T , ATCC 19262 ^T	M. Akagawa-Matsushita

^a ATCC, American Type Culture Collection, Rockville, Md.; IAM, Institute of Molecular and Cellular Biosciences (formerly Institute of Applied Microbiology), University of Tokyo, Bunkyo-ku, Tokyo, Japan; KMM, Collection of Marine Microorganisms, Pacific Institute of Bio-Organic Chemistry, Vladivostok, Russia; LMG, Collection of Bacteria of the Laboratory of Microbiology, University of Ghent, Ghent, Belgium; NCIMB, National Collection of Industrial and Marine Bacteria, NCIMB, Ltd., Torry Research Station, Aberdeen, Scotland; NCTC, National Collection of Type Cultures, London, England.

^b T = type strain.

mented with 0.1% [wt/vol] L-tyrosine). We also used synthetic medium S, which contained 0.05% (wt/vol) KH₂PO₄, 0.05% (wt/vol) K₂HPO₄, 0.005% (wt/vol) MgSO₄ · 7H₂O, 0.06% (wt/vol) CuSO₄, 0.005% (wt/vol) CaCl₂, 20 mg of L-methionine, 20 mg of DL-tryptophan, 10 mg of α-asparagine, 10 mg of DL-phenylalanine, 0.2% (wt/vol) yeast extract, and 0.1% (wt/vol) (NH₄)₂SO₄ (pH 7.6); medium ST was medium S supplemented with 0.1% (wt/vol) L-tyrosine. Basal medium BT was also used with inhibitors of melanogenesis (L-cysteine, EDTA, ascorbic acid). All inhibitors were added at final concentrations of 1 to 10 mM. The solubility of pigments was determined with 0.5-ml portions of cultural fluid in 2-ml aliquots of various solvents. Pigments were considered soluble if the solvent was colored after agitation and insoluble if no color was imparted to the solvent.

RESULTS AND DISCUSSION

Phenotypic characteristics and G+C contents of DNAs. The bacteria isolated from Far Eastern mussels were motile gram-negative rods; each cell had a polar flagellum. Electron micrographs of the bacteria have been published elsewhere (13). Some cells were surrounded by a capsule. No strain accumulated poly-β-hydroxybutyrate as an intracellular reserve material and had an arginine dihydrolase system. All strains were strictly aerobic (negative in the oxidation-fermentation test) and oxidase positive and required Na⁺ ions or seawater for growth. The strains which we studied differed from the type strain by exhibiting amylase activity and having a wider temperature range for growth. The G+C contents of the DNAs ranged from 38.5 to 40.2 mol%. All of these data suggested that the strains which we isolated belonged to the genus *Alteromonas*. The results of an investigation of the phenotypic properties of these organisms (Table 2) allowed us to distinguish them from the nonpigmented species that have similar DNA G+C contents (*A. haloplanktis*, *A. tetraodonis*, *Alteromonas atlantica*, *Alteromonas undina*, *Alteromonas espejiana*) and from *Alteromonas carrageenovora*. The differential characteris-

tics of these species are shown in Table 3. Most of the nine strains isolated from mussels differed from members of other *Alteromonas* species by utilizing mannose, fructose, sucrose, pyruvate, glycerol, and rhamnose. The strains which we studied had some phenotypic characteristics that differentiated them from the type strain of *A. nigrifaciens*. All of them produced amylase, and most of them grew at 35°C, utilized mannitol, adonitol, L-ornithine, and L-phenylalanine, and did not utilize citrate and glutarate.

DNA relatedness. The results of our DNA-DNA hybridization experiments are shown in Table 4. The levels of DNA-DNA relatedness among the strains isolated from mussels ranged from 61 to 100%. These data indicate that the nine strains isolated from mollusks belong to the same species. The levels of DNA-DNA similarity between these organisms and the type strains of *Alteromonas macleodii*, *A. haloplanktis*, and *A. tetraodonis* ranged from 12 to 41%. In contrast, the levels of DNA-DNA homology between the type strain *A. nigrifaciens* and the strains isolated from mussels were 66 to 70%. On the basis of the generally accepted criterion (27), we concluded that the strains isolated from mussels were *A. nigrifaciens* strains.

Production of melanin-like soluble pigment. A distinctive feature of *A. nigrifaciens* is its ability to produce a melanin-like pigment (as reflected by the name of the species). The strains which we isolated did not synthesize any pigment when they were grown on common media, such as marine agar 2216 or medium B. We also examined the formation of pigments on tyrosine-containing media that are optimal for melanogenesis by the type strain of *A. nigrifaciens* and on the same media in the presence of inhibitors of melanogenesis, including cysteine, EDTA, and ascorbic acid (Table 5). We found that all of the

TABLE 2. Characteristics of nine strains of *A. nigrifaciens* isolated from marine mollusks and *A. nigrifaciens* IAM 13010^T

Characteristic	KMM 160	KMM 159	KMM 158	KMM 161	KMM 155	KMM 153	KMM 156	KMM 154	KMM 294	<i>A. nigrifaciens</i> IAM 13010 ^T	
										Study of Baumann et al. ^a	This study
Polar flagellum	+	+	+	+	+	+	+	+	+	+	+
Oxidase activity	+	+	+	+	+	+	+	+	+	+	+
Pigmentation	—	—	—	—	—	—	—	—	—	+	+
Na ⁺ required for growth	+	+	+	+	+	+	+	+	+	+	+
Requirement for organic growth factors	—	—	—	—	—	—	—	—	—	+	—
Arginine dihydrolase activity	—	—	—	—	—	—	—	—	—	—	—
Denitrification	—	—	—	—	—	—	—	—	—	—	—
Production of:											
Gelatinase	+	+	+	+	+	—	+	+	+	+	+
Amylase	+	+	+	+	+	+	+	+	+	—	—
Lipase	+	+	+	+	+	+	+	+	+	+	+
Chitinase	—	—	—	—	—	—	—	—	—	—	—
Growth at:											
4°C	—	—	+	+	+	—	+	—	+	+	+
10°C	+	+	+	+	+	+	+	+	+	ND	+
28°C	+	+	+	+	+	+	+	+	+	ND	+
35°C	v	v	—	—	+	v	—	+	—	—	—
41°C	—	—	—	—	—	—	—	—	—	—	—
Utilization of:											
D-Glucose	+	+	+	+	+	+	+	+	+	ND	+
D-Ribose	+	+	+	+	+	—	+	+	+	ND	+
D-Xylose	—	+	—	—	—	—	+	+	+	—	+
D-Arabinose	+	—	—	—	+	+	+	+	—	—	+
D-Rhamnose	—	+	—	—	+	—	+	+	+	—	+
D-Mannose	+	—	—	+	+	—	+	+	+	—	+
D-Galactose	+	+	+	+	+	+	+	+	+	+	+
D-Fructose	+	+	+	+	+	—	+	+	—	+	+
Sucrose	+	+	+	+	+	+	—	+	+	—	+
Trehalose	+	+	—	+	+	+	+	+	+	ND	+
Maltose	+	+	+	+	+	+	—	+	+	ND	+
Cellobiose	—	—	—	—	+	+	+	+	—	—	+
Lactose	+	—	+	+	—	—	—	+	+	+	+
Gluconate	+	+	—	—	—	—	+	+	+	+	+
Salicin	—	—	—	—	—	—	—	+	+	—	—
Acetate	+	—	—	—	—	—	+	+	+	ND	+
Propionate	+	—	+	+	+	+	+	+	+	ND	+
Butyrate	+	+	+	+	—	—	+	+	+	+	+
Valerate	+	—	+	+	+	+	+	+	+	+	+
Caproate	+	—	—	+	+	+	+	+	+	+	+
Succinate	+	+	—	+	—	—	+	+	+	+	+
Fumarate	+	—	—	—	+	—	—	—	+	+	—
α-Glutarate	—	—	—	—	—	—	—	—	+	ND	+
DL-Lactate	+	+	+	+	+	+	+	+	+	+	+
Citrate	—	—	—	—	—	—	—	—	—	+	+
α-Ketoglutarate	—	—	—	—	—	—	—	—	+	ND	—
Pyruvate	+	+	+	+	+	+	+	+	+	ND	+
Aconitate	—	+	+	+	+	+	+	+	+	+	+
Glycerol	+	—	+	—	—	—	+	+	—	+	+
Caprylic acid	—	—	—	—	+	+	+	+	—	ND	—
Pelargonic acid	—	—	—	—	—	—	—	—	—	ND	—
Glycogen	+	+	+	+	+	+	+	+	+	ND	+
Mannitol	+	+	+	+	+	+	+	+	+	—	—
D-Sorbitol	+	+	+	+	+	+	+	+	+	ND	+
Inositol	+	+	+	+	—	+	+	+	+	ND	—
Adonitol	+	+	+	+	—	—	—	—	+	ND	—
Ethanol	+	+	—	+	—	+	+	+	+	+	+
p-Hydroxybenzoate	+	+	+	—	+	+	+	+	+	ND	+
Phenylacetic acid	+	+	+	+	—	+	+	+	+	ND	+
L-α-Alanine	+	+	+	+	+	+	+	—	+	+	+
L-Threonine	+	+	+	+	+	+	+	+	+	—	+
Aspartic acid	—	—	—	—	—	—	—	—	—	ND	—
Glutamic acid	+	—	+	+	+	+	—	+	+	ND	+
L-Lysine	+	+	+	+	+	+	+	+	+	—	+
L-Arginine	+	+	+	—	+	+	+	+	+	—	+
L-Ornithine	+	+	+	+	+	+	+	+	+	—	—

Continued on following page

TABLE 2—Continued

Characteristic	KMM 160	KMM 159	KMM 158	KMM 161	KMM 155	KMM 153	KMM 156	KMM 154	KMM 294	<i>A. nigrifaciens</i> IAM 13010 ^T	
										Study of Baumann et al. ^a	This study
L-Histidine	+	+	—	+	—	—	—	+	+	—	+
L-Proline	+	—	+	+	+	+	+	—	+	—	+
L-Tyrosine	+	+	+	+	+	+	+	+	—	+	+
L-Phenylalanine	+	+	+	+	+	+	+	—	—	—	—
Susceptibility to:											
Rifampin (15 µg)	+	ND	+	+	+	+	+	ND	ND	ND	+
Ristomycin (150 µg)	—	ND	—	w	+	—	w	ND	ND	ND	—
Kanamycin (10 µg)	w	ND	—	—	w	—	w	ND	ND	ND	—
Ampicillin (10 µg)	+	ND	+	+	+	+	+	ND	ND	ND	+
Benzyl-penicillin (10 µg)	—	ND	+	—	—	+	+	ND	ND	ND	+
Streptomycin (15 µg)	+	ND	w	w	w	+	+	ND	ND	ND	+
Neomycin (15 µg)	w	ND	—	—	—	+	+	ND	ND	ND	w
Erythromycin (15 µg)	+	ND	+	+	+	+	+	ND	ND	ND	+
Gentamicin (10 µg)	+	ND	+	+	+	+	+	ND	ND	ND	+
Oxacillin (20 µg)	—	ND	—	—	—	—	—	ND	ND	ND	—
Cephalexin (10 µg)	—	ND	—	—	—	—	—	ND	ND	ND	—
Polymyxin (50 µg)	+	ND	+	+	+	+	+	ND	ND	ND	+
Lincomycin (10 µg)	—	ND	—	—	—	—	—	ND	ND	ND	—
Ofloxacin (10 µg)	+	ND	+	w	w	+	+	ND	ND	ND	+

^a Data from reference 6.^b +, positive; —, negative; v, variable; ND, no data available; w, weakly susceptible.

nonpigmented strains isolated from mussels except strain 2 MC12 produced pigment on media containing tyrosine and were colorless on the media that contained the inhibitors. The pigment extracted from cells with a 0.5 N NaOH solution formed a brown solution. The alkaline pigment solution reacted qualitatively like melanin; it became colorless when H₂O₂ was added, and addition of KMnO₄ resulted in a green solution. The absorption maximum of the pigment (225 nm) was close to the absorption maximum for synthetic melanin (Sigma) (223 nm). The data in Table 6 confirmed the melanin-like nature of the pigments tested.

The differences in pigment production and in utilization of some sources of carbon between the strains associated with mussels and other representatives of *A. nigrifaciens* might depend on the habitats of the strains. We found that bacterial

associates of mussels had more active hydrolytic enzymes (amylases, gelatinases, lipases, alkaline phosphatases, α-galactosidase, etc.) than bacteria isolated from seawater. The most likely reason for these differences is symbiotrophic relationships between bacteria and mussels.

Antimicrobial agent susceptibility. The nine strains of *A. nigrifaciens* examined were susceptible to rifampin, ampicillin, erythromycin, gentamicin, polymyxin, and ofloxacin but were not susceptible to lincomycin, oxacillin, and cephalexin.

Description of *A. nigrifaciens* strains. Only one strain of *A. nigrifaciens* has been described previously (6). This strain was isolated by White (28) and was designated "*Pseudomonas nigrifaciens*," and it was later renamed because of its close relationship with species belonging to the *A. haloplanktis* rRNA cistron group (26). Detailed phenotypic, genetic, and chemo-

TABLE 3. Differential phenotypic characteristics of *Alteromonas* species

Characteristic	<i>A. haloplanktis</i> IAM 12915 ^T	<i>A. tetraodonis</i> IAM 14160 ^T	<i>A. atlantica</i> IAM 12975 ^T	<i>A. carrageenovora</i> IAM 12662 ^T	<i>A. espejiana</i> IAM 12640 ^T	<i>A. undina</i> IAM 12922 ^T	<i>A. nigrifaciens</i>
Melanin-like dark pigment ^a	+ ^b	—	—	+	—	—	d
Production of:							
Amylase	—	—	+	—	+	+	+
Chitinase	—	—	—	—	—	+	—
Growth at:							
4°C	—	—	—	+	—	+	d
35°C	+	+	+	+	+	+	d
Utilization of:							
D-Mannose	—	—	+	—	—	—	+
D-Fructose	+	—	+	+	+	—	+
Sucrose	—	+	+	+	+	+	+
Pyruvate	+	+	+	—	+	—	+
Fumarate	+	+	+	+	+	—	—
Glycerol	—	—	+	+	+	—	d
Rhamnose	—	—	—	—	—	—	d
Citrate	+	—	+	+	+	—	—

^a Data from reference 3.^b +, positive; —, negative; d, 11 to 89% of the strains are positive.

TABLE 4. Levels of DNA relatedness among strains

Strain	G+C content (mol%)	% Hybridization with DNA from:							
		KMM 160	KMM 159	KMM 158	KMM 161	KMM 155	KMM 153	KMM 156	KMM 298
<i>A. nigrifaciens</i> IAM 13010 ^T	39.2	66				70			
KMM 160	39.3		61			83		92	
KMM 159	40.2	61							
KMM 158	38.5							93	
KMM 161	39.6						100		
KMM 155	39.0	83						85	
KMM 153	38.5							65	
KMM 156	39.2	92		93					74
KMM 154	39.2			61		100			
KMM 298	39.5							74	
<i>A. macleodii</i> ATCC 27126 ^T	44.2				22		16	12	
<i>A. haloplanktis</i> IAM 12915 ^T	43.4					16		19	
<i>A. tetraodonis</i> IAM 14160 ^T	36.1	29				37		41	
<i>A. atlantica</i> IAM 12927 ^T	41.5								40
<i>A. carrageenovora</i> IAM 12662 ^T	38.9								36

taxonomic characteristics of *Alteromonas nigrifaciens* strains isolated from marine animals have not been described previously. The intraspecific variation of the species is discussed below, and an emended description of *A. nigrifaciens* is presented below.

There are no data about capsulated representatives of the genus *Alteromonas* available. Recently, we described the diversity of the polysaccharides produced by the *Alteromonas* strains that have been studied (13). The following monosaccharides of *A. nigrifaciens* capsules were identified: D-glucolactylic acid; L-gulosamine uronic acid; L-galactosamine uronic acid; bacillosamine, 4-aminoquinovose acylated with an alanine residue; and 3-aminofucose acylated with a 4-hydroxybutyryl residue. The last N-acyl sugar derivative has not been found previously as a constituent of bacterial polysaccharides. We also examined the susceptibility of cells to antibiotics. The strains which we studied were susceptible to rifampin, ampicillin, erythromycin, gentamicin, polymyxin and ofloxacin but were not susceptible to lincomycin, oxycillin, and cephalixin.

The overall levels of polyamines in *A. nigrifaciens* were low, but putrescine, cadaverine, and spermidine were present (3a). Small quantities (0.2 to 0.6 $\mu\text{mol/g}$ [dry weight]) of 2-hydroxyputrescine and spermine were detected in two strains. Svetashev et al. (24) reported that palmitoleic acid [16:1(n-7)], palmitic acid (16:0), heptadecenoic acid (17:1), and vaccenic acid [18:1(n-7)] were the most abundant fatty acids in strains of *A. nigrifaciens*. They also showed that low levels of the branched-chain fatty acids and certain hydroxy fatty acids and the absence of cyclopropane acids are charac-

teristic features of these strains as well as of other *Alteromonas* species.

On the basis of the properties mentioned above, we present the following emended description of *A. nigrifaciens*.

Emended description of *Alteromonas nigrifaciens* Baumann, Baumann, Bowditch, and Beaman 1984. *Alteromonas nigrifaciens* (ni.gri.fa'ci.ens. L. adj. *niger*, black; L. v. *facio*, to make; M.L. part. adj. *nigrifaciens*, blackening). Cells are gram-negative, strictly aerobic, straight rods that are 0.8 to 1.2 μm wide and 1.8 to 2.3 μm long. They are motile by means of a single flagellum at one pole. Some cells are encapsulated. Cells grow at 4 to 30°C. Some strains grow at 35°C, but no strain grows at 41°C. A melanin-like dark pigment is produced by almost all strains in L-tyrosine-containing media, particularly at low temperatures. Produces oxidase and lipase. Sodium ions are required for growth. Growth factors are not required. Chitinase and arginine dihydrolase negative. Does not denitrify. Grows on D-glucose, D-galactose, DL-lactate, pyruvate, D-sorbitol, L-threonine, and L-lysine. Pelargonate and aspartic acid are not used as sole carbon sources. Susceptible to rifampin, ampicillin, erythromycin, gentamicin, polymyxin, and ofloxacin, but not to lincomycin, oxacillin, and cephalixin. Other biochemical characteristics are shown in Table 3. The major polyamines are putrescine, cadaverine, and spermidine. The major cellular fatty acids are 16:1(n-7), 16:0, 17:1, and 18:1(n-7). Isolated from seawater and mussels, as well from salted food. The G+C content of the DNA is 39 to 41 mol%.

The type strain is strain KMM 661 (= LMG 2227 = IAM 13010 = ATCC 19375 = NCTC 10691).

TABLE 5. Production of melanin-like dark pigments by *A. nigrifaciens* strains in different media

Medium	KMM 160	KMM 159	KMM 158	KMM 161	KMM 155	KMM 153	KMM 156	KMM 154	KMM 294	<i>A. nigrifaciens</i> IAM 13010 ^T
B	— ^a	—	—	—	—	—	—	—	—	+
BT	w	—	w	+	—	+	+	w	w	+
S	—	—	—	—	—	—	—	—	—	+
ST	w	w	w	+	—	w	+	w	—	+
BT + cysteine (1–10 mM)	—	—	—	—	—	—	—	—	—	+
BT + EDTA (1–10 mM)	—	—	—	—	—	—	—	—	—	—
BT + ascorbic acid (1–10 mM)	—	—	—	—	—	—	—	—	—	—

^a —, no pigment produced; +, pigment produced; w, weak brown pigment produced.

TABLE 6. Characterization of pigments produced by *A. nigrifaciens* strains isolated from mollusks

Organism	Color of pigment	Solubility of pigment in ^a :		Precipitation of pigment			Peak(s) in absorption spectrum (350–650 nm)	Tyrosinase activity
		Water	NaOH (0.1 M)	Blackberg-Wanger technique	From NaOH by HCl	By FeCl ₃		
<i>A. nigrifaciens</i> IAM 13010 ^T	Brown	I	S	ND ^b	+	+	ND	ND
KMM 160 (= 2MC41)	Brown	I	S	ND	+	+	ND	ND
KMM 159 (= 2MC45)	Brown	I	S	ND	+	+	ND	ND
KMM 158 (= 2MM6)	Brown	I	S	ND	+	+	ND	ND
KMM 161 (= 3GM32)	Brown	I	S	ND	+	+	225	ND
KMM 156 (= 2ML26)	Brown	I	S	ND	+	+	ND	ND
<i>Aspergillus nidulans</i> ^c	Black	I	S	ND	+	+	480, 535	ND
<i>Shewanella colwelliana</i> ^d	Brown	I	S	+	+	+	223, 264, 406	+

^a I, insoluble; S, soluble.^b ND, no data available.^c Data from reference 25.^d Data from reference 15.

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ADDENDUM

The members of the “*A. haloplanktis*” rRNA cluster and [*Pseudomonas*] *piscicida* have been reclassified as separate species of the genus *Pseudoalteromonas* (10a).

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