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Characterization of *Alteromonas denitrificans* sp. nov.

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Eleven strains of gram-negative, denitrifying, prodigionine-producing marine bacteria with sheathed flagella were recovered from the fjord system off the Norwegian west coast. These isolates form a physiologically and morphologically very homogeneous group. Apart from their ability to denitrify and having sheathed flagella, the strains correspond closely to the current description of the genus *Alteromonas*. Their strictly respiratory metabolism precludes their inclusion in the genus *Vibrio*, and the low guanine-plus-cytosine contents of their deoxyribonucleic acids preclude their inclusion in the genus *Pseudomonas* or the genus *Marinomonas*. Despite their ability to denitrify, we suggest that these organisms belong to the genus *Alteromonas* and constitute a new species, *Alteromonas denitrificans*. A detailed description of the type strain of *Alteromonas denitrificans*, Nygaard 1977, is given. The type strain has been deposited with the American Type Culture Collection, Rockville, Md., as strain ATCC 43337.

In conjunction with studies on bacterial degradation of excess sperm produced during spawning of marine organisms (4), we have for several years been working with a group of prodigionine-producing, arginine-degrading, denitrifying marine bacteria with sheathed flagella. In the past we felt unable to assign our strains unequivocally to any established genus, but the recent suggestion (15) that bacteria with sheathed flagella be admitted to the genus *Alteromonas* prompted us to suggest that bacteria capable of vigorous denitrification but otherwise largely conforming to the current description of *Alteromonas* also might be admitted to this genus.

Baumann et al. (2) stated that the genus *Alteromonas* should be regarded as a temporary refuge for a number of common marine bacterial species whose evolutionary relationships are, for the most part, unknown. Recognizing the heterogeneity of this genus, van Landshoot and De Ley (18) proposed, on the basis of biochemical properties, ribosomal ribonucleic acid hybridization, and deoxyribonucleic acid (DNA) guanine-plus-cytosine (G+C) contents, the formation of the new genus *Marinomonas*, containing two former members of the genus *Alteromonas*. The *Marinomonas* species have G+C contents in the range from 46 to 49 mol%. The nonfermentative nature of our strains precludes their inclusion in the genus *Vibrio*, and the low G+C contents of their DNAs preclude their inclusion in the genus *Pseudomonas* or the genus *Marinomonas*. Despite the fact that these organisms have sheathed flagella and the fact that they are capable of effective denitrification with gas formation, our results suggest that these strains should be included in the genus *Alteromonas*. The alternative, to suggest the formation of a new genus, seems unwarranted at this stage. However, our strains do differ in sufficient characteristics from previously described strains of *Alteromonas* to warrant inclusion in a new species, *Alteromonas denitrificans*.

MATERIALS AND METHODS

Media. Enrichment medium contained (final concentrations) 2.1 g of L-arginine per liter, 7.3 g of L-glutamine per liter, and 10 ml of an iron phosphate stock solution (8.71 g of K_2HPO_4, 2.75 g of $FeSO_4$, and 32.7 g of disodium ethylenediaminetetraacetate in 1,000 ml of distilled water) per liter made up in the seawater samples to be enriched. Stock solutions of L-arginine, L-glutamine, and iron phosphate were sterilized by filtration. Growth medium contained (final concentrations) 0.5 g of Bacto-Peptone (Difco Laboratories) per liter, 0.5 g of tryptone (Difco) per liter, and 0.5 g of yeast extract (Difco) per liter made up in dilution water (200 g of tap water in 800 g of aged seawater). This medium was adjusted to pH 8 after autoclaving; 15 g of Bacto-Agar (Difco) was added for solid media.

Isolation, cultivation, and storage. Water samples were taken with Niskin samplers at depths ranging from 90 to 100 m and were used for enrichment cultures. The enriched samples were incubated at 10°C aerobically in the dark. Subsamples were taken daily, suitably diluted, plated onto solid growth medium, and incubated at 10°C. Prodigionine-producing bacteria were isolated, and the pure cultures were maintained either on agar slants of growth medium (requiring monthly transfers) or as diluted cell suspensions in unsupplemented seawater.

Electron microscopy. To study flagellar morphology, cells from dense cultures in liquid medium were placed on carbon- and Formvar-coated grids, washed with distilled water, and negatively stained with a uranyl acetate solution (5 g/liter in distilled water) for 4 min. After staining the preparations were viewed with a JEOL model 100S transmission electron microscope. To study internal structure, the cells were fixed with glutaraldehyde, stained with OsO_4, embedded in Spurr resin, and sectioned with an LKB Ultrotome III ultramicrotome as described by Hayat (8).

Assays for Gram stain, for catalase, oxidase, alginase, agarase, amylase, chitinase, and gelatinase activities, and for acetoin production. Assays were performed as described by Skerman (17), with minor adaptions made necessary by the marine nature of the bacteria. Cells for the assays were grown on either solid or liquid growth medium.

Assay for deoxyribonuclease activity. The assay for deoxyribonuclease activity was performed as described by Jeffries et al. (10), using aged seawater instead of 2% (wt/vol) NaCl.

Assay for lipase. The assay for lipase was performed as described by Sierra (16), using solid growth medium supplemented with 10 g of polyoxyethylene sorbitan monooleate (Tween 80) per liter.

Differentiation between oxidative and fermentative carbohydrate metabolism. Oxidative carbohydrate metabolism and

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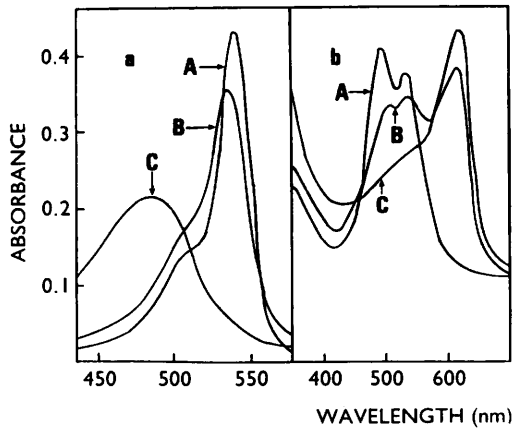

FIG. 1. (a) Absorption spectra of pigment extracts from *A. denitrificans* ATCC 43337^T. Spectrum A, Chloroform extract, pH 4; spectrum B, ethanol extract, pH 4; spectrum C, ethanol extract, pH 10. (b) In vivo absorption spectra of an *A. denitrificans* ATCC 43337^T culture grown under aerobic conditions at 6°C. Spectrum A, Blue-colored culture grown on arginine as a sole source of carbon; spectrum B, purple-colored culture grown on glutamine as a sole source of carbon; spectrum C, red-colored culture grown on growth medium.

fermentative carbohydrate metabolism were differentiated as described by Hugh and Leifson (9), using dilution water in making up the media. All carbohydrate substrates were made up as 100-g/liter stock solutions and were sterilized by filtration prior to use. Aerobic and anaerobic cultures were set up in triplicate.

Ability to utilize single compounds as sources of energy, carbon, and nitrogen. Organic carbon sources (final concentrations, 1 g/liter) were added from filter-sterilized stock solutions to a mineral base consisting of 10 ml of iron phosphate stock solution and 1.3 g of $(NH_4)_2SO_4$ in 1 liter of dilution water. The mineral base was adjusted to pH 7.5 prior to autoclaving. When we assayed for the ability to utilize nitrogen-containing compounds, $(NH_4)_2SO_4$ was omitted from the media.

Assays for arginine dihydrolase activity. Assays for arginine dihydrolase activity were carried out as described by Dundas and Halvorson (5), with adaptations made necessary by the marine nature of the bacteria. Whole washed cells and cell-free extracts were incubated with L-arginine, and the rates of formation of citrulline, ornithine, urea, and ammonium were measured.

Requirement for growth factors. A medium identical to that used in determining the ability to use glucose as the single source of energy, but made up with synthetic seawater (RILA), was used. Vitamin-free Casamino Acids (Difco) were added to a final concentration of 20 μg/ml.

Denitrification. Denitrification was assayed in growth medium supplemented with 2 g of KNO_3 per liter. Assay tubes were closed with semisolid paraffin. Nitrite was detected as described by Skerman (17).

Effect of temperature on growth rate. The effect of temperature on growth rate was studied by using a previously described temperature gradient incubator (14). Cells were grown in growth medium in 20-ml optically calibrated tubes fitted with screw caps. Filtered air was bubbled through aerobic cultures, and 2 g of KNO_3 per liter was added to growth medium in paraffin-capped anoxic cultures. Growth was measured turbidimetrically, and gas production in anoxic cultures was registered visually. All assays were carried out in triplicate.

Effect of salinity on growth rate. The effect of salinity on growth rate was studied by using growth medium made up with synthetic seawater (RILA) instead of natural seawater. Salinity was controlled by diluting the synthetic seawater with distilled water and by adding NaCl to the desired salt concentration. The cultures were incubated on a shaker at 6°C in 250-ml flasks with optically calibrated side arms. Growth was measured turbidimetrically.

DNA isolation. DNA was isolated by using the method of Marmur (13), with minor modifications. Cells were harvested from growth medium by centrifugation. Saline ethylenediaminetetraacetate supplemented with 0.5 M NaCl was used to prevent premature lysis of cells. Incubation with a mixture containing 50 μg of bovine pancreatic ribonuclease type II-A (Sigma Chemical Co.) and 0.05 ng of ribonuclease T_1 (Sigma) per ml was carried out to ensure complete ribonucleic acid degradation. DNA from *Escherichia coli* B was prepared as described by Marmur (13) and was used as a standard. The DNA base composition, expressed as G+C content, was calculated both from the melting temperature (11) and from buoyant density (12).

Sensitivity to ultraviolet irradiation. Cultures of cells in the late exponential growth phase were diluted 100-fold with sterile seawater to a concentration of about 10^6 cells per ml. The cell suspensions were exposed to irradiation from two lamps (Phillips type 57417 P/40A 6T UV 15W) placed at a distance of 70 cm. Subsamples were taken at appropriate intervals. Immediately after exposure, the suspensions were serially diluted in seawater and plated onto solid growth medium. Parallel series of plates were incubated in the dark after illumination with a 100-W incandescent light placed at a distance of 2 m. Cells of *E. coli* B were exposed to identical irradiation doses for calibration purposes.

Pigment spectroscopy. In vivo spectra of liquid cultures and spectra of pigment extracts were determined by using a Shimadzu model MPS 50 recording spectrophotometer and glass cuvettes with a 1-cm light path. Pigments were ex-

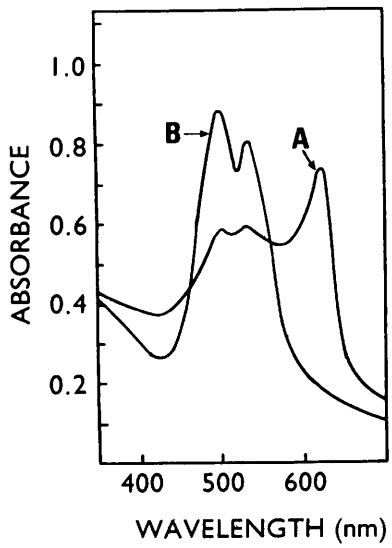

FIG. 2. Effect of temperature on the absorption spectrum of a culture of *A. denitrificans* ATCC 43337^T grown under aerobic conditions at 6°C. Spectrum A, Culture grown on medium with glutamine as a source of carbon; spectrum B, same culture after 10 min of exposure to a temperature of 40°C.

TABLE 1. Characteristics differentiating previously described species of *Alteromonas* and *Marinomonas* from *A. denitrificans* sp. nov.[a]

Characteristic	*A. macleodii*	*A. haloplanktis*	*A. espejiana*	*A. undina*	*A. hanedai*	*A. rubra*	*A. luteo-violacea*	*A. citrea*	*A. aurantia*	*M. communis*	*M. vaga*	*A. denitri-ficans*
Luminescence	−	−	−	−	+	−	−	−	−	−	−	−
Pigmentation												
Prodigionine	−	−	−	−	−	+	−	−	−	−	−	+
Other pigments	−	−	−	−	d[b]	−	+	+	+	−	−	+
Growth at 4°C	−	−	−	d	+	−	d	−	+	−	−	+
Growth at 35°C	+	d	d	−	−	+	+	d	−	+	+	−
Reduction of NO_3^- to NO_2^-	−	d	−	−	+	−	−	−	−	−	−	+
Denitrification with gas formation	−	−	−	−	−	−	−	−	−	−	−	+
Utilization of:												
L-Glutamate	d	d	−	−	+		−	−	−	+	+	+
L-Arginine	d	d	d	d	−		d	−	+	+	d	+

[a] Adapted from reference 2. Data from references 2 and 18.
[b] d, Strains vary within species.

tracted with ethanol or chloroform from centrifuged pellets obtained from liquid cultures and from colonies harvested from solid media.

Resistance and susceptibility to antibiotics. Resistance and susceptibility to antibiotics were assayed by placing antibiotic-impregnated disks on bacterial lawns developing on solid growth medium. Disks containing penicillin G (10 IU; Oxoid), chloramphenicol (30 μg; Oxoid), tetracycline (30 μg; Oxoid), furazolidone (50 μg; Oxoid), and vibriostatic agent 0/129 (30 μg; Sigma) were used. Cells were scored as susceptible when clear zones of complete growth inhibition were observed in dense lawns of growing bacteria.

RESULTS

Natural habitat, isolation, and maintenance. Strains of the new species were isolated in 1977, 1978, 1979, 1981, and 1984 from water samples taken at depths of 90 to 100 m in the fjord system off the west coast of Norway. Natural abundance was too low for direct isolation, and the organisms could not be enriched from sediments or from surface water in the fjord system. Cultures maintained on agar slants tended to die off rapidly and were subcultured monthly. Cells suspended in sterile, unsupplemented seawater remained viable for several years if they were stored at stable temperatures between 5 and 10°C.

Pigmentation. The formation of bright red-pigmented colonies on solid growth medium was the basis for selection from the enriched water samples. Crude acidic acetone or ethanol extracts of the pigments from either red, purple, or blue liquid cultures exhibited a single sharp absorption maximum at 537 nm, whereas acidic chloroform extracts exhibited a single maximum at 542 nm (Fig. 1) identical to results reported for extracts of prodigiosine from *Serratia marcescens* (7). Ethanolic extracts at pH 10 exhibited a single broad absorption maximum at 483 nm, similar but not identical to the spectrum of *S. marcescens* extracts. The in vivo pigmentation of the cells in liquid growth medium varied from bright blue to red, depending on growth medium (Fig. 2). Exposure of blue-pigmented cells to room temperature changed their in vivo pigmentation to red (Fig. 2).

Morphology. The cells are short rods that are 2 to 4 μm long with a diameter of 0.5 to 0.7 μm and have rounded ends (Fig. 3). Cells are flagellated with a single polar sheathed flagellum (Fig. 3). Cells in young cultures are very actively motile; older cultures exhibit less motility and contain many cells with involution forms. Young colonies on solid growth medium are pink, turning red and darkening with age. The morphology of young colonies is generally smooth with sharp edges, whereas older colonies often exhibit lobed edges and develop a distinctive metallic surface sheen.

Physiological characteristics. The results of some physiological assays of 11 strains of *A. denitrificans* are summarized in Table 1.

Resistance and susceptibility to antibiotics. All strains were resistant to vibriostatic agent 0/129, tetracycline, and penicillin. All strains were susceptible to furazolidone.

G+C contents. The G+C contents of three of the strains, calculated on the basis of determinations of denaturation temperature, ranged from 36.5 to 37.1 mol%. DNA from *E. coli* was always used as a standard.

The proposed type strain of *A. denitrificans*, strain Nygaard 1977, was subjected to a more detailed study, giving the results described below.

Physiological characteristics. Both aerobically and anaerobically grown (denitrifying) cells exhibited maximal growth rates at 14.5°C, but growth rates were higher for aerobic cultures than for anaerobic cultures at the same temperature (Table 2). The optimal salinity for growth was about 25 g of NaCl per liter, with no growth registered at or below a concentration of 15 g of NaCl per liter and at or above a concentration of 55 g of NaCl per liter (Fig. 4). Denitrification and aerobic growth were maximal at a KNO_3 concentration of 2 g/liter, whereas no growth was registered in the presence of 5 g of KNO_3 per liter. No production of citrulline or ornithine was detected in an extensive set of experiments when L-arginine was used as the substrate for whole washed cells or cell-free extracts. The only degradation products from L-arginine were urea and ammonium, indicating the absence of any arginine dihydrolase activity. In the absence of organic nitrogen, the addition of minute quantities (20 μg/ml) of vitamin-free Casamino Acids (Difco) markedly enhanced growth on nitrogen-free single carbon sources when media containing ammonium sulfate were made up with synthetic seawater instead of natural seawater. Acid production indicated oxidative metabolism of some carbohydrates, whereas no carbohydrate tested supported fermentative metabolism (Table 3).

The type strain did exhibit oxidase activity, but no catalase activity. Agarase activity was not detected.

In addition to the physiological properties shown in Table 3, strain Nygaard 1977^T utilized butyrate, DL-alanine, L-

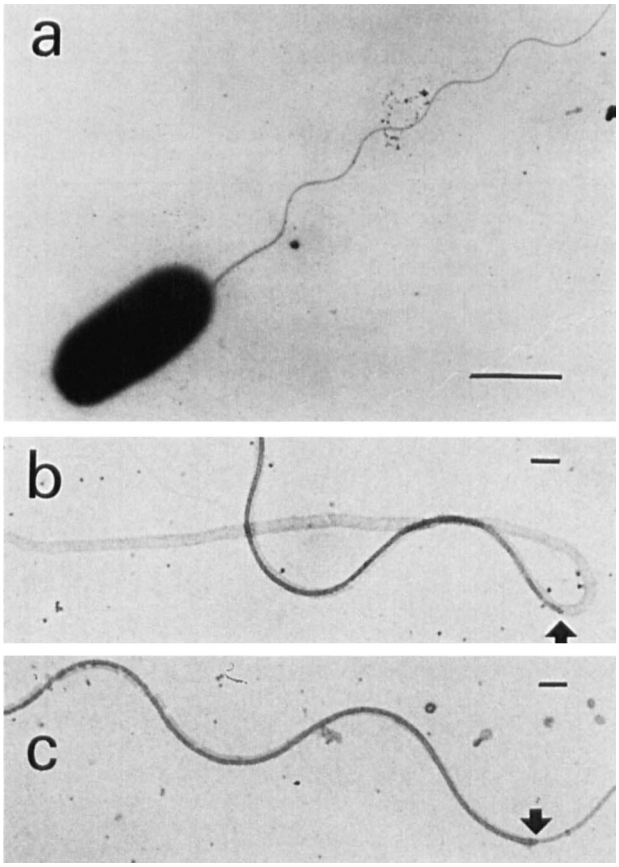

FIG. 3. (a) Cell of *A. denitrificans* ATCC 43337^T. Negative staining. Bar = 1 μm. (b) Sheathed flagellum end with sheath protruding beyond flagellum. Bar = 0.1 μm. (c) Sheathed flagellum end with flagellum protruding beyond sheath. Bar = 0.1 μm.

asparagine, L-glutamate, L-glutamine, and protamine as single sources of energy and carbon. When grown on butyrate, DL-alanine, L-asparagine, and L-glutamate in liquid cultures, the cells exhibited red pigmentation. When grown on L-glutamine and protamine in liquid cultures, the cells exhibited a purple or blue color. The strain was unable to utilize malonate, fumarate, citrate, glycolate, ethanol, L-cysteine, or L-tryptophan as a single source of carbon and energy.

Resistance and susceptibility to antibiotics. In addition to the antibiotic resistance properties mentioned above, strain Nygaard 1977^T was also susceptible to chloramphenicol.

Ultraviolet sensitivity. The cells were very sensitive to ultraviolet irradiation, with an inactivation rate roughly 50 times higher than that of *E. coli* B subjected to the same irradiation doses (Fig. 5). No photoreactivation was registered.

G+C content. The G+C content, calculated on the basis of four independent determinations of denaturation temperature and one determination of buoyant density, all utilizing *E. coli* DNA as a standard, was found to be 36.80 mol% (standard deviation, 0.3 mol%).

DISCUSSION

Genus assignment. The strains which we studied all correspond closely to the description given for the genus *Alteromonas* by Baumann et al. (2) in *Bergey's Manual of Systematic Bacteriology*, with a couple of distinctive differences. Our bacteria are all vigorous denitrifiers, a flagellar sheath was demonstrated for most strains, and they have G+C contents slightly lower than those of any of the strains currently included in this genus. Our strains also have many of the traits required for inclusion in the genus *Vibrio*, notably the presence of sheathed flagella, but none of our strains is fermentative and they all grow anaerobically, producing gas with nitrate as a terminal electron receptor. Although the G+C contents of our strains are not so low as to disallow inclusion in the genus *Alteromonas*, they are much too low to allow placing these organisms in the genus *Pseudomonas* or in the genus *Marinomonas*. The absence of fermentative metabolism and the ability to denitrify seem sufficient to preclude inclusion in the genus *Vibrio*. Flagellar sheaths are not necessarily easy to visualize, and their apparent absence is not a strong taxonomic criterion. We do not regard the presence of a flagellar sheath as decisive for exclusion of our strains from the genus *Alteromonas*, supporting the suggestion of Novic and Tyler (15) that the species *Alteromonas luteoviolacea* remain in the genus *Alteromonas* despite the flagellar sheath found in some strains. Currently, the genus *Alteromonas* is described as not including any strains able to denitrify. Negative characteristics are intrinsically weak taxonomic determinants. Assay methods for denitrification often employ concentrations of KNO_3 of 5 g/liter or even higher. Such concentrations effectively inhibit denitrification by at least one of our strains, which consequently was originally supposed to be

TABLE 2. Generation time of *A. denitrificans* ATCC 43337^T grown at different temperatures under aerobic conditions in growth medium and under anaerobic conditions in growth medium supplemented with 3 g of KNO_3 per liter

Temp (°C)	Culture generation time (h)	
	Aerobic	Anaerobic
2.5	NG[a]	NG
3.5	20.4	NG
7.5	4.7	16.5
12.0	2.7	8.0
13.5	2.3	7.3
14.5	1.9	6.6
16.0	4.2	6.9
18.5	4.7	7.2
19.5	4.0	10.7
21.5	5.1	NG
22.0	10.0	NG
23.0	NG	NG

[a] NG, No dectectable growth.

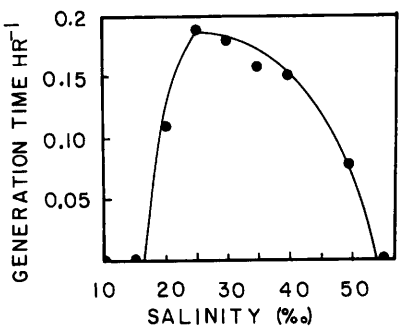

FIG. 4. Effect of salinity on aerobic growth of *A. denitrificans* ATCC 43337^T on growth medium at 6°C.

TABLE 3. Characteristics of previously described species of *Alteromonas* and *Marinomonas* and the proposed type strain of *A. denitrificans*[a]

Characteristic	*A. macleodii*	*A. haloplanktis*	*A. espejiana*	*A. undina*	*A. hanedai*	*A. rubra*	*A. luteo-violacea*	*A. citrea*	*A. aurantia*	*M. communis*	*M. vaga*	*A. denitri-ficans* ATCC 43337^T
Pigmentation	−	−	−	−	d[b]	+	+	+	+	−	−	+
Requirement for growth factors	−	d	+	+	+	+	+	+	+	−	−	+
Production of:												
Amylase	+	d	+	d	−	+	+	+	+	−	−	+
Gelatinase/lipase	+	+	+	+	+	+	+	+	+	−	−	+
Alginase	d	−	+	−	−				−	−	−	+
Chitinase	−	d	−	+	+	−	−	d	−	−	−	+
Deoxyribonuclease						+	+	+	+			+
Utilization of:												
D-Galactose	+	d	+	−	d	−	−	−	−	d	+	−
D-Fructose	+	d	d	−	−	−	−	+	+	+	+	−
Sucrose	+	+	+	+	−	−	−	−	−	−	−	−
Maltose	+	+	+	+	−	−	+	−	d	d	d	+
Lactose	+	−	+	−	−	−	−	−	−	−	−	−
D-Gluconate	+	−	−	−	d	−	−	−	−	+	+	−
N-acetylglucosamine	d	+	−	+	+		+		+	−	+	−
Succinate/fumarate	−	+	−	+	−	−	−	−	−	+	+	−
DL-Lactate	d	−	−	−	−	−	d	−	−	+	+	−
D-Mannitol	d	d	+	−	−	−	−	−	−	+	+	−
Glycerol	+	−	−	−	−	−	−	−	−	+	+	−
L-Tyrosine	+	+	+	+	d		d		+	−	−	+
Putrescine	−	−	−	−	+					+	+	−
D-Sorbitol	−	−	−	−	−	−	−	−	−	+	+	−
L-Arabinose	−	d	−	−	−		−			d	+	−
D-Glucose	+	+	+	+	d	+	+	+	+	+	+	+
Acetate	+	+	+	+	+	−	−	−	−	+	+	+
Propionate	+	+	+	+	+					d	d	+
DL-β-OH-butyrate	d	d	d	+	−		−			+	d	−
Pyruvate	+	+	+	−	+	−	d	−	−	+	+	+
p-OH-benzoate	−	−	−	−	−		−	−	−	+	+	−
L-Ornithine	−	−	d	d	−		−	−	−	+	+	−
L-Citruline	−	−	+	d	−				d	d	d	−
L-Proline	−	d	+	d	−		d	−	−	+	+	+
DNA content G+C (mol%)	44-47	41-45	43-44	43-44	43-44	46-48	40-45	41-45	38-43	46-50	46-50	37
No. of characteristics differing from *A. denitrificans* ATCC 43337^T	14	7	11	10	9	8	7	9	10	22	24	0

[a] Data for previously described species adapted from reference 2.
[b] d, Strains vary within species.

unable to denitrify. Of the five alternative generic assignments for our strains, *Pseudomonas*, *Vibrio*, *Marinomonas*, *Alteromonas*, and a new genus, we find that assignment to the genus *Alteromonas* is the most appropriate.

Species assignment. A strain of *Vibrio psychroerythrus*, placed incertae sedis in the genus *Photobacterium* (1, 3), has many characteristics in common with our strains, including the production of prodigionine pigments (7). The ability of *V. psychroerythrus* to ferment glucose and the fact that it is specifically described as having an unsheathed flagellum seem sufficient to preclude assigning our strains to this species. When compared as to 38 different characteristics (Table 3), the proposed type strain of *A. denitrificans* differs from previously described species of *Alteromonas* by 7 to 24 characteristics (Table 3). Two previously described species, *Alteromonas haloplanktis* and *Alteromonas hanedai*, differ from *A. denitrificans* by only seven and nine characteristics, respectively, and contain strains which reportedly are able to reduce nitrate to nitrite, but none of these strains has been reported to use nitrate for anaerobic growth. It is also difficult to assign our strains to either *A. haloplanktis* or *A. hanedai* because the lowest G+C contents reported for strains of these species are 41 and 43 mol%, respectively, *Alteromonas rubra* produces prodigionines (6), but it differs from *A. denitrificans* by eight characteristics and has a DNA base composition as high as 46 mol% G+C (Table 3). Although *A. luteoviolacea* contains strains with sheathed flagella (15) and may exhibit a visually similar color to that of blue-colored cultures of *A. denitrificans* in liquid cultures with L-arginine, the blue pigment of *A. luteoviolacea* is violacein (15), whereas only red prodigionine pigments are extracted even from blue-colored cultures of *A. denitrificans* (Fig. 1a). The only previously described species of *Alteromonas* containing strains with G+C contents as low as 38 mol% is *Alteromonas aurantia*, but this species differs from the proposed type strain of *A. denitrificans* by 10 character-

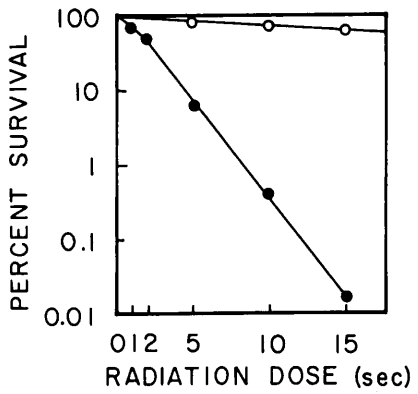

FIG. 5. Survival of *A. denitrificans* ATCC 43337^T (●) and *E. coli* B (○) during irradiation with ultraviolet light. The irradiation dose is given as exposure time.

istics (Table 3). Accordingly, we propose that our strains be assigned to a new species and that *A. denitrificans* Nygaard 1977 (= ATCC 43337) be accepted as the type strain of the species.

Description of type strain. The cells of type strain Nygaard 1977 of *A. denitrificans* sp. nov. (de. ni. tri' fi. cans. M.L. inf. *denitrificare*, to denitrify; M.L. part. adj. *denitrificans*, denitrifying) are rod shaped, 2 to 4 μm long and 0.5 to 0.7 μm wide in logarithmic growth phase. Involution forms are common in dense populations in stationary growth phase. Motile by means of a single sheathed polar flagellum, very rarely with a tuft of two or three flagella. Gram negative. Produces prodigionine pigments, giving a bright blue, purple, or red color in liquid cultures, depending on medium composition and culture conditions. Young colonies on agar media are pink, turning bright red and darkening with age. Old colonies are deep red, often with a green metallic sheen. Produces autotoxic substances which kill cells and inhibit further growth in dense cultures. Survives for at least 7 years at low cell densities in sterile unsupplemented seawater stored at 7 to 8°C.

Psychrotrophic, growing at temperatures ranging from 4 to 22°C. Halophilic; unable to grow at salinities lower than 1.5% or higher than 5.5%. Aerobic, nonfermentative metabolism; able to denitrify with gas production. Produces a series of exoenzymes, notably deoxyribonuclease and chitinase. Degrades protamine, growing luxuriantly on released arginine. Subcultures tend to increase the upper temperature limit for growth and to decrease production of autotoxic substances after prolonged subculturing in the laboratory. Very sensitive to ultraviolet irradiation. The G+C content of the type strain DNA determined from measurements of thermal denaturation temperatures in four independent subcultures and from density gradient equilibrium measurement of DNA from one subculture is 36.80 ±0.3 mol%. The type strain has been deposited with the American Type Culture Collection as strain ATCC 43337.

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LITERATURE CITED

1. **Baumann, P., and L. Baumann.** 1984. Genus *Vibrio*, p. 539–545. *In* N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol 1. The Williams & Wilkins Co., Baltimore.
2. **Baumann, P., M. J. Gauthier, and L. Baumann.** 1984. Genus *Alteromonas*, p. 343–352. *In* N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol 1. The Williams & Wilkins Co., Baltimore.
3. **D'Aoust, J. Y., and D. J. Kushner.** 1972. *Vibrio psychroerythrus* sp.n.: classification of the psychrophilic marine bacterium NRC 1004. J. Bacteriol. **111**:340–342.
4. **Dundas, I.** 1986. Fate and possible effects of excessive sperm released during spawning. Mar. Ecol. Prog. Ser. **30**:287–290.
5. **Dundas, I. E. D., and H. O. Halvorson.** 1966. Arginine metabolism in *Halobacterium salinarium*, an obligately halophilic bacterium. J. Bacteriol. **91**:113–119.
6. **Gauthier, M. J.** 1976. *Alteromonas rubra* sp. nov. New marine antibiotic-producing bacterium. Int. J. Syst. Bacteriol. **26**:459–466.
7. **Gerber, N. N.** 1975. Prodigiosine-like pigments. Crit. Rev. Microbiol. **3**:469–485.
8. **Hayat, M. A.** 1976. Principles and techniques of electron microscopy. Biological applications, vol 1. Van Nostrand Reinhold Co., New York.
9. **Hugh, R., and E. Leifson.** 1953. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram-negative bacteria. J. Bacteriol. **66**:24–26.
10. **Jeffries, C. D., D. F. Holteman, and D. G. Guse.** 1957. Rapid method for determining the activity of microorganisms on nucleic acids. J. Bacteriol. **73**:590–591.
11. **Mandel, M., and J. Marmur.** 1968. Use of ultraviolet absorbance-temperature profile for determining the guanine plus cytosine content of DNA. Methods Enzymol. **12B**:195–206.
12. **Mandel, M., G. L. Schildkraut, and J. Marmur.** 1968. Use of CsCl density gradient analysis for determining the guanine plus cytocine content of DNA. Methods Enzymol. **12B**:184–195.
13. **Marmur, J.** 1961. A procedure of isolation of deoxyribonucleic acid from microorganisms. J. Mol. Biol. **3**:208–218.
14. **Norland, S., M. Heldal, and D. Dagestad.** 1977. Discontinuous gradients of light and temperature for culturing microalgae in liquid cultures. Lab. Pract. **26**:863.
15. **Novic, N. J., and M. E. Tyler.** 1985. Isolation and characterization of *Alteromonas luteoviolacea* strains with sheathed flagella. Int. J. Syst. Bacteriol. **35**:111–113.
16. **Sierra, G.** 1957. A simple method for the detection of lipolytic activity of microorganisms and some observations on the influence of contact between cells and fatty substrates. Antonie van Leeuwenhoek J. Microbiol. Serol. **23**:15–22.
17. **Skerman, V. B. D.** 1967. A guide to the identification of the genera of bacteria. The Williams & Wilkins Co., Baltimore.
18. **van Landschoot, A., and J. De Ley.** 1983. Intra- and intergeneric similarities of the rRNA cistrons of *Alteromonas*, *Marinomonas* (gen. nov.) and some other Gram-negative bacteria. J. Gen. Microbiol. **129**:3057–3074.