A Halophilic Denitrifier, *Bacillus halodenitrificans* sp. nov.

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A new halotolerant denitrifier, which was isolated from a solar saltern by enrichment culture in liquid medium supplemented with 1.06 M (9%) NaNO₃, grew optimally in media containing 0.5 to 1.35 M NaCl, survived and multiplied in media ranging in salinity from 0.35 to 4.25 M NaCl, and tolerated high nitrite ion concentrations, as well as high nitrate ion concentrations. The salt requirement could be provided by 1 M KNO₃ or KCl instead of NaCl. For this nonfermentative organism, nitrate and nitrite were the only electron acceptors tested that supported anaerobic growth on a complex medium. Washed cells reduced both nitrate and nitrite at significant rates. The isolate lacked a nitrous oxide reductase activity, utilized a variety of substrates as carbon and energy sources, and required both growth factors and organic (reduced) sulfur. Ammonia served as a nitrogen source for growth, but nitrate did not. Despite the failure of the organism to sporulate, assignment to the genus *Bacillus* appeared to be consistent with results of cell constituent analyses and partial 16S ribosomal ribonucleic acid sequencing. We propose the name *Bacillus halodenitrificans* for this organism. A type culture has been deposited with the American Type Culture Collection, Rockville, Md., as strain ATCC 49067.

Denitrification is a process by which bacteria reduce nitrate to a gaseous end product, either elemental nitrogen (N₂) or, in some cases, nitrous oxide (29). Wastewater engineers exploit this process for removal of nitrate from freshwaters, thus preventing eutrophication of lakes and eliminating threats to health (18). Some industrial effluents contain large amounts of ammonium, calcium, or sodium nitrates. For example, discharges from uranium extraction plants contain nitrate at concentrations exceeding 50 g of NO₃⁻-N per liter. Biological nitrate removal from effluents with high nitrate concentrations usually requires large dilutions to obviate inhibition of denitrifying bacteria. Both nitrate ions and sodium ions may be toxic to the denitrifiers (10). To overcome such difficulties, we set about isolating a strongly denitrifying bacterium that is capable of growth at high sodium nitrate concentrations. Since this isolate is to be active under varying conditions, flexibility with regard to salt concentration is necessary. *Bacillus licheniformis*, a bacterium repeatedly isolated with enrichment media containing 0.7 M (7%) KNO₃, might seem to be an ideal choice, but this choice is obviated by the presence of only weak nitrite reductase activity in this organism (31). *Paracoccus halodenitrificans* denitrifies vigorously but requires chloride ions for growth and is extremely sensitive to accumulation of nitrite, the first intermediate in denitrification (34). *Halobacterium denitrificans*, an extreme halophile, lyses at salt concentrations below 1.5 M (37) and therefore does not allow much flexibility. Enrichment culturing of the bacteria in material from a solar saltern in an anaerobic medium containing 1.06 M (9%) sodium nitrate yielded the desired isolate. In this paper we describe the halotolerant denitrifying bacterium which we obtained.

MATERIALS AND METHODS

Media and cultural methods. Our yeast extract medium contained (per liter of distilled water) 3.8 g of Na₂HPO₄, 1.43 g of NaH₂PO₄ or 1.3 g of KH₂PO₄, 1 g of (NH₄)₂SO₄, 1 g of yeast extract (Difco Laboratories, Detroit, Mich.), 1 g of Mg(NO₃)₂ · 6H₂O, and NaCl or NaNO₃ at the concentrations specified below. The pH was adjusted to 7.2 with

KOH. This basal medium was supplemented with different carbon sources. The complex medium (NY medium) contained (per liter of distilled water) 8 g of nutrient broth (Difco), 5 g of yeast extract, 1 g of MgSO₄ · 7H₂O, and NaCl or NaNO₃ at different concentrations; the pH was adjusted to 7.4 with KOH. For anaerobic use, media were cooled under argon or nitrogen after autoclaving. Denitrification studies with growing cultures were performed in 150-ml rubber-capped and stoppered bottles containing 50 ml of culture medium under a gas phase of helium. Stock cultures of the selected isolate were transferred every 6 months on agar slants of YA medium (see below) containing 1.7 M (10%) NaCl and were kept at room temperature.

Isolation. Samples collected from a solar evaporation pond (Berre L'étang, France) were injected into Hungate tubes containing yeast extract medium supplemented with 1.06 M NaNO₃ and one of the following compounds: 1% sodium acetate · 3H₂O (YA medium) 10 ml of sodium lactate per ml (YL medium) 1% tryptone (YT medium), or 15 ml of methanol per liter (YM medium). After 1 to 2 weeks of incubation at 37°C, growth was observed in the tubes containing YA, YL, and YT media. The three cultures produced significant amounts of N₂O, as determined by gas chromatography (29). After one transfer, after which good growth could be obtained after 1 day, the liquid enrichment cultures were streaked onto YA medium containing 1.7 M NaCl and 1.5% agar, and the preparations were incubated aerobically at 37°C. The four strains obtained in axenic culture reduced nitrate to nitrous oxide but not N₂, whether acetylene was present or not (1). The isolate that produced the most N₂O from nitrate in liquid culture was chosen for further study.

Morphology. Flagellar arrangement was examined by using the method of Mayfield and Inniss (28) and was checked by electron microscopy in which we used negatively stained cells prepared by the method of Beuscher et al. (2). Gram type was determined by using the polymyxin B reaction (42); gram-negative *Pseudomonas stutzeri* was used as a positive control for bleb formation.

Nitrogen source. YL medium lacking (NH₄)₂SO₄ and containing 30 g of NaCl per liter and 0.1 g of yeast extract per liter was used for nitrogen source tests. The nitrogen compounds tested were NaNO₃, NH₄Cl, and vitamin-free

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Casamino Acids, each at a concentration of 1 g/liter. Medium which contained 0.1 g of yeast extract per liter as the sole nitrogen source was used as a control. The requirement for a nutritional growth factor(s) was assayed by using an identical medium (no yeast extract) supplemented with either 1 g of vitamin-free Casamino Acids per liter or 1 g of yeast extract per liter.

Carbon and energy source determinations. Yeast extract medium containing 70 g of NaCl per liter, 0.2 g of yeast extract per liter, and 0.1 g of methionine per liter was used for carbon and energy source determinations. Most of the substrates tested were added (after neutralization when necessary) at concentrations of 1% (wt/vol); the exceptions were propionate (20 ml/liter), butyrate (20 ml/liter), methanol (20 ml/liter), ethanol (20 ml/liter), glycerol (20 ml/liter), and lactate (16 ml/liter [60%]). The cultures were incubated at 30°C on a rotary shaker, and growth was assayed turbidimetrically at 660 nm after 1 and 2 days. A test was considered positive when the turbidity was at least twice that of the control cultures lacking an added carbon source.

Anaerobic growth. Anaerobic growth was assayed in NY medium containing 1.2 M NaCl, 1.5% agar, and MgCl₂ rather than MgSO₄. Electron acceptors were added to concentrations of 1 g/liter, and sugars were added to concentrations of 10 g/liter. Culture dishes were incubated at 35°C in an anaerobic chamber and inspected daily for 2 weeks.

Miscellaneous biochemical tests. Standard biochemical tests were performed by using the methods of Cowan and Steel (8) and Claus and Berkeley (6) and media containing 0.35 M NaCl.

Deoxyribonucleic acid base composition. Deoxyribonucleic acid was extracted and purified by the method of Heath et al. (13) from cells grown aerobically on NY medium containing 1.2 M NaCl. The guanine-plus-cytosine content was determined chemically by using the method of Whitman et al. (41).

Growth studies. The isolate was grown under conditions permitting denitrification in culture tubes containing 10 ml of medium. Turbidity at 660 nm was assayed at intervals and was related to the effects of various pH values, salinity values, and temperatures. For determining the effects of different pH values, tubes of NY medium containing 1.2 M NaCl and 12 mM NaNO₃ were inoculated and incubated at 32°C. HCl or KOH was used to adjust the pH values. To measure the salinity effect, tubes of NY medium containing 12 mM NaNO₃ (pH 7.4) were inoculated and incubated at 35°C. The NaCl concentrations varied between 0 and 4.8 M. The different concentrations took account of the volumes and the salt concentrations of the inocula, which were taken from a culture without added NaCl for media containing 0 to 2.0 M NaCl and from a culture containing 1.6 M NaCl for media supplemented with 2.4 to 4.8 M NaCl. For assays to determine the effects of different temperatures, tubes of NY medium containing 0.6 M NaNO₃ (pH 7.4) were inoculated and incubated in a temperature gradient incubator (Scientific Industries, Inc., Bohemia, N.Y.) set to provide a gradient ranging from 3 to 68°C. The design of this apparatus required that anaerobic Hungate tubes be used instead of culture tubes.

Preparation of rRNA. The glassware used to prepare ribosomal ribonucleic acid (rRNA) was oven baked overnight (160°C), and pipette tips and Eppendorf tubes were sterilized by autoclaving. Cells grown on NY medium containing 0.5 M NaCl were lysed by fast mixing in a solution containing 8 M urea, 50 mM tris(hydroxymethyl)aminomethane hydrochloride (pH 7.5), 350 mM NaCl, 20 mM

disodium ethylenediaminetetraacetic acid, 2% sarkosyl, and 5% phenol. The resulting emulsion was subjected to two extractions with phenol-chloroform-isoamyl alcohol (3:1:1/24) and one ethanol precipitation. High-molecular-weight ribonucleic acids were further purified by LiCl (2 M) precipitation, after which most of the preparation comprised intact 16S and 23S rRNAs, as determined by agarose gel electrophoresis. The rRNA samples were stored at concentrations of 1.5 mg/ml in 10 mM tris(hydroxymethyl)aminomethane hydrochloride (pH 8.0) at -70°C. Sequencing of 16S rRNA was accomplished by a modification of the method described by Lane et al. (21). The primer was annealed to the rRNA at 95°C for 5 min, and this was followed by slow cooling over a period of 45 min to 50°C. The sequencing reaction proceeded at 50°C for 1 h, with a chase time of 15 min at the same temperature. The reaction products were separated by electrophoresis on 8% polyacrylamide-8 M urea buffer gradient gels. The following two 15-mer primers were used: 920 primer (5'-ATTCCTTTGAGTTTC-3') and 1400 primer (5'-ACGGGCGGTGTGTAC-3'). The numbering corresponds to the locations of the sequences complementary to these primers in *Escherichia coli* 16S rRNA.

Denitrification studies. Cultures were grown anaerobically in YA medium containing 0.6 M NaNO₃ for 24 h. The cells were harvested, washed twice with 20 mM phosphate buffer (pH 7.0) containing 0.85 M NaCl, and then suspended in the identical buffer. Reactions were carried out in 8-ml rubber-capped and stoppered vials containing 1 ml of cell suspension, 50 µmol of sodium acetate, 20 µmol of NaNO₃, and 10 µmol of NaNO₂ or 100 µmol (4.46 µmol) of N₂O; the gas phase was helium. The products of the reaction were analyzed by gas chromatography (29). Specific activities were expressed in nanomoles of N₂O produced per milligram (dry weight) per hour. Dry weights were determined from a standard curve relating dry weight and the optical densities of cell suspensions at 660 nm.

RESULTS

Morphology. Cells were rod shaped (0.6 to 0.8 by 2.5 to 4.0 µm) and occurred singly or in small chains. A number of anaerobically grown cells were often more elongated and flexuous, with lengths of up to 15 µm. Young cells displayed frequent spinning and twisting in wet mounts. A few flagella were found in both polar and lateral arrangements (Fig. 1). Colonies appeared to be entire, smooth, semitranslucent, cream colored, and approximately 2 mm in diameter on solid complex medium. After 24 h, the colonies began to spread up to 3 to 4 mm in diameter and to have darker centers. Colonies were 0.5 to 1 mm in diameter and reached a maximum of 2 mm in diameter (after prolonged incubation) on solid YA medium. The isolate was gram variable, as determined by conventional staining techniques. The polymyxin B reaction technique, which depends upon binding of polymyxin B to the lipopolysaccharide of the outer membrane of gram-negative bacteria, revealed no bleb formation even at the highest polymyxin B concentration (1,000 U). In contrast, control cells of *Pseudomonas stutzeri* yielded a positive reaction with 250 U of polymyxin B. The bacterium failed to sporulate either in liquid enrichment media or on the solid medium during isolation. Use of solid NY medium supplemented with 50 µM MnCl₂ and 0.7 mM CaCl₂, as well as a soil extract nutrient agar (6), also failed to promote sporulation.

Physiological and biochemical properties. The isolate was catalase and cytochrome oxidase positive. It hydrolyzed

FIG. 1. Electron micrograph of a negatively stained cell showing the flagellar arrangement. Bar = 1 μm .

gelatin and casein, but not starch, esculin, or Tween 80. It was β -galactosidase positive and urease, deoxyribonuclease, lysine decarboxylase, ornithine decarboxylase, arginine dihydrolase, phenylalanine deaminase, and lecithinase negative. It produced acid (weakly) but no gas from glucose. Acid was also produced from mannitol, but not from L-arabinose, inositol, sorbitol, rhamnose, melibiose, sucrose, or amygdalin (results from rapid API 20E tests in the presence of 0.35 M NaCl). The bacterium did not produce dihydroxyacetone from glucose or hydrogen sulfide from cysteine. The indole and Voges-Proskauer tests were negative. Tyrosine was not decomposed. The isolate was susceptible to streptomycin (10 μg), chloramphenicol (30 μg), tetracycline (30 μg), and novobiocin (30 μg), but not to penicillin G (10 U), polymyxin B (300 U), or vibriostatic agent 0/129. An additional test of penicillin G resistance in liquid NY medium yielded growth after 2 days in the tubes containing 50 and 100 U/ml and after 4 days in the tube containing 250 U/ml. The cells resumed growth immediately when they were transferred into a medium lacking penicillin G. Such behavior suggests synthesis of an inducible penicillinase (15).

The requirement of the isolate for a nutritional growth factor(s) was satisfied by yeast extract but not by amino acids alone. Only Casamino Acids served as a nitrogen source in YL medium containing 0.1 g of yeast extract per liter as a source of growth factors. However, further testing showed that this effect was attributable to an organic (reduced) sulfur requirement. When the same medium (with 0.1 g of yeast extract per liter) was supplemented with either cysteine or methionine (0.1 g/liter), the bacterium thrived in the presence of ammonia. Glutamine also served as a nitrogen source, but arginine, nitrate, and urea did not. The bacterium utilized a variety of substrates as sole carbon and energy sources (see below). It did not grow on complex NY medium in the absence of oxygen or another acceptable oxidant. Among the possible terminal electron acceptors and potentially fermentable sugars tested (nitrate, nitrite, sulfate, thiosulfate, tetrathionate, fumarate, glucose, and sucrose), only nitrate and nitrite supported anaerobic growth. Nitric oxide did not serve as an electron acceptor for anaerobic growth. This compound was toxic; growth stopped following the addition of NO (10% of the gas phase) to a mid-log-phase culture.

Growth characteristics. Optimum growth occurred near 38°C. No growth occurred at temperatures above 45°C or below 10°C (Fig. 2). Growth took place over a pH range of pH 5.8 to 9.6 (highest pH tested); the approximate optimum pH was pH 7.4 (Fig. 3). Growth was rapid (Fig. 4a) at the lowest NaCl concentrations tested (0 [actually containing 1.2 mM NaNO_3] and 0.2 M), but the cells lysed as soon as they reached the late log phase. After a few hours, the medium contained mainly spheroplasts. The cells were more resistant to lysis at NaCl concentrations of 0.35 M and above. The lag phase lengthened as the NaCl concentration approached 2.6 M, increased to 40 h for the cultures containing 3.8 M NaCl, and reached 2 weeks for cultures containing 4.35 M NaCl, which was the highest concentration tolerated. The specific growth rate was maximal in media containing 0.09 to 0.6 M NaCl and remained high with NaCl concentrations up to 1.35 M, while maximal cell densities were reached at 0.5 to 1.7 M NaCl (Fig. 4b). Taking into consideration the three sets of data (resistance to lysis, growth rate, and cell density), the optimal salt concentration for growth can be considered to range between 0.5 and 1.35 M (3 to 8%) NaCl. The lysis observed at low salt concentrations was confirmed by incubating the organism on solid media not supplemented with NaCl, on which the bacterium failed to

FIG. 2. Effect of temperature on growth in liquid complex medium.

FIG. 3. Effect of pH on growth in liquid complex medium.

grow. The isolate grew well in liquid media containing 1 M KCl or KNO₃, showing that the salt requirement was not specific for sodium or chloride ions. The bacterium tolerated NaNO₃ concentrations up to 1.65 M (14%) and sodium nitrite concentrations up to 0.29 M (2%) in anaerobic cultures and 0.58 M (4%) in aerobic cultures.

Sequence of the 16S rRNA. The sequence obtained with the 1400 primer (Fig. 5) was similar to the sequences of known *Bacillus* species; the levels of similarity with sequences from *Bacillus subtilis* (11) and *Bacillus brevis* (20) were 91.2 and 90.1%, respectively. For comparison, the level of sequence similarity with *E. coli* (4) was 80.3%, and the level of sequence similarity with *Halobacterium volcanii* (12) was 71.6%. Sequencing rRNA with the 920 primer did not provide transcripts long enough for reliable analysis. As expected, signatures for gram-positive bacteria were found at positions 1,207 (cytosine) and 1,198 (adenine) (43).

Cellular constituents. The fatty acids found in the membrane lipids were of the iso/anteiso type. Cell wall preparations contained *meso*-diaminopimelic acid and an excess of glutamic acid. The isolate yielded a menaquinone of the MK-7 type (analysis provided by the Deutsche Sammlung von Mikroorganismen). The presence of large quantities of type *b* and *c* cytochromes gave a pinkish orange color to concentrated cell suspensions. Unlike the cytochrome *cd*₁ nitrite reductase produced by many denitrifiers (29), the nitrite reductase isolated from our bacterium was found to be a nonheme copper protein (Denariáz, Payne, and Le Gall, unpublished data). The guanine-plus-cytosine content of the deoxyribonucleic acid was 38 mol%, as determined by chemical analysis.

Denitrifying activity of whole cells. Washed cells reduced both nitrate and nitrite to N₂O (Fig. 6). The specific activities were 960 nmol of N₂O produced per h per mg (dry weight) (electron acceptor NO₃⁻) and 1,200 nmol of N₂O produced per h per mg (dry weight) (electron acceptor NO₂⁻), values comparable to those of other denitrifiers (Table 1); 2 mol of NO₂⁻ yielded 1 mol of N₂O. N₂O was not reduced to N₂ by washed cells.

DISCUSSION

Use of a selective medium containing 1.06 M (9%) NaNO₃ permitted isolation of a new denitrifying bacterium that was capable of tolerating high concentrations of both sodium and nitrate ions. Unlike *B. licheniformis*, which also grows under such conditions, our new isolate denitrified as actively, as measured by the production of N₂O from nitrate and nitrite, as several other denitrifiers. Thus, this isolate may participate in the anaerobic treatment of industrial effluents containing high concentrations of sodium nitrate. A possible drawback to service in such processes might be its lack of N₂O-reducing capacity. However, it has been suggested that high concentrations of nitrate are inhibitory to N₂O-reducing activity in denitrifiers (3, 19), and one could expect to get high N₂O/N₂ ratios under these conditions. The potential problem of N₂O production can be overcome by using a second denitrifier that can remove this compound in the gas phase by reduction to N₂ (Denariáz, Payne, and LeGall, FEMS Symp., in press).

Impressively, the isolate grew in media containing up to 0.58 M (4%) sodium nitrite, which is often a toxic intermediate in denitrification. Resistance to nitrite is not common in denitrifiers, except for the *Bacillus* spp. strains isolated by Pichinoty et al. (32). More typically, Eddy and Ingram (9) showed that a *Bacillus* species was 100-fold more sensitive to nitrite during anaerobiosis than during aerobiosis. The difference which we found for our isolate was less dramatic, suggesting that these two organisms probably deal with nitrite differently.

Most of the morphological and physiological characteristics of our isolate were typical of *Bacillus* species. Only one significant characteristic, sporulation, was not exhibited. Sporulation is a property that is easily lost by some *Bacillus* spp. strains (6), perhaps because of the involvement of several operons (at least 50 operons in *B. subtilis*) that are subject to mutation and regulation (24). Moreover, most bacilli reportedly fail to sporulate during anaerobiosis. The few strains that are able to do so seem to lose this capacity when they are placed in the presence of high concentrations of nitrate (7), the conditions used for our enrichment cultures.

The identities of the cell wall constituents, the lipids present in the cytoplasmic membrane, and the type of quinone recovered from the cells are consistent with placement of the isolate in the genus *Bacillus* (6, 23). rRNA sequencing (43) revealed an unequivocal indication of evolutionary relatedness between known *Bacillus* species and our isolate, with levels of similarity of more than 90%.

With a salinity range for growth between 0.35 and 4.35 M NaCl (based on cell resistance to lysis) and an optimum salinity range between 0.5 and 1.35 M NaCl, our isolate is a moderate halophile (22, 38). Unlike the extremely halotolerant organism *Halomonas elongata* (39) and an unidentified halophile (26), our bacterium does not maintain itself in stationary phase in liquid media with NaCl concentrations below 0.35 M, although its initial growth rate is nearly optimal in this range. Our *Bacillus* sp. strain is another example of the physiological tolerances found in the genus *Bacillus*, which includes haloalkaliphilic species (40), as well as halotolerant-thermotolerant species (30).

Our strain is the first denitrifying *Bacillus* sp. strain whose nitrite reductase has been identified. The presence of a copper-containing enzyme in a bacterium in the genus *Bacillus* suggests that this type, as opposed to the cytochrome *cd*₁ type, might be more common among denitrifiers than

FIG. 4. (a) Growth in complex medium at different NaCl concentrations. The numbers next to the curves indicate NaCl concentrations (in moles per liter). Note the change in scale on the abscissa. (b) Effect of NaCl on the growth rate and cell density in complex liquid medium. A₆₆₀, Absorbance at 660 nm.

frequently assumed. The copper enzyme has so far been found in two *Alcaligenes* species (17, 25), in *Achromobacter cycloclastes* (16), in *Pseudomonas aureofaciens* (44), and in *Rhodopseudomonas sphaeroides* f. sp. *denitrificans* (35), all of which are gram-negative bacteria.

Among the previously described *Bacillus* species, *Bacillus firmus* matches our isolate most closely, but this species differs in that it hydrolyzes starch, does not require salt for

growth, does not grow in the presence of 10% NaCl, and does not denitrify (6, 36). With a range of 36.1 to 47.4 mol% for 42 strains, the guanine-plus-cytosine content of the deoxyribonucleic acid of *B. firmus* cannot be used as a decisive means for identification (6). *B. firmus* also reportedly fails to utilize ammonium ions as a nitrogen source, information that should be viewed with caution (see above). Consequently, our bacterium may reasonably be considered

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1180      5'-NNNNNNNNNU GACGUCAAAAU CAUCAUGCCC CUUAUGACCU GGGNUACACA
1230      CGUGCUACAA UGGAUGGAAC AAAGGGNNGC AAAACCGCCA GGUNAAGCAA
1280      AUCCCAUNAA ACCAUUCUNA GUUCGGAUUG CAGGCUGCAA CUCGCCUNCA
1330      UGAAGCCGGA AUCGCUAGUA AUCGCGGAUC AGCAUGCCGC GGUGAAANCG-3'

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FIG. 5. Partial 16S rRNA sequence obtained with the 1400 primer. N, Residue assignment not clear. The two residues in boxes correspond to gram-positive signatures at positions 1,198 (adenine) and 1,207 (cytosine).

FIG. 6. Gas production by washed cells. Symbols: ○, from nitrate (20 μmol); △, from nitrite (10 μmol). The cell concentration was 2.5 mg (dry weight) per ml. The total volume was 1 ml.

a new species. We propose the name *Bacillus halodenitrificans* for this organism because of its salient physiological properties (i.e., halophilism and denitrification).

Description of *Bacillus halodenitrificans* sp. nov. *Bacillus halodenitrificans*. (ha.lo.de.ni.tri'fi.cans. Gr. n. *halos*, salt, the sea; M.L. v. *denitrifico*, to denitrify; M.L. part. adj. *halodenitrificans*, salt [-requiring] and denitrifying). Cells are rod shaped (0.6 to 0.8 by 2.5 to 4.0 μm) and occur singly or in short chains. Anaerobically grown cells are often more elongated and flexuous, with lengths of up to 15 μm. Motile with frequent spinning and twisting. Only a few flagella are observed in both lateral and polar arrangements. Gram stain variable. Asporulated. On solid, complex media containing 10% salt, colonies are entire, smooth, semitranslucent, cream colored, and about 2 mm in diameter; darkening and limited spreading occur after extended incubation.

Facultative anaerobe capable of anaerobic growth only when nitrate (or nitrite) is present. Reduces nitrate to nitrite and nitrous oxide; no dinitrogen produced. Moderate halophile. The salinity range for growth is between 0.35 and 4.35 M, with an optimum between 0.5 and 1.35 M NaCl. Salt requirement not specific for sodium or chloride ions. Grows in anaerobic liquid media containing up to 1.65 M (14%) NaNO₃ or 0.29 M (2%) NaNO₂. The pH range for growth is pH 5.8 to 9.6 (optimum pH, around 7.4). Grows at temperatures ranging from 10 to 45°C (optimum temperature, around 38°C).

Requires growth factors and an organic (reduced) sulfur

TABLE 1. Gas production by whole cells by denitrifying bacteria^a

Organism	Gas production (μmol of gas per h per mg [dry wt]) from:		Reference
	NO ₃ ⁻	NO ₂ ⁻	
<i>B. halodenitrificans</i>	0.96 ^b	1.2 ^b	
<i>Bacillus</i> sp. strain RS3	1 (3.9) ^c	1.9 (5.1)	32
<i>Bacillus</i> sp. strain RS14	0.5 (3.3)	0.6 (6.9)	32
<i>B. licheniformis</i>	0–0.1 ^b	0–0.12 ^b	31
<i>Pseudomonas stutzeri</i>	2.8	1.74	5
<i>Pseudomonas aeruginosa</i>	1.35	0.28 ^d	5
<i>Paracoccus denitrificans</i>	1–1.9	1.76	5
<i>Alcaligenes faecalis</i>	ND ^e	2.12	27
<i>Corynebacterium nephridii</i> ^f	0.5–1.6 ^b	0.42–1.4 ^b	33

^a Values from references 31 through 33, which were originally given per milligram of cell nitrogen, were converted to milligrams of dry weight by assuming that cell nitrogen is 15% of the dry weight. Microliters of gas were converted to micromoles by using the following conversion: 1 mol = 22.4 liters.

^b The gas produced was N₂O.

^c The values in parentheses are values for nitrite-grown cells.

^d The gas produced was mostly NO.

^e ND, Not determined.

^f This bacterium is more likely an "*Achromobacter*" species (14).

source. Utilizes ammonia and glutamine as nitrogen sources, but not nitrate, arginine, or urea. Grows well on fructose, mannose, galactose, gluconate, glucose, maltose, sucrose, fumarate, lactate, L-malate, pyruvate, succinate, glycerol, mannitol, alanine, asparagine, aspartate, glutamine, glutamate, serine, threonine, and tryptophan as carbon and energy sources and more slowly on lactose, acetate, histidine, isoleucine, leucine, proline, and valine. Does not grow on xylose, D- and L-arabinose, butyrate, citrate, formate, propionate, ethanol, methanol, sorbitol, arginine, cysteine, glycine, lysine, methionine, and phenylalanine. Produces acid (weakly) but no gas from glucose. Catalase and cytochrome oxidase positive. Hydrolyzes gelatin and casein but not starch, esculin, or Tween 80. β-Galactosidase positive. Urease, deoxyribonuclease, lysine decarboxylase, ornithine decarboxylase, arginine dihydrolase, phenylalanine deaminase, and lecithinase negative. Indole negative. Does not produce dihydroxyacetone from glycerol or hydrogen sulfide from cysteine. Voges-Proskauer reaction negative. Does not decompose tyrosine.

Susceptible to streptomycin (10 μg), chloramphenicol (30 μg), tetracycline (30 μg), and novobiocin (30 μg), but not to penicillin G (10 U), polymyxin B (300 U), or vibriostatic agent O/129.

Produces a menaquinone of the MK-7 type. Contains large amounts of type *b* and *c* cytochromes that confer a pinkish orange color to concentrated cell suspensions. Synthesizes a nonheme, copper-containing nitrite reductase.

Isolated from a solar evaporation pond (saltern) in Berre l'étang, France.

The guanine-plus-cytosine content of the deoxyribonucleic acid is 38 mol%.

A subculture of *B. halodenitrificans* has been deposited in the American Type Culture Collection, Rockville, Md., as strain ATCC 49067; this is the type strain by monotypy.

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