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Identification and Characterization of *Bdellovibrio bacteriovorus*, a Predator of *Burkholderia glumae*

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Abstract Six strains of an obligate predatory bdellovibrio isolate that preys on *Burkholderia glumae* in rice paddy field water and rhizosphere soil, were identified and characterized. The numbers of *Bdellovibrio* cells varied from 3.2×10^3 to 9.2×10^3 plaque-forming unit/g after enrichment in cells of *B. glumae*. Prey range tests with six *Bdellovibrio* strains and 17 prey strains of rice-pathogenic, antibiosis-related, or nitrogen-fixing bacteria resulted in unique predation patterns in related prey cells. Strain BG282 had the widest prey range on 7 plant pathogenic bacteria among the 17 prey strains tested. However, no predation occurred with strains of *Azospirillum brasilense*, *Paenibacillus polymyxa*, *Pseudomonas fluorescens*, *P. putida*, and *Serratia marcescens* that are associated with antibiosis or nitrogen fixation in the rice ecosystem. Identification was confirmed by the presence of typical bdelloplast in the prey cells of *B. glumae* and by a PCR assay using *B. bacteriovorus*-specific primers. Furthermore, 16S rDNA sequencing of the six bdellovibrio strains showed a homology range of 97.2% to 99.2% to the type strain of *B. bacteriovorus*.

Key words: Obligate predatory, *Bdellovibrio bacteriovorus*, *Burkholderia glumae*, plant pathogen

Bdellovibrio bacteriovorus is a small, comma-shaped, Gram-negative, predatory bacterium that obligately preys upon a wide variety of susceptible Gram-negative bacteria. This predatory bacterium is characterized by two distinct stages in its life cycle including a predatory attack-phase and a parasitic growth-phase. The biphasic growth cycle includes a free-swimming attack, entry stage, and an intraperiplasmic proliferative stage that exists within a bdelloplast. This unique growth cycle distinguishes *Bdellovibrio* from all other bacterial parasites [13]. The presence of the intraperiplasmic growth phase forming the bdelloplast within an invaded bacterium is the most unique and important taxonomic characteristic of *Bdellovibrio* species [3]. Bdellovibrios are a heterogeneous group which include this type of biphasic bacteria, but a recent proposal suggested transferring two *Bdellovibrio* species, *B. stolpii* and *B. starii*, into a new genus, *Bacteriovorax*. These organisms are ubiquitous in soil and can also often be found in a wide range of other habitats, including water and water-related ecosystems [17, 19, 20, 23], and the gut of some vertebrates [11, 29]. However, the relationship of these organisms to other bacterial communities within the same ecological niche is mostly unknown.

Unlike the highly specific host range of bacteriophages, the prey range of *B. bacteriovorus* includes many Gram-negative species. The wide prey range and the lethal attack of these bacterial pathogens make them attractive candidates for controlling plant pathogenic bacteria. Attempts have been made to suppress the population of several phytopathogenic bacteria, including *Xanthomonas oryzae* pv. *oryzae* [26], *Erwinia carotovora* subsp. *carotovora* [9], and *Pseudomonas glycinea* [22], but none have gained acceptance.

In this study, we characterize several *B. bacteriovorus* strains with potential for the biological control of *B. glumae*, the causal agent of rice grain rot [10].

Materials and Methods

Bacterial Strains, Isolation Procedure, and Maintenance

The *Bdellovibrio* strains and prey strains are shown in Tables 1 and 2, respectively. All *Bdellovibrio* strains were isolated from rice ecosystems such as paddy field water or rhizosphere soil collected in Korea in 2002 (Table 1). Bacteria used as prey strains were obtained from several culture collections (Table 2).

All *Bdellovibrio* strains were isolated after enrichment as described below. Prey cells of *B. glumae* were cultured overnight in nutrient broth-yeast extract (NBY) and adjusted to 1.0 at OD_{600} in phosphate-buffered saline (PBS, pH7.5) using a OPRON 2500 spectrophotometer (Hanson Technology,

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Table 1. Strain of *Bdellovibrio* retrieved from water of paddy field and from rhizosphere soil of rice and substrate, *Burkholderia glumae*, and quantification of enriched *Bdellovibrio* plaques on each prey cells[a].

Origin	*Bdellovibrio* strain no.	Substrate strain no.	10^3 pfu[b]	Total bacterial counts (cfu)[b]
Paddy field water	BG260	OG321	3.2±0.65	3.7×10^7
	BG278	OG344	6.8±0.55	3.3×10^7
	BG279	OG321	6.3±0.12	2.9×10^7
Rhizosphere soil	BG273	OG321	2.3±0.26	6.1×10^7
	BG282	OG344	9.2±0.82	3.8×10^7
	BG287	OG344	3.3±0.09	6.3×10^7

[a]The paddy field water and rhizosphere soil samples of rice used were randomly collected in Korea in 2002 and bdellovibrio cells were isolated by using prey strains and enrichment method described in Materials and Methods. Isolates were purified by three successive subcultures on the same prey cells and the strains were designated to BG.
[b]Plaque-forming unit (PFU) and colony-forming unit (CFU) on NBY agar plate are expressed per gram (dry weight) of soil and per milliliter of paddy field water.

Korea). Ten milliliters of diluted cells were seeded into a soil sample (40 g of rhizosphere soil mixed in 30 ml of PBS or 40 ml of paddy field water, respectively) in a 100-ml Erlenmyer flask and incubated at 30°C for 24 h. After incubation, the total volume was adjusted to 50 ml with PBS and the sample was centrifuged at 3,000 ×g for 5 min at 4°C. The supernatant was filtrated through double layers of cheesecloth followed by a 0.45 μm membrane filter and finally centrifuged at 12,000 ×g for 20 min at 4°C. After resuspending the pellet in 500 μl of PBS, the suspension was serially diluted to 10^{-4} in PBS, and 300 μl of each dilution was mixed with 1.0 ml of the cell suspension of a potential prey organism such as *B. glumae* OG321 or OG344 in 0.7% molten diluted NBY agar. The mixture was spread onto a NBY agar (1.5% agar) plate, which was incubated at 30°C. Unlike those of bacteriophages, which appeared after a 1-day incubation, typical bdellovibrio plaques were detected after a 2 to 3-day incubation. Developing plaques were examined microscopically at 1,000× to confirm the presence of typical small bdellovibrio cells. Individual bdellovibrio plaques were cloned by three successive subculturings on lawns of *B. glumae* cells.

To prepare a lysate, an agar plug, containing one plaque and surrounding lawn of prey cells, was removed with a corkborer from a double-layered plate and suspended in 5× (w/v) volume of PBS with vigorous vortexing. The suspension was then incubated at 30°C on a rotary shaker at 200 rpm until the suspension was cleared. *Bdellovibrio* strains were stored as plaques on a double-layered NBY plate or lysates in PBS for up to 2 weeks at 4°C for short-term use. For long-term storage, aliquots of a 25% glycerol suspension of concentrated fresh lysate were stored at -80°C.

Determination of Prey Range and Lysis Kinetics

Cultures of the prey bacteria tested were grown in NBY broth to the stationary stage and then pelleted by centrifugation at 10,000 ×g for 10 min at 4°C. Each pellet was washed

Table 2. Prey ranges of *Bdellovibrio* strains[a].

Prey	Origin[b]	Prey range of BG:					
		260	273	278	279	282	287
Acidovorax avenae subsp. *avenae* COA1	1	+[c]	-	-	-	-	-
Agrobacterium tumefaciens 10298	1	-	-	-	-	-	+
Azospirillum brasilliens 10429	2	-	-	-	-	-	-
Burkholderia cepacia 102301	1	-	-	-	-	-	-
Burkholderia glumae OG321	1	+	+	+	+	+	+
B. glumae OG344	1	-	+	+	-	+	+
B. glumae OG366	1	-	-	+	-	+	-
Burkholderia plantarii 301723	3	-	+	-	-	+	-
Paenibacillus polymyxa 100601	1	-	-	-	-	-	-
Pantoea herbicola CEh1	1	-	-	-	-	-	-
Pseudomonas fluorescens 104101	1	+	-	-	-	-	-
Pseudomonas fuscovaginae 301177	3	+	-	-	-	+	-
Pseudomonas maginalis pv. *marginalis* 301173	3	-	-	+	-	+	-
Pseudomonas putida 101301	1	-	-	-	-	-	-
Pseudomonas syringe pv. *aptata* 301008	3	-	-	-	-	-	-
Serratia marcescens SDS7	1	-	-	-	-	-	-
Xanthomonas oryzae pv. *oryzae* OK2102	1	-	+	-	-	+	-

[a]Rice pathogenic, nitrogen-fixing, or plant growth-promoting strains in rice ecosystem were used as substrate bacteria.
[b]Origin of prey strain: 1, Laboratory collection; 2, KACC, Korean Agricultural Culture Collection, Suwon, Korea; and 3, MAFF, Ministry of Agriculture, Forestry and Fisheries of Japan, Tsukuba, Japan.
[c]Symbols: +, lysis; -, no lysis occurred.

once with PBS containing 2 mM $CaCl_2 \cdot 2H_2O$ (pH 7.8), resuspended in the same buffer, and adjusted to 0.5 at OD_{560}.

Fresh lysates of the bdellovibrio strains were filtered through a 0.4-μm-pore-size membrane filter (Whatman Paper Ltd., England) to remove residual prey cells, centrifuged at 10,000 ×g for 15 min at 4°C, and resuspended in PBS to 0.1 at OD_{560}. The supernatant was filtered through a 0.2-μm-pore-size membrane filter and used as a control.

One milliliter of a *Bdellovibrio* suspension was mixed into 3.0 ml of prey suspension in a screw-cap test tube. The controls included bdellovibrio-free prey suspensions and prey suspensions amended with filtered lysates. The mixtures were incubated overnight at 30°C with shaking at 200 rpm. The resulting optical density was measured at 560 nm as described above. Triplicates of each parasite-prey combination were prepared and tested.

Using the same procedures, the lysis kinetics of BG273 and BG282 were monitored with *B. glumae* OG321 and OG344, respectively, in a triplicate of screw-cap test tubes. The control contained prey cells without bdellovibrio cells. Optical densities were determined every 6 h.

Electron Microscopy

Transmission electron microscopy of negatively stained cell preparations of developing plaque was used to measure the size of strain BG282 cells. After adding a single plaque to 100 ml PBS and gentle mixing, one drop of the suspension was spotted onto a microscope grid. Excess liquid was removed by blotting with filter paper, and the sample was counterstained with a 1% (w/v) solution of uranylacetate for 30 min, air-dried, and examined with a JEOL model 100-CX transmission electron microscope.

Primers and PCR Conditions

A universal forward primer 63F [16] and *B. bacteriovorus*-specific reverse primer 434R [12] designed for amplifying 16S ribosomal DNA (rDNA) were used for the PCR-based detection and identification of *B. bacteriovorus*. For the preparation of the sequencing template, 63F [16] and *Bdellovibrio* specific 842R (5'-CGWCACTGAAGGGGT-CAA-3') [12] and 799F (5'-GGTAGTCCACGCCGTAA-ACGATG-3') and 1492R [1, 4, 5, 18] primer sets were used in PCR-amplification. The lysate cell suspensions obtained after filtration as above, resuspended in 10 mM Tris-HCl (pH 7.5), and adjusted to 0.1 at OD_{600} or individual plaque suspensions in 10 mM Tris-HCl (pH 7.5), were used for PCR amplification with whole cells after denaturation at 95°C for 10 min. In the latter case, plaques were resuspended in 100 μl of sterile 10 mM Tris-HCl (pH 7.5) and thoroughly vortexed. After a brief centrifugation, the liquid phase was transferred to a new tube and boiled in water for 15 min and the tubes were cooled on ice.

PCR (25-μl mixtures) was performed by using a PE9600 thermocycler (Perkin Elmer, Cetus, U.S.A). Each reaction mixture contained 1.5 mM $MgCl_2$, 2.5 mM each dNTP, 2.5 μl 10× reaction buffer, 1.0 U of Ampli*Taq* Gold, and 4 pmole each primer. Amplification was started after a 10-min denaturation by using 34 cycles of 94°C for 30 sec, 50°C for 30 sec, and 72°C for 1 min and a final elongation step of 72°C for 7 min.

DNA Sequencing, Sequence Analysis, and Phylogenetic Analysis

PCR-amplified products with a primer set of 63F and 842R or with primers 799F and 1492R and denatured whole cell suspensions of 6 *Bdellovibrio* strains were obtained by using GeneClean kit (BIO101, U.S.A.). Sequencing reaction was done by using a Big Dye terminator kit (Perkin-Elmer Inc., Branchburg, N.J., U.S.A.) and these primer pairs following the manufacturer's instructions. The sequences were determined by using a model Prism 377 DNA sequencer (Applied Biosystem Inc., U.S.A.).

16S rDNA sequence analyses, alignments, and phylogenetic analyses were performed by using the MagAlign program from Lagergene package (DNASTAR, WI, U.S.A.). The alignment was refined manually by visual inspection and by secondary-structure analysis. Final sequences were BLAST-searched for the species identification of *Bdellovibrio* candidates. Multiple sequence alignments with a selection of the available sequences of 16S rDNA from GenBank were performed using the MegAlign program and a phylogenetic tree was constructed with the Clustral algorithm of the program.

Results and Discussion

Enrichment, Isolation, and Quantification of Paddy Field Water and Rhizosphere *Bdellovibrio* Strains

Six strains were isolated from rhizosphere soil and paddy field rice water (Table 1). The isolation procedure resulted in the efficient enrichment and separation of target bdellovibrios from plaque-forming bacteriophages. The presence of very small microscopic vibrioid cells from tested plaques was regarded as proof of bdellovibrio-induced activity rather than bacteriophage.

Three paddy water strains and three rhizosphere soil strains isolated by preying on *B. glumae* OG321 and OG344, respectively, were tested to determine whether they exhibited activity with each of three substrate *B. glumae* strains, OG321, OG344, and OG366, in liquid culture. While BG278 and BG282 induced lysis-plaques of three *B. glumae* strains, OG321, OG344, and OG366, strains BG260 and BG279 did not prey on *B. glumae* OG344 and OG366. Strains BG273 and BG287 were inactive only, when *B. glumae* OG366 was used as a prey. Table 1 shows the numbers of bdellovibrio plaques retrieved from the paddy field water and the rhizosphere rice soil after the enrichment.

Bdellovibrios should be isolated and grown only in two-member cultures with selected prey organisms because of their obligate parasitism [21]. This characteristic makes estimating bdellovibrio population sizes and isolating large numbers of strains difficult. A high prey density has often been shown to be necessary for bdellovibrio survival, and various authors have reported that prey concentrations of at least 10^5 to 10^6 cfu/g soil or per ml are required for bdellovibrio survival [15, 27]. Moreover, high densities of other fast-growing bacteria coexisted in the tested paddy field water and rhizosphere soil at different levels. Furthermore, substantial losses of bdellovibrio cells resulted after filtration and differential centrifugation in an attempt to reduce unwanted bacterial background. The low recovery of bdellovibrios from the samples suggested that the target bdellovibrios preying on *B. glumae* were only a small fraction of the total bacterial population in the rhizosphere soil and paddy water samples of rice. Therefore, direct isolation of target bdellovibrio without the enrichment procedure using a high density of prey cells was not often successful in finding target bdellovibrio plaques on the test plates. To circumvent these problems, we used the enrichment method in this study, and the numbers of bdellovibrio plaques per gram or milliliter of sample only after the enrichment steps were enough to circumvent the recovery problems resulting from the low density of bdellovibrio preying on *B. glumae* in the samples (data not shown). Although the numbers of bdellovibrios have been reported to fluctuate seasonally in water to below detectable levels during the colder months [14, 30, 31], by using our enrichment procedure, we have observed that the numbers of bdellovibrio cells are relatively stable within the rhizosphere soil and paddy-field water.

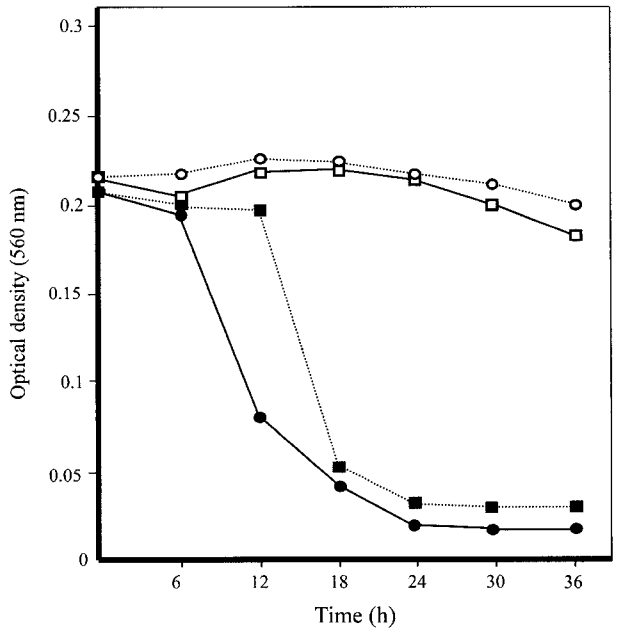

Fig. 1. Predation kinetics of *Burkholderia glumae* OG321 and OG344 by bdellovibrio strains BG273 and BG282, respectively. Mixture of BG282 and OG344 cells (■) and mixture of BG273 and OG321 (●) were tested and the control, OG344 cells (□) and OG321 cells (○), contained prey cells without bdellovibrios.

Determination of Prey Range and Kinetics of Prey Lysis

The *Bdellovibrio* strains isolated from paddy field water (BG260, BG278, & BG279) and rhizosphere (BG273, BG282, & BG287) lysed a wide range of selected bacteria from the rice-related ecosystem. Rhizosphere strain, BG278, preyed on *B. glumae* OG344 showed a wider prey range than most other strains. Strains BG260 and BG279 preyed on *B. glumae* OG321 and strains BG273 and BG287 preyed on *B. glumae* OG321, and OG344. Strains BG278 and BG282 preyed on *B. glumae* OG321, OG344, and OG366, respectively. Strains BG278 and BG282 preyed on all strains of *B. glumae* tested, while BG282 induced lysis of both *B. glumae* and *X. oryzae* pv. *oryzae*, which are major bacterial pathogens of rice.

The strain with the widest prey range was BG282, which preyed on 7 of the 17 prey strains tested. *B. glumae* OG321 supported the growth of all six bdellovibrio strains,

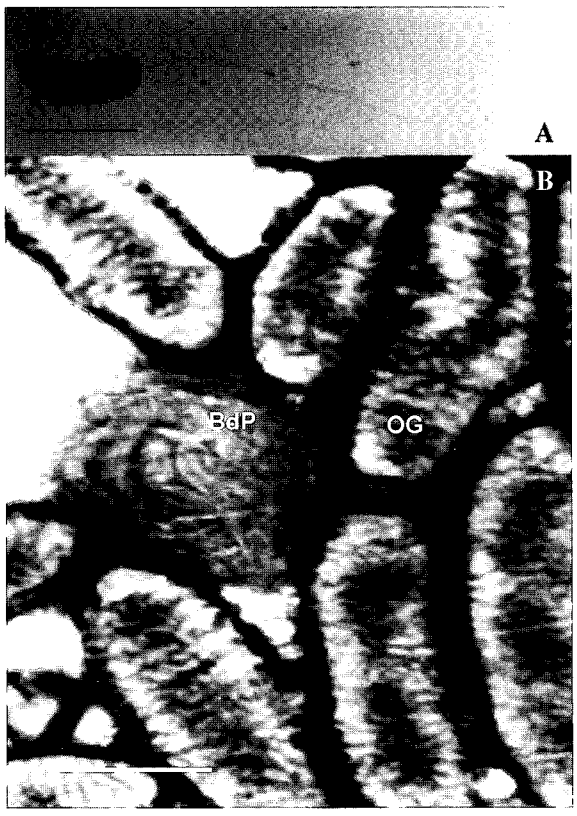

Fig. 2. Electron micrographs of *Bdellovibrio* strain BG282. A, Attack-phase cell; B, bdelloplast by *Bdellovibrio* BG282 (BdP) near *B. glumae* OG344 (OG). Bars indicate 0.5 μm.

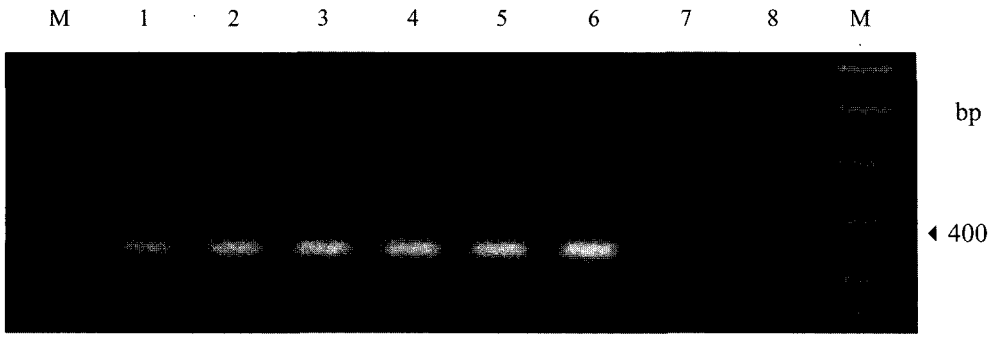

Fig. 3. PCR-amplified products (370 bp) obtained by using *Bdellovibrio bacteriovorus*-specific primer set, 63F & 434R, and *Bdellovibrio* strains from rice paddy field water and rhizosphere.
Lanes: M, PCR marker (Promega, USA); 1, BG260; 2, BG273; 3, BG278; 4, BG279; 5, BG282; 6, BG287 strain; 7, *B. glumae* OG321; and 8, *B. glumae* OG344.

while *Burkholderia cepacia*, *Paenibacillus polymyxa*, *Pantoea herbicola*, *Pseudomonas syringae* pv. *aptata*, and *Serratia marcescens* were resistant to predation by all the *Bdellovibrio* strains tested (Table 2). Large differences were observed in this prey range analysis, even when the substrate strains used were from the same species. While *B. glumae* OG321 was preyed on by all strains, *B. glumae* OG344 and 366 were not. Similarly, the bdellovibrio strains exhibited unique preying abilities on the prey substrates of other rice-pathogenic bacteria used. *Acidovorax avenae* subsp. *avenae*, *Agrobacterium tumefaciens*, *Azospirillium brasilense*, *Pseudomonas fluorescens*, and *Pseudomonas putida* were resistant to all of the bdellovibrio strains except one of each prey strain, respectively. Strains BG273, 278, 282, and 287 did not prey on the tested beneficial bacteria such as *Azospirillium brasilense*, *Paenibacillus polymyxa*, *P. fluorescens*, *P. putida*, and *Serratia marcescens*, which are related to antibiosis or nitrogen fixation.

The lysis kinetics of BG273 and BG282 against substrate cells of *B. glumae* OG321 and OG344 were determined as

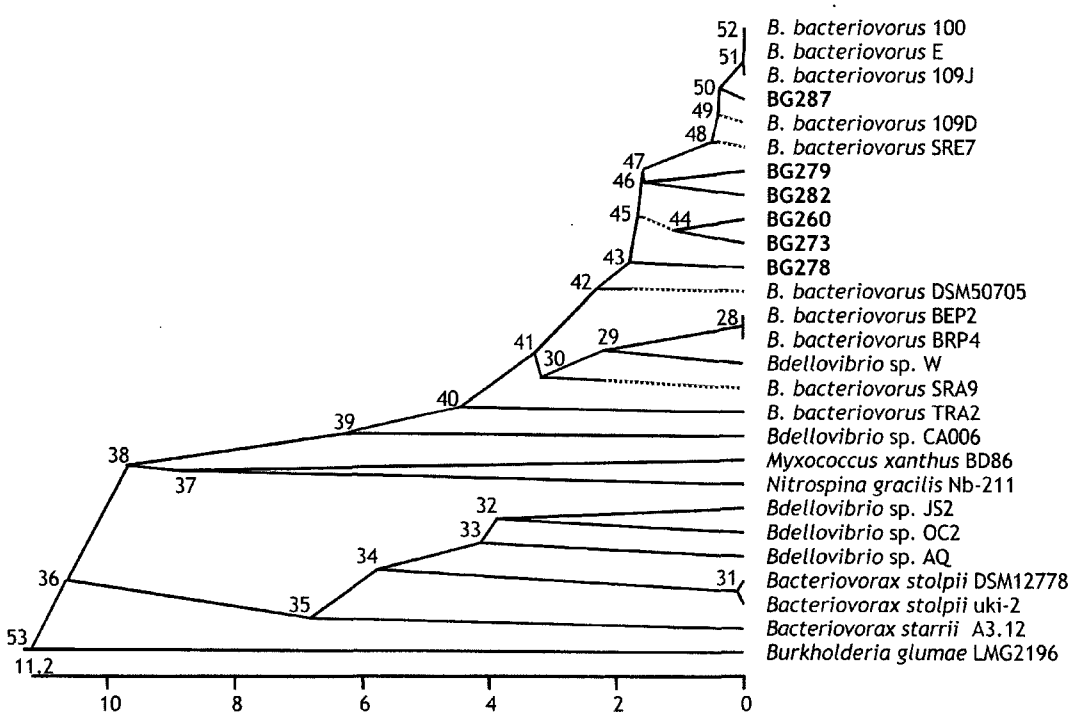

Fig. 4. Phylogenetic tree based on 16S rDNA sequences.
The position of bdellovibrio strains designated to BG and the representatives of some other bdellovibrios and related taxa published previously. The tree was constructed with the Clustral algorithm of the MegAlign program (DNASTAR, WI, U.S.A.). The length of each pair of branches represents the distance between sequence pairs. The scale beneath the tree measures the distance between sequences. Units indicate the numbers of substitution events.

shown in Fig. 1. Two bdellovibrio strains behaved similarly even though they did not have the same prey range. The mixed cultures showed similar patterns. Although lysis of OG344 and BG282 and OG321 and BG273 occurred after 6 h and 12 h, respectively, they were all lysed after 30 h. When preying on cells in *B. glumae* OG321 in agar plates, strain BG282 developed plaques more slowly than strain BG282. The lysis zones were up to 1 mm in diameter after 2 days, while BG273 plaques were rarely 2.0 to 3.5 mm in diameter when these strains were used as prey cells.

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100     GTGGCGCACGGGTGAGGAACGCGTGGATAATCTGCCTTAGAGTGGGGGATAACTAGTCGAAAGATTAGCTAATACCGCATAAGACCACAGGAGCTGCGGC   100
BG279   ................T..C.....................C.A.....................G.....................................   100
BG260   ..........................................A.....................TT.................T.................T   100
BG287   ..........C.....................................................G.....................................   100
BG273   ..................................................GGT.....................................................   100
BG278   ..........................................T.....A.............G.....................GA.................T   100
BG282   ..................................TC.A.....................................................................   100

100     TCTAGGGGTCAAAGGTTTTTCGCTCTAAGATGAGTCCGCGTAAGATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGATCTTTAACTGGTCTGA   200
BG279   ..C..............A.....................................................T.....................A.....   200
BG260   ..C..............A.................GC.....................................G.................A.......   200
BG287   ..................................................................................................   200
BG273   ..................A.................G.....................A.....................C.....................   200
BG278   ............T..............T.....................................C.....................................   200
BG282   ..........................................A.....................G.....................................   200

100     GAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTAGGGAATATTGCACAATGGAGGAAACTCTGATGCAGCGAC   300
BG279   ............A.....................................................................................   300
BG260   ..................................................................................................   300
BG287   ...AT..............................................................................................   300
BG273   ..................................................................................................   300
BG278   .........G.....................................T....T.............................................   300
BG282   ............A.....................C.....................................................................   300

100     GCCGCGTGAGTGATGAAGGCCTTCGGGTCGTAAAGCTCTGTCGCAGGGGAATAACACAATGAATGTACCCTGTAAGAAAGGATCGGCTAACTTCGTGCCA   400
BG279   ....................A.....................................................................................   400
BG260   ..................................................................................................   400
BG287   ..................................................................................................   400
BG273   ..................................................................................................   400
BG278   ..................................................................................................   400
BG282   ..............T.C.....................................................................................   400

100     GCAGCCGCGGTAAGACGAGGGATCCTAGCGTTGTTCGGAATTATTGGGCGTAAAGCGGATGTAGGTGGCTTTGTAAGTCAGATGTGAAAGCCCAGGGCTC   500
BG279   ..................................................................................................   500
BG260   ..................................................................................................   500
BG287   ..................................................................................................   500
BG273   ..................................................................................................   500
BG278   ..................................................................................................   500
BG282   ....................C.....................................................................................   500

100     AACCCTGGAAGTGCATTTGATACTGCGAAGCTTGAGTGTCGGAGAGGTTACTAGAATTGTTGGTGTAGTGGTGAAATACGTAGATATCAACAGGAATACC   600
BG279   ..................................................................................................   600
BG260   ..................................................................................................   600
BG287   ..................................................................................................   600
BG273   ..................................................................................................   600
BG278   ..................................................................................................   600
BG282   ..................................................................................................   600

100     GGAGGCGAAGGCGGGTAACTGGCCGAACACTGACACTGAGATCCGAAAGCGTGGGGATCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGAT   700
BG279   ..................................................................................................   700
BG260   ..................................................................................................   700
BG287   ........T.....................................................................................   700
BG273   ..................................................................................................   700
BG278   ..................................................................................................   700
BG282   ..................................................................................................   700

100     GGATACTTGTTGTTGGAGGTATTGACCCCTTCAGTGACGAAGCTAACGCGTTAAGTATCCCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGAAA   800
BG279   ..........................G.....................AA.....................................................   800
BG260   ..........................T.....................A.....................................................   800
BG287   ..............A.....................................................................................   800
BG273   .......A.....................................................G.....................................   800
BG278   ..............................T.C.....................................A.....................T.   800
BG282   ..........A...C.....................................................................G.A.............   800

100     TTGACGGGGGCCCGC-ACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTAGGCTTGACATGTACTGGAAGACTGGCAGAAA   899
BG279   ..............-.......C.....................................................................G.....   899
BG260   ..............-.....................G.....................................T.T.....................   899
BG287   ..............-.....................................TG.....................................................   899
BG273   ..............-..................................................................................   899
BG278   .....C........-..................................................................................   899
BG282   ..............C.....................C.....................CC.......G.....................................   900

100     TGTCGTCGCCCGCAAGGGTCGGTACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCCTGC   999
BG279   .....A.....................................................................................   999
BG260   ..................................................................................................   999
BG287   ..................................................................................................   999
BG273   ..................A.....................A.....................................................   999
BG278   A.......G.....................C.......A.....................................................   999
BG282   ..................C.....................A.....................................................   1000

100     ATTTAGTTGCCAGCATTCAGTTGGGCACTCTAGATGGACTGCCGGTGTTAAACC                                          1053
BG279   ..........................................                                                       1053
BG260   ..........................................                                                       1053
BG287   ..........................................                                                       1053
BG273   ..........................................                                                       1053
BG278   ..........................................                                                       1053
BG282   ..........................................                                                       1054
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Fig. 5. Comparison of partial 16S rDNA sequence of bdellovibrio strains aligned with that of *Bdellovibrio bacteriovorus* 100^T (GenBank accession no.: AF084850).

Sequences were aligned by using MegAlign software (DNASTAR, WI., U.S.A.) with the Clustral algorithm. Identical sequence is indicated by a dot.

However, no differences were observed in the kinetics of lysis for the strains in a screw-cap test tube (data not shown).

The range of susceptible prey cells varies with the *Bdellovibrio* strain but is confined to Gram-negative bacteria. The wide prey range remains one of the unsolved enigmas of *Bdellovibrio* spp. as intracellular parasitic organisms. The prey range is presumed to be determined by some specificity for attachment, but a receptor has not been identified. The mechanism for prey specificity or preference is not known, although it has been suggested that the R antigen is involved [28]. However, *B. glumae* and *X. oryzae* pv. *oryzae* differ markedly in this respect, although both of these organisms are lysed by strain BG282.

Electron Microscopy

Cells of BG282 were the smallest (0.55 by 0.3 μm) (Fig. 2A). A typical bdellovibrio-containing bdelloplast that is unique at the parasitic stage of *Bdellovibrio* sp. was often observed (Fig. 2B).

PCR Amplification and Sequence Analysis

A PCR fragment (370 bp) was obtained from all of the six strains in this study by using a set of *B. bacteriovorus*-specific primer, 434R [12], and bacterial universal primer, 63F [16], designed from 16S rDNA for PCR-based identification of *B. bacteriovorus* (Fig. 3).

The partial 16S rRNA gene sequences of six bdellovibrio strains originated from rhizosphere soil and paddy field rice water exhibited homology to the 16S rRNA gene of *Bdellovibrio* spp. *Bdellovibrio* sequence data obtained from GenBank were used to construct a reliable phylogenetic tree based on the 16S rRNA gene sequence and these sequences include recently completed 16S rRNA sequences of bdellovibrio strains [2]. Phylogenetic analysis of the bdellovibrio isolates confirmed that these strains are members of the delta subclass of the class *Proteobacteria* [32], and that these organisms are related to previously reported *Myxococcus xanthus* and *Nitrospina gracilis* [6, 7, 13] as described in Fig. 4. The bdellovibrio strains could be divided into 4 groups using 16S rDNA sequences. However, this did not agree with the results of the prey range pattern. Although BG278 and BG282 had identical activity to *B. glumae* strains, BG278 showed a low level of homology (94.9%) to the 16S rDNA sequence of BG282. Strain BG278 belonged to the *B. bacteriovorus* group based on the 16S rDNA sequence homology. However, the strain showed a low level of homology and it was more distantly related to other strains, exhibiting 97.2% identity with type strain *B. bacteriovorus* 100 (Fig. 5). BG287 showed the highest homology (99.2%) to the type strain, but showed a low homology to other strains, TRA2 (91.5%) and SRA9 (95.9%), of distantly related *B. bacteriovorus* group. Strains BG273 and BG282 had unidentical activity to *B. glumae* strains and the low sequence homology (96.6%), but they were found to be very similar in growth kinetics in liquid and solid media, and prey range.

The results obtained for prey ranges and phylogenetic relationships, based on partial 16S rDNA sequences, suggest that the bdellovibrio strains tested were not closely related. Even though a phylogenetic relationship cannot be inferred on the basis of a prey range that also depends on experimental conditions [25], such information still provides a useful tool for characterizing and distinguishing among bdellovibrios isolated from a particular environment and for assessing the impact of these predators on bacterial communities. In this study, rhizosphere strains BG278 and BG282 exhibited the same activity in all *B. glumae* strains tested, but had different prey spectra in spite of the fact that they were isolated with the same substrate cells.

The role of bdellovibrios in nature is not clear [24]. It would seem that they might be important in the regulation of prey populations in specialized habitats [8, 26]. Although bdellovibrios will not eliminate a prey population under these conditions, they could adversely affect the activity of prey bacteria. The characteristic lethal attack by the predatory bacteria on prey bacteria has generated interest in their impact on bacterial mortality in nature and their potential as biological agents or as useful candidates for proteomics in the search for new lysis-related proteins. The increasing emphasis on the search for effective means of biocontrol over bacterial pathogens has stimulated additional research into the activity of bdellovibrios.

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