Diversity and evolution of *Bdellovibrio*-and-like organisms (BALOs), reclassification of *Bacteriovorax starrii* as *Peredibacter starrii* gen. nov., comb. nov., and description of the *Bacteriovorax*–*Peredibacter* clade as *Bacteriovoracaceae* fam. nov.

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A phylogenetic analysis of *Bdellovibrio*-and-like organisms (BALOs) was performed. It was based on the characterization of 71 strains and on all consequent 16S rRNA gene sequences available in databases, including clones identified by data-mining, totalling 120 strains from very varied biotopes. Amplified rDNA restriction analysis (ARDRA) accurately reflected the diversity and phylogenetic affiliation of BALOs, thereby providing an efficient screening tool. Extensive phylogenetic analysis of the 16S rRNA gene sequences revealed great diversity within the *Bdellovibrio* (> 14% divergence) and *Bacteriovorax* (> 16%) clades, which comprised nine and eight clusters, respectively, exhibiting more than 3% intra-cluster divergence. The clades diverged by more than 20%. The analysis of conserved 16S rRNA secondary structures showed that *Bdellovibrio* contained motifs atypical of the δ -*Proteobacteria*, suggesting that it is ancestral to *Bacteriovorax*. While none of the *Bdellovibrio* strains were of marine origin, *Bacteriovorax* included separate soil/freshwater and marine-specific groups. On the basis of their extensive diversity and the large distance separating the groups, it is proposed that *Bacteriovorax starrii* be placed into a new genus, *Peredibacter* gen. nov., with *Peredibacter starrii* A3.12^T (= ATCC 15145^T = NCCB 72004^T) as its type strain. Also proposed is a redefinition of the *Bdellovibrio* and the *Bacteriovorax*–*Peredibacter* lineages as two different families, i.e. '*Bdellovibrionaceae*' and a new family, *Bacteriovoracaceae*. Also, a re-evaluation of oligonucleotides targeting BALOs is presented, and the implications of the large diversity of these organisms and of their distribution in very different environments are discussed.

INTRODUCTION

The obligate Gram-negative predatory bacteria belonging to the *Bdellovibrio*-and-like organisms (BALOs) are ubiquitously found in soil, in association with plant roots, in sea water, fresh water, biofilms and sewage (Jurkevitch *et al.*, 2000; Varon & Shilo, 1980; Williams *et al.*, 1995). They were recently detected in the faeces of animals and humans (Schwudke *et al.*, 2001). BALOs exhibit a unique biphasic

life cycle in which a free-swimming cell infects the periplasmic space of a Gram-negative prey. Thereafter, the intracellular predator grows into a filament from which, at a later stage, progeny cells differentiate, lyse the host cell and start a new cycle (Jurkevitch, 2000). The molecular basis of this biphasic cycle and the determinants of the prey range (which varies greatly between BALO isolates) are unknown.

BALOs consist of two poorly defined genera, *Bdellovibrio* and *Bacteriovorax* (Baer *et al.*, 2000), which are affiliated with the δ -*Proteobacteria* (Woese, 1987). Recently, the classification of these two genera in the family '*Bdellovibrionaceae*' under the order '*Bdellovibrionales*' was proposed (Garrity *et al.*, 2002). To date, only three BALO species have been defined: *Bdellovibrio bacteriovorus*, *Bacteriovorax stolpii* and *Bacteriovorax starrii* (Baer *et al.*, 2000; Seidler *et al.*, 1972).

Abbreviations: ARDRA, amplified rDNA restriction analysis; BALO, *Bdellovibrio*-and-like organism.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences determined in this study are AY294207–AY294225.

Details of sequences of environmental clones exhibiting homology to BALOs, and 16S rRNA secondary-structure models, are available in IJSEM Online.

Although BALOs were discovered 40 years ago (Stolp & Starr, 1963), and earlier works have yielded an important understanding of their fascinating cellular and physiological capabilities, we still know little of the extent of their diversity, their evolutionary history is not resolved, and our knowledge of their taxonomy remains fragmentary. Knowledge acquired by studying these aspects of BALO biology would certainly help in the development of new approaches for ecological, physiological and genome-based studies, as well as enabling a better assessment of their role in nature, which is still only rather poorly understood.

Previous studies of the phylogeny of BALOs (Baer *et al.*, 2000; Donze *et al.*, 1991; Jurkevitch *et al.*, 2000; Schwudke *et al.*, 2001; Snyder *et al.*, 2002) were mostly restricted to certain habitats, made use of partial 16S rRNA gene sequences and were all based on a limited number of BALO strains (up to 33). Hence, a comprehensive study of BALO phylogeny based on the analysis of 16S rRNA gene sequences from predatory bacteria originating from very varied habitats is needed to enable a better classification of these organisms. Such a study could then be used as a framework for the reconstruction of their natural history.

To achieve these goals, BALO strains originating from various habitats and geographical zones were analysed using the 16S rRNA gene as a phylogenetic marker. Using previously published BALO 16S rRNA gene sequences (Baer *et al.*, 2000; Jurkevitch *et al.*, 2000; Schwudke *et al.*, 2001; Snyder *et al.*, 2002) and sequence data available from environmental 16S rRNA gene clone libraries, we analysed the accumulated data from 120 BALO strains.

Our results reveal the very large diversity of BALOs and provide support for the definition of a new genus and for the reclassification of BALOs into two families. We also propose a putative scenario for the complex natural history of these organisms. Consequences for culture-independent studies of BALOs are presented together with a discussion of the implications of these results in a broader microbial ecological context.

METHODS

Bacterial strains, isolation procedure, media and maintenance.

The BALO isolates grown in the laboratory and their habitats are listed in Table 1. BALOs were isolated from the environment on double-layer agar as described previously (Jurkevitch *et al.*, 2000), except in the cases of freshwater and sewage isolates, for which 250 ml samples were centrifuged at 27 000 *g* for 20 min at 4 °C or homogenized and centrifuged at 800 *g* for 5 min, respectively, then processed like the samples from other habitats.

Media, maintenance and growth conditions for BALO and prey cultures were as described previously (Jurkevitch *et al.*, 2000), except for BALO strains PNEc1, PNEc2 and PNEc3, which were grown at 18 °C. The presence of very active, swimming, small, usually vibrioid cells and interactions with prey cells, as observed by using phase-contrast microscopy, established that the lysates obtained from all the plaques tested resulted from BALO-predatory activity.

Amplification of the 16S rRNA gene from BALO isolates. To remove residual prey but keep predatory cells, late-stage BALO-induced lysates were filtered (0.45 µm pore size), centrifuged at 10 000 *g* for 10 min and resuspended in sterile double-distilled water. The suspensions were subjected to three cycles of freezing in liquid nitrogen followed by 3 min of heating in boiling water, cooled on ice and 10 % DMSO added. BALO-specific primer 842R (Jurkevitch & Ramati, 2000) was used in conjunction with *Bacteria*-domain-targeted primer 63F (Marchesi *et al.*, 1998), to yield an approximately 800 bp fragment of the 16S rRNA gene. When no product could be obtained, *Bacteria*-domain-targeted primer 907R (Teske *et al.*, 1996) was used instead of primer 842R. In that case, the lysates were filtered twice through 0.45 µm membranes to ensure that no prey DNA would be amplified. Also, the 16S rRNA gene of the prey in pure culture was similarly amplified.

Amplification was performed as described previously (Jurkevitch *et al.*, 2000), apart from the addition of 12.5 µg bovine serum albumin per 50 µl reaction. Amplification products were purified using the High Pure PCR purification kit (Roche Molecular Biochemicals) prior to further analysis.

Amplified rDNA restriction analysis (ARDRA). To select the most distinguishing restriction enzymes, all available BALO 16S rRNA gene sequences were subjected to simulated digestion with almost all commercially available restriction enzymes. Three enzymes, *Hae*III, *Hinf*I and *Rsa*I, were selected. *Eco*RI was used for further analysis of specific groups. Each PCR product (approx. 15 µl) was separately digested with each restriction enzyme according to manufacturers' instructions. Separation of the digested PCR fragments was performed by electrophoresis in 3 % (w/v) NuSieve GTG agarose (FMC) or in 2.5 % (w/v) agarose gel in TAE buffer (40 mM Tris/acetate, 1 mM EDTA) containing 0.5 µg ethidium bromide ml⁻¹.

Amplification products obtained with prey cultures were analysed by ARDRA and the resulting patterns were compared with those of BALO products obtained with the *Bacteria*-domain-targeted primer pair, to ensure that the latter originated from the predators alone.

The combination of the restriction patterns defined the ARDRA group affiliation of each strain. Restriction maps were inferred from comparison of the resulting fragment patterns with the available sequences. Twenty-nine different restriction sites were identified. Restriction-site differences were analysed by using the maximum-likelihood approach for analysis of restriction sites (Felsenstein, 1992), available with the PHYLIP package (J. Felsenstein, University of Washington, Seattle).

DNA sequencing and phylogenetic analysis. The 16S rRNA gene of selected isolates was first partially sequenced (450–750 bp). When sequences diverged from any other known sequence by more than 1.5 %, the gene was sequenced along almost its entire length (more than 1400 bp), using internal primers (Jurkevitch *et al.*, 2000) or new custom-designed primers, as needed for primer walking. DNA sequencing was carried out with an ABI Prism 337 DNA sequencer (Applied Biosystems).

16S rRNA gene sequences were added to the rRNA gene sequence database of the ARB phylogenetic program package (Strunk *et al.*, 1999). The ARB-ALIGN tool was used for sequence alignment. The resulting alignments were checked and corrected manually on the basis of both primary and secondary structure considerations.

Framework phylogenetic trees were constructed on the basis of positions 69–1404 (*Escherichia coli* numbering) of the BALO 16S rRNA gene sequences, using the ARB neighbour-joining, parsimony and maximum-likelihood methods, in combinations with different filters (excluding different degrees of variable positions), different

Table 1. BALO strains used in this study and their ARDRA group

Strain(s)	Host	Origin	Reference/source	ARDRA group*
HEA	<i>Escherichia coli</i> ML35	Soil, Rehovot, Israel	This study	B
ESA, ESB, ESC	<i>Pseudomonas syringae</i>	Soil, spring bank, Eyn-hanaziv, Israel†	This study	U
EPA	<i>Pseudomonas corrugata</i> PC	Soil, spring bank, Eyn-hanaziv, Israel†	This study	L
EPB, EPC2, EPC4	<i>Pseudomonas corrugata</i> PC	Soil, spring bank, Eyn-hanaziv, Israel†	This study	U
EPC3	<i>Pseudomonas corrugata</i> PC	Soil, spring bank, Eyn-hanaziv, Israel†	This study	K
ETA, ETB	<i>Agrobacterium tumefaciens</i>	Soil, spring bank, Eyn-hanaziv, Israel†	This study	C
ETC	<i>Agrobacterium tumefaciens</i>	Soil, spring bank, Eyn-hanaziv, Israel†	This study	P‡
EEA	<i>Escherichia coli</i> ML35	Soil, spring bank, Eyn-hanaziv, Israel†	This study	P
ECB, ECC	<i>Erwinia carotovora</i> subsp. <i>carotovora</i>	Soil, spring bank, Eyn-hanaziv, Israel†	This study	P
MPA	<i>Pseudomonas corrugata</i> PC	Soil, Munich, Germany	This study	F
MTA	<i>Agrobacterium tumefaciens</i>	Soil, Munich, Germany	This study	C
PC2	<i>Pseudomonas corrugata</i> PC	Soil, Rehovot, Israel	This study	K
Pt1	<i>Pseudomonas syringae</i>	Soil, Rehovot, Israel	This study	B
Reactor1, Reactor2	<i>Escherichia coli</i> ML35	Aeration reactor, Shafdan Wastewater Treatment Plant, Israel	This study	O
RAS1, RAS2, RAS3	<i>Escherichia coli</i> ML35	Return activated sludge, Shafdan Wastewater Treatment Plant, Israel	This study	O
SRE11, SRE13	<i>Erwinia carotovora</i> subsp. <i>carotovora</i>	Soil, Rehovot, Israel	This study	A
SRE14	<i>Erwinia carotovora</i> subsp. <i>carotovora</i>	Soil, Rehovot, Israel	This study	B‡
SNE	<i>Erwinia carotovora</i> subsp. <i>carotovora</i>	Soil, Nes-Ziyona, Israel	This study	A
SJE	<i>Erwinia carotovora</i> subsp. <i>carotovora</i>	Soil, Jerusalem, Israel	This study	B
FCE	<i>Erwinia carotovora</i> subsp. <i>carotovora</i>	Strawberry root, Rehovot, Israel	This study	M
DPE	<i>Erwinia carotovora</i> subsp. <i>carotovora</i>	Drainage water from hydroponically grown pepper, Beit-dagan, Israel	This study	H
PRE	<i>Erwinia carotovora</i> subsp. <i>carotovora</i>	Root extract of hydroponically grown pepper, Beit-dagan, Israel	This study	H
PNEc1, PNEc2, PNEc3	<i>Escherichia coli</i> ML35	Fresh water, pond, Tromso, Norway§	This study	M
Ec13, Ec14, Ec15, Ec16, Ec17	<i>Escherichia coli</i> ML35	Soil, Surata Lake, Uttar Pradesh, India	This study	D
R0, R1	<i>Agrobacterium tumefaciens</i>	Soil, Surata Lake, Uttar Pradesh, India	This study	C
R4	<i>Agrobacterium tumefaciens</i>	Soil, Surata Lake, Uttar Pradesh, India	This study	I
BEP2	<i>Pseudomonas corrugata</i> PC	Total root extract, Rehovot, Israel	Jurkevitch <i>et al.</i> (2000)	D

Table 1. cont.

Strain(s)	Host	Origin	Reference/source	ARDRA group*
SRP8E	<i>Pseudomonas corrugata</i> PC	Soil, Rehovot, Israel	Jurkevitch <i>et al.</i> (2000)	A
SRP20	<i>Pseudomonas corrugata</i> PC	Soil, Rehovot, Israel	Jurkevitch <i>et al.</i> (2000)	A
SRE8	<i>Erwinia carotovora</i> subsp. <i>carotovora</i>	Soil, Rehovot, Israel	Jurkevitch <i>et al.</i> (2000)	A
TRA2	<i>Agrobacterium tumefaciens</i>	Total root extract, Rehovot, Israel	Jurkevitch <i>et al.</i> (2000)	I
CHI	<i>Escherichia coli</i> AB6	Fresh water, Murcia, Spain	Kindly donated by F. Torrella (Universidad de Murcia, Spain)	A‡
F2	<i>Escherichia coli</i> AB6	Fresh water, Murcia, Spain	Kindly donated by F. Torrella	O
ARL-1, ARL-2	<i>Pseudomonas fluorescens</i>	Sewage, Pushchino, Russia	Kindly donated by A. Afinogenova (previously at the Russian Academy of Sciences, Pushchino, Russia)	G
ARL-11, B-608	<i>Erwinia carotovora</i> subsp. <i>carotovora</i>	Sewage, Pushchino, Russia	Kindly donated by A. Afinogenova	A
B-610	<i>Escherichia coli</i> K-12	Sewage, Pushchino, Russia	Kindly donated by A. Afinogenova	A
ARL-12	<i>Alcaligenes faecalis</i>	Oka River, Russia	Kindly donated by A. Afinogenova	H
P	<i>Escherichia coli</i> K-12	Fresh water, Pushchino Pond, Russia	Kindly donated by A. Afinogenova	A
L	<i>Pseudomonas fluorescens</i>	Sewage water, St Petersburg, Russia	Kindly donated by A. Afinogenova	G
Viet	<i>Escherichia coli</i> B	Anaerobic layer, rice field, Vietnam	Kindly donated by A. Afinogenova	A
NM1	<i>Proteus mirabilis</i>	Chicken gut, Germany	Schwudke <i>et al.</i> (2001)	A
Pf	<i>Proteus mirabilis</i>	Horse gut, Germany	Schwudke <i>et al.</i> (2001)	A
S1	<i>Proteus mirabilis</i>	Human gut, Germany	Schwudke <i>et al.</i> (2001)	A
SD1	<i>Citrobacter freundii</i>	Sewage, Leipzig, Germany	Schwudke <i>et al.</i> (2001)	N
100 ^T	<i>Erwinia amylovora</i>	Soil, California, USA	Stolp & Starr (1963)	A
109D	<i>Escherichia coli</i> ML35	Sewage, California, USA	Kindly donated by A. Afinogenova	A
109J	<i>Escherichia coli</i> ML35	Sewage, California, USA	American Type Culture Collection (Manassas, VA, USA)	A
W	<i>Rhodospirillum rubrum</i>	Sewage, Freiburg, Germany	American Type Culture Collection	E
Uki2 ^T	<i>Escherichia coli</i> B	Soil, Kentucky, USA	American Type Culture Collection	M
A3.12 ^T	<i>Pseudomonas fluorescens</i>	Soil, California, USA	Stolp & Starr (1963)	K
6-5-S	<i>Escherichia coli</i> ML35	London, Ontario, Canada	Kindly donated by A. Afinogenova	A
JSS	<i>Caulobacter crescentus</i>	Sewage, London, Ontario, Canada	Kindly donated by S. Koval (University of Western Ontario, Canada)	J

*See Fig. 1 and Table 2 for ARDRA patterns.

†Isolates retrieved in the same isolation event: ESA, ESB, ESC, EPA, EPB, EPC2, EPC3, EPC4, ETA, ETB, ETC, EEA, ECB, ECC.

‡In some cases, complex restriction patterns were exhibited (i.e. the total length of the restriction pattern bands was above the expected 800 bp) with enzyme *Hae*III (SRE14), *Hinf*I (CHI) or with both enzymes (ETC).

§Isolated and grown at 18 °C.

corrections and different outgroups. The consistency of the tree was also verified by bootstrapping ($n=1000$) for neighbour-joining and parsimony. Shorter sequences (less than 1100 bp) were then added to the tree using the parsimony and maximum-likelihood methods. It was verified that these additions did not alter the overall tree structure.

Identification of similarity to BALO sequences in environmental clones. 16S rRNA gene sequences from environmental clone libraries appearing in BLAST (<http://www.ncbi.nih.gov/blast/>) and FASTA (<http://www.ebi.ac.uk/fasta33/>) in similarity searches using BALO sequences were included in the phylogenetic trees as described above. Sequences for which phylogenetic positions could not be unambiguously determined, because of their short length or

their large divergence from other sequences, were not included in our analysis. In total, 11 almost-complete sequences and nine shorter sequences from environmental clones were selected (see Table A in IJSEM Online). Nine of these sequences exhibited similarity to the *Bdellovibrio* lineage and 11 showed similarity to the *Bacteriovorax* lineage.

Identification of differences in evolutionary rates as a source of uncertainty. We used the following criteria to identify differences in evolutionary rates and the long-branch attraction effect (Philippe & Laurent, 1998) on selected branches in our phylogenetic trees: (1) with a lower filter, the branch tended to move towards the root; (2) the more distant the outgroup used to root the

tree, the further the branch moved towards the root; (3) with parsimony or neighbour-joining as treeing methods, the branch tended to move towards the root, in comparison with maximum likelihood; and (4) the sequence under analysis shared conserved insertions or deletions (i.e. rare genomic changes) with sequences on other distantly related branches (the Hennigian approach; Philippe & Laurent, 1998).

Analysis of 16S rRNA secondary structure. Secondary structures were deduced using the European database on small-subunit rRNA (Wuyts *et al.*, 2002), the Comparative RNA Web Site (Cannone *et al.*, 2002) and previous work (Schwudke *et al.*, 2001). The results were confirmed by computational investigation using MFOLD (Mathews *et al.*, 1999).

Nucleotide sequence accession numbers. Sequences determined during this study are available from GenBank under accession numbers AY294207–AY294225. Environmental clone sequences are available in the GenBank database under accession numbers AY177783, AY168736, AF445705, AF445695, AF385539, AF332269, AF314449, AF314450, AY157087, AJ290009, AJ290002, AF030776, AF030781, AJ240999, AB054175, AJ518802, AF211265, AF392700, AF431402 and AY341110. Other BALO isolate sequences used in this study are available from GenBank under accession numbers AF084852, AF084850, M34125, AF263833, AF263832, AF148938–AF148941, AJ278145, AJ288899, AJ292759, AJ292760, AJ292518, AJ278146, AY094106–AY094131 and AF084851–AF084863.

Clone descriptions and corresponding accession numbers are given in Table A in IJSEM Online.

RESULTS AND DISCUSSION

All previously available BALO 16S rRNA sequence data, as well as data from new isolates, but excluding short and ambiguous sequences, were used in this study. Included in this were data for 71 isolates grown in the laboratory, sequence data from another 29 isolates (mostly published) found in the GenBank database, and 20 sequences from environmental clones, which were identified by data-mining.

ARDRA

Seventy-one BALO isolates (Table 1), originating from soil, root extracts, fresh water, sewage and animal faecal samples, spanning geographical regions from the tropics to almost arctic areas, and isolated using various Gram-negative bacteria as prey, were used to construct a phylogenetic screen using ARDRA. Restriction profiles, most of which are presented in Fig. 1(a), were obtained using *Hae*III, *Hinf*I and *Rsa*I. Combination of these profiles yielded 17 different ARDRA groups (Table 1 and Table 2). An *in-silico* analysis of another 18 sequences of BALOs isolated revealed four

Fig. 1. (a) Restriction patterns of amplified 16S rRNA gene fragments of BALOs after digestion with *Hae*III (I), *Hinf*I (II) and *Rsa*I (III). M, 100 bp molecular size marker. The combination of the patterns defined the different ARDRA groups. (b) Dendrogram of relatedness of the ARDRA groups. The tree was constructed using the maximum-likelihood method (RestML) based on the presence or absence of each restriction site in the different ARDRA groups. Bar, 10% estimated differences.

Table 2. Patterns obtained by ARDRA of BALOs

Group	ARDRA banding pattern*			Number of isolates	
	<i>Hae</i> III	<i>Hinf</i> I	<i>Rsa</i> I	This study	From database
A	1	1	1	19	3
B	1	3	2	4	—
C†	2	2	1	5	1
D†	2	2	1	6	1
E	10‡	1	1	1	—
F	3	1	1	1	—
G	1	2	2	3	—
H	1	1	2	3	1
I	9‡	9‡	1	2	—
J	4	4	1	1	—
K	5	5	3	3	1
L	5	5	7	1	—
M	6	6	4	5	—
N	6	6	5	1	1
O	6	6	6	6	—
P§	7	7	2	4	—
Q	11‡	10‡	8‡	—	5
R	12‡	6	9‡	—	2
S	13‡	6	10‡	—	2
T	6	6	11‡	—	1
U§	8	8	7	6	—

*Numbers in columns relate to the lanes in Fig. 1(a). Groups Q, R, S and T are based on *in-silico* digestions with sequences retrieved from GenBank.

†Distinguishable by restriction with *Eco*RI (for patterns, see Jurkevitch *et al.*, 2000).

‡Lanes not shown in Fig. 1(a).

§Amplicon obtained using primer 907R instead of 842R.

(Q, R, S, T) additional ARDRA groups. Groups Q, R and S were exclusively composed of marine strains.

On the basis of these data, a dendrogram was constructed (Fig. 1b). Two clades consisting of groups A–J and K–T, which completely overlapped the *Bdellovibrio* and the *Bacteriovorax* lineages and their internal complexities, respectively (see below), could be calculated. ARDRA group U formed a separate group (Fig. 1b). The taxonomic resolution of ARDRA is considered to be at the genus or species level (Heyndrickx *et al.*, 1996; Vandamme *et al.*, 1996) but, to date, BALOs have only been assigned to two genera and three species.

Fourteen strains isolated from Eyn-hanaziv soil in one isolation event (Table 1) belonged to five different ARDRA groups and were affiliated to *Bdellovibrio*, *Bacteriovorax starrii*, *Bacteriovorax stolpii* and to the unclassified U group, indicating that the same habitat supports a great diversity of BALOs.

16S rRNA gene sequence and phylogenetic analyses

The 16S rRNA genes of representative strains from different ARDRA groups were sequenced. In some cases, a number of strains from the same group but of different origins were selected to evaluate the resolution of ARDRA. Also included in the phylogenetic analysis were all BALO sequences available in databases and 20 sequences of environmental clones identified by similarity searches.

When long-branch attraction criteria were applied, a number of branches, most of which were in the *Bdellovibrio* lineage, behaved as if large differences in evolutionary rate existed between them and the rest of the lineage. Therefore, their phylogenetic positions varied considerably with different combinations of treeing methods, filters and outgroups. Strains included in ARDRA group U did not belong to either the *Bdellovibrio* or the *Bacteriovorax* lineage. Strain JSS clustered within the *Bdellovibrio* lineage but exhibited a unique morphology. Both will be discussed in other publications (Y. Davidov & E. Jurkevitch, unpublished).

Bdellovibrio phylogeny

Forty-two 16S rRNA gene sequences were included in the analysis of the *Bdellovibrio* clade (Fig. 2a). *Bdellovibrio* isolates and environmental clones originated from soil, roots, fresh water, sewage and faeces, but none were from marine environments. Using different treeing methods, filters and outgroups, nine stable monophyletic clusters were identified within the *Bdellovibrio* lineage (Fig. 2a). Sequence similarity between members of the different clusters was always below 97 %, while sequence similarity between members of the same cluster was always above 97.5 %. The definition of a bacterial species requires a polyphasic approach (Rossello-Mora & Amann, 2001; Vandamme *et al.*, 1996). However, strains that are more than 3 % divergent in their 16S rRNA gene sequences are nearly always members of different species (Stackebrandt & Goebel, 1994). The larger distances observed between the clusters (≥ 3 %) suggested that each represents at least one putative species. Strains that are less than 3 % divergent may or may not be members of different species (Fox *et al.*, 1992; Cohan, 2002), so more data are needed to determine in-cluster systematic affiliations. Although the phylogenetic positions of environmental strains PHOS-HC31, PHOS-HE81 and WB-66, represented by short (≤ 600 bp) sequences, could not be determined unequivocally (Fig. 2a), the degree of their divergence from other sequences supported a putative affiliation to currently unrecognized additional clusters. Despite the unambiguous strain composition of each cluster, and a stable internal structure in most, the relative positions of the clusters were often uncertain. The following description of the clusters supports the information obtained by ARDRA and by sequencing (Fig. 3a).

Cluster 1 was the largest BALO cluster, including 32 isolates. It comprised the species *Bdellovibrio bacteriovorus* and some of the first BALOs isolated (e.g. strain 100^T) and 109J, the best-studied BALO (Stolp & Starr, 1963). Although subgroups could be differentiated, the overall internal heterogeneity was below 2.2%. Whole genome DNA–DNA hybridization data are available for five of the strains in this cluster: 100^T, 109J, E, Ox92 and 2484Se2 (Baer *et al.*, 2000). A high degree of homology (75–100%) was observed between the four former strains, while lower values (33–55%) were obtained between strain 2484Se2 and the four other strains (Baer *et al.*, 2000). These results are in accordance with the relative positions of these strains, as determined by 16S rRNA gene sequence analysis (Fig. 2a). On the basis of the standard of 70% homology adopted for determining whether two strains should be considered as different species (Wayne *et al.*, 1987), the data supported the existence of more than one species within this cluster. Isolates found in this group varied in origin (Table 1). However, none of the environmental clones clustering with BALOs belonged to this group, suggesting a bias due to the isolation method employed. Cluster 2 consisted of six isolates and one environmental clone from soil and fresh water. The long distance separating it from its closest neighbour, which may be due to different evolutionary rates, hampered an unambiguous resolution of its position. All of the six strains constituting cluster 3 were isolated on *Agrobacterium tumefaciens* from four different soils. Clusters 4 and 5 were neighbouring clusters and included strains originating from sewage and soil, respectively. Strain W, the sole strain in cluster 4, is the only recognized BALO isolate exhibiting a resting stage (Hoeniger *et al.*, 1972). It is not currently known if the seven strains in neighbouring cluster 5 also exhibit this property. Clusters 6 and 7 are represented by a unique isolate and an environmental clone, respectively, the latter originating from an arsenite-oxidizing biofilm. Two environmental clones obtained from hot-spring travertine depositions in a 55 °C, slightly basic water column (G. Bonheyo, personal communication) formed cluster 8. These environmental sequences represented additional, hitherto unrecognized *Bdellovibrio* groups, but predatory behaviour could not be confirmed because the organisms were not isolated. These sequences showed clear signs of unusually rapid evolution and of long-branch attraction. One soil strain and one plant-root strain, each from different geographical origins, but both having been isolated on *A. tumefaciens*, constituted cluster 9. No identifiable phenotypic differences in predation dynamics, plaque development or predator–prey interactions could be detected between the members of this phylogenetically distant cluster and other members of the *Bdellovibrio* clade. Using accepted standards for genus definition (Ludwig *et al.*, 1998; Vandamme *et al.*, 1996), clusters 1–7, 8 and 9 (Fig. 3a) may represent different genera. On the basis of its outlying phylogenetic position and unknown morphology, the inclusion of oral clone CA006 as a true BALO is tentative, although it clearly clustered with the *Bdellovibrio* lineage.

***Bacteriovorax* phylogeny**

Forty-one 16S rRNA gene sequences were included in this analysis. Using different treeing methods, filters and outgroups, eight stable monophyletic clusters were identified within the *Bacteriovorax* lineage (Fig. 2b). Sequence similarity between members of the different clusters was always below 96%. Internal cluster similarity was above 98.5%, except for cluster I, in which heterogeneity was 96.4%. These eight clusters formed four stable monophyletic groups (Fig. 2b). Environmental clones HOC41 and Sva0447 may define additional, unrecognized putative *Bacteriovorax* groups, but because of their outlying phylogenetic and unstable position, as well as an unknown morphology, their affiliation to the BALOs is not certain. Because of the low quality of their sequences and long-branch attraction, environmental clones Adriatic33 and Adriatic91, which clearly clustered within the *Bacteriovorax* lineage, were not designated clusters. The following description of the clusters supports the information obtained by ARDRA and by sequencing (Fig. 3b).

Cluster I consisted of seven isolates, including *Bacteriovorax stolpii* Uki2^T, and six additional environmental clones from five different samples. Isolates and clones were mainly obtained from fresh water, but also came from sewage, soil, rhizospheres and uranium mining waste. The large 16S heterogeneity (3.6%) and the clear branching order within this cluster (Fig. 2b) showed that it may be composed of more than one species, but because other specific phenotypic or molecular characteristics were lacking, these phylotypes cannot yet be characterized as new species. The relatively high frequency of environmental clones in this cluster suggested that members of this group are more abundant than is reflected by the isolation methods. Cluster II consisted of seven freshwater and sewage isolates; together with cluster I, they formed the Uki2^T group (Fig. 2b).

Only marine strains were found in clusters III, IV and V. Together, they formed a monophyletic group consisting of 18 members (including clones Adriatic33 and CE46) with an inside sequence heterogeneity of more than 8% (the marine group, Fig. 2b).

Cluster VI was made up of four isolates of soil origin, and exhibited 12.2% divergence from the closest other BALOs. Together, the Uki2^T, marine and ETC groups formed a stable, distinct monophyletic lineage.

Clusters VII and VIII were composed of five soil isolates in total, including *Bacteriovorax starrii* A3.12^T. These organisms formed a very distinct monophyletic group (the A3.12^T group), with an inside sequence heterogeneity of 4%, and exhibited a minimal dissimilarity of more than 12.5% to the closest known group.

Since (1) the divergence separating the A3.12^T group from the other *Bacteriovorax* members was large, with a minimum distance of more than 12.5% and a maximum distance of more than 16%, (2) they formed two separated

Fig. 2. Phylogenetic 16S rRNA tree of the *Bdellovibrio* (a) and *Bacteriovorax* (b) lineages. The tree is based on maximum-likelihood (FastDNAmI) analysis, using a 50% conservation filter. Listed beside each strain or clone name is the GenBank accession number (in parentheses). Branch points supported by parsimony bootstrap values (1000 replicates) of >90, >75 and >50% are indicated by solid black, solid grey and open circles, respectively, while branch points that were not resolved (bootstrap values of <50%) are depicted without circles. Partial sequences (<600 bp), which did not provide sufficient information for an unequivocal phylogenetic position, are indicated by dotted lines. *Bacteriovorax* strains Uki2^T, A3.12^T and ETC were used as outgroups for treeing the *Bdellovibrio* lineage, while *Bdellovibrio* strains 100^T, HEA and TRA2 were used as outgroups for treeing the *Bacteriovorax* lineage. Bars, 5% estimated sequence divergence.

	Cluster	ARDRA groups	In-cluster divergence (%)	Divergence with closest outgroup (%)
(a)	1	A, G, H	1.4	3.0
	2	B	2.5	6.6
	3	C	1.3	3.0
	4	D	0	3.6
	5	E	—	4.6
	6	F	—	3.6
	7	—	—	7.1
	8	—	0.5	11.6
	9	I	0.9	7.6
(b)	I	M, N, T	3.6	5.3
	II	O	1.3	5.3
	III	S	0.7	7.8
	IV	R	1.4	6.8
	V	Q	0.1	6.8
	VI	P	0	12.2
	VII	K	1.5	4.0
	VIII	L	—	4.0

Fig. 3. Schematic relationships of phylogenetic clusters with ARDRA groups in the *Bdellovibrio* (a) and *Bacteriovorax* (b) lineages. The environmental clones composing clusters 7 and 8 defined two additional, different ARDRA patterns. Multifurcations indicate that a relative order could not be unambiguously determined by applying different treeing methods or outgroups.

monophyletic groups with high bootstrap values, (3) the 16S rRNA secondary structure formed at positions 184–193 in this group was atypical of *Bacteriovorax* (Schwudke *et al.*, 2001; and see below), and (4) the DNA–DNA hybridization values obtained between strains *Bacteriovorax starrii* A3.12^T and *Bacteriovorax stolpii* Uki2^T were very low (< 5%; Baer *et al.*, 2000), we propose the creation of a new genus, *Peredibacter* gen. nov., and *Peredibacter starrii* comb. nov., with A3.12^T as the type strain.

Using accepted standards for genus definition (Ludwig *et al.*, 1998; Vandamme *et al.*, 1996), clusters I–II, III–V and VI (Fig. 3b) may signify additional different genera.

Many marine-specific and some freshwater-specific bacterial phylogenetic clusters have been proposed (Giovannoni *et al.*, 1990; Glöckner *et al.*, 2000). Phylogenetic analysis of the *Bacteriovorax* lineage suggested the existence of a separate marine group. Members of the Uki2^T group (Fig. 2b) were isolated from soil rhizospheres but especially from freshwater and sewage environments. The position of the marine group compared to the ETC and the A3.12^T groups, which are solely composed of soil isolates, suggested that BALOs adapted from soil to fresh water and then to marine environments. The fact that all the *Bdellovibrio*

strains known to date originated from terrestrial environments also lends support to that assumption (see below).

BALO clusters comprising at least one isolated strain will be characterized further to describe the putative new species and genera identified in this work.

ARDRA versus sequencing

The results obtained by ARDRA showed that by using an appropriate combination of restriction enzymes and primers this relatively rapid method enabled an efficient screening of BALO isolates that accurately reflected the diversity and phylogeny of these organisms. Members of the same ARDRA group always exhibited sequence heterogeneity of less than 3.2%. There was a complete overlap between the ARDRA groups and the 16S sequence clusters, except in the cases of clusters 1 and I, both of which consisted of three ARDRA groups (Fig. 3).

BALO phylogeny

The analysis confirmed the conclusion of previous studies, namely that *Bdellovibrio* and *Bacteriovorax* are members of the δ -Proteobacteria (Jurkevitch *et al.*, 2000; Snyder *et al.*, 2002; Woese, 1987). However, neither of these groups formed a stable association with any other δ - or ϵ -proteobacterial groups (Fig. 4). Most of the δ/ϵ -Proteobacteria orders form clear clades ('*Desulfobacterales*' are a prominent exception; Castro *et al.*, 2000). Nevertheless, the relative position of the clades is not resolved. Each of the *Bdellovibrio* and *Bacteriovorax* lineages formed a very stable clade (using different treeing methods, bootstrap values were always above 90%), but together they did not form a monophyletic group, and the phylogenetic distance between them was large (>20%). Long-branch attraction appeared to contribute to the uncertainty in the relative positions of the BALO clades. While the *Bacteriovorax* lineage clearly clustered with other δ -Proteobacteria, the *Bdellovibrio* lineage seemed to be distinct, indicating a very early split or a fast evolutionary rate. This is in accordance with the finding that, by omitting *Bdellovibrio* from the δ -Proteobacteria analysis, bootstrap values supporting the δ -Proteobacteria as a monophyletic clade were improved considerably (Wright *et al.*, 1997). When analysed by BLAST or FASTA, the best hits obtained with *Bdellovibrio* 16S rRNA gene sequences outside their own clade mostly belonged to the α -Proteobacteria, providing yet another indication of the separation of the two BALO lineages and the occurrence of an early split of *Bdellovibrio* from other δ -Proteobacteria.

The occurrence of BALO sequences forming a separate cluster (*Bdellovibrio* cluster 8) in libraries of 16S rRNA from hot-springs bacteria suggested that the bacterial predators can also be active in thermophilic environments. Psychrophilic BALOs were represented by *Bacteriovorax stolpii* strains PNEc1, PNEc2 and PNEc 3, and by clone Sva0447, isolated from a pond in Northern Norway, and

from arctic marine sediments, respectively. BALOs (or BALO sequences) have now been isolated from habitats covering a wide range of environmental conditions. The large phylogenetic diversity uncovered suggested that the actual variety of BALOs may be orders of magnitude above that which is known at present, as only very few culturable prey have been used for their isolation and many more habitats have yet to be probed. Moreover, as BALOs are not dominant populations, BALO clones are seldom found in environmental 16S rRNA gene libraries. DNA extraction biases may be another reason for the low occurrence of BALOs in cultivation-independent surveys: under starvation conditions in a marine environment, the formation of stable bdelloplasts with increased stress resistance was observed (Sanchez-Amat & Torrella, 1990), and these bdelloplasts may be more recalcitrant to DNA extraction. Although still sporadic, the examination of cultivation-independent data as opposed to cultivation-dependent data suggested that isolation-based studies were biased towards or against certain clusters (e.g. *Bdellovibrio* cluster 1 and *Bacteriovorax* cluster I, respectively). BALO groups that can grow at the expense of a commonly used prey such as *Escherichia coli* were probably over-represented in cultivation-based studies, while others remained undetected. Similarly, BALOs requiring 'non-standard' isolation and growth conditions (temperature, salinity, pH, etc.) were certainly under-represented.

What are the implications of the wide distribution and great diversity of BALOs? It has been suggested (1) that marine viruses influence ecological and biogeochemical processes such as bacterial biodiversity, dynamics and species distributions, primary productivity and nutrient cycling (Fuhrman, 1999), (2) that predatory protists are major contributors to the control of microbial community structure (Jürgens *et al.*, 1999; Ronn *et al.*, 2002), and (3) that predation acts as a major factor driving prokaryotic diversity (Torsvik *et al.*, 2002). As BALOs exhibit host-specificity, a small increase in mortality induced by predation in a group of organisms may have a profound effect on the relative proportions and functions of different key species in the community. The abundance and diversity of BALOs in nature, barely investigated to date, suggest that, like to other bacterial predators, BALOs may have a large effect on the structure and dynamics of microbial communities.

Since no other known group clustered between the *Bdellovibrio* and *Bacteriovorax* lineages, and given their morphological similarity, the occurrence of common specificities such as predatory behaviour, and their peculiar life cycle, it is reasonable to hypothesize that most of these shared unique characteristics originated from the same common ancestor. Predatory behaviour, and, in particular, predatory behaviour resulting in cell invasion, is a complex trait not commonly encountered in the domain *Bacteria*. Close investigation of different BALO clades might provide us with new insights about the evolution of predation in

Fig. 4. Phylogenetic tree, based on 16S rRNA gene sequences, reflecting the relationships between the BALO lineages and the *Proteobacteria*. Represented in the tree are all families of the δ - and ϵ -*Proteobacteria* and representatives of the α -, β - and γ -*Proteobacteria*. The tree is based on maximum-likelihood (FastDNAmI) analysis, using a 50 % conservation filter. Bootstrap values are indicated as described in Fig. 2. The following collection of non-*Proteobacteria* was used as the outgroup for treeing: *Spirochaeta litoralis*, *Bacillus subtilis*, *Deinococcus radiodurans*, *Microcystis aeruginosa*, *Mycobacterium leprae*, *Fibrobacter succinogenes*, *Cytophaga hutchinsonii*, *Chlamydia psittaci*, *Aquifex pyrophilus* and *Thermotoga maritima*. Bar, 10 % estimated sequence divergence.

prokaryotes. BALOs can be viewed as representing a greatly simplified example of intracellular parasitism (Martin, 2002; Moulder, 1985) and can therefore serve as a model for other intracellular prokaryotes exhibiting predatory, symbiotic or pathogenic behaviours. Given the proposed early δ -*Proteobacteria* branch location for *Bdellovibrio*, it can be hypothesized that the predatory behaviour of myxobacteria (the order *Myxococcales* is located within the δ -*Proteobacteria*) may have also originated from such a common early δ -proteobacterial predatory ancestor.

16S rRNA secondary-structure analysis

Analysis of the 16S rRNA molecule confirmed that specific regions formed secondary structures that were clearly distinguishable between the *Bdellovibrio* and the *Bacteriovorax* clades (Schwudke *et al.*, 2001). Helices formed at nucleotide positions 198–219 and 446–488 (*Escherichia coli* numbering) were considerably shorter in all the *Bdellovibrio* sequences than in all the *Bacteriovorax* sequences (see Fig. Aa in IJSEM Online). Long helices at both sites were typical of most other members of the δ -*Proteobacteria*, but

some intermediate forms, i.e. short helices at one site, were also found (data not shown). Almost all of the β - and γ -*Proteobacteria* bore the longer structures, while shorter helices were found in almost all of the members of the α - and ε -*Proteobacteria*. Helix 198–219 was proposed by Woese (1987) as a tool for distinguishing between proteobacterial classes: the α -*Proteobacteria* version was shorter than any of the two β/γ - or δ -*Proteobacteria* versions. As a short helix was the common structure found outside *Proteobacteria*, it may represent the ancestral state. Up-to-date information available in the databases (Cannone *et al.*, 2002; Wuyts *et al.*, 2002) supported these assumptions and suggested a similar development in helix 446–488. These structures appeared to interact and to form a tertiary structure (Gutell *et al.*, 2002), so therefore their co-evolution is plausible. These data lent support to the hypothesis that *Bdellovibrio* was an early δ -*Proteobacteria* lineage possessing ancient 16S rRNA secondary structures atypical of latter δ -*Proteobacteria*, while the more recent evolution of the *Bacteriovorax* clade was reflected by the presence of structures common to the other members of the δ -*Proteobacteria*.

Comparative analysis of other secondary structures enabled better determination of the internal relationships within the *Bdellovibrio* and *Bacteriovorax* lineages: a signature that was found in helix 184–193 (Schwudke *et al.*, 2001) was considerably shorter in all the members of the *Bacteriovorax starrii* group (the A3.12^T group) than in all the other members of the *Bacteriovorax* lineage. In addition, the structure found in strain ETC (*Bacteriovorax* cluster VI) at positions 198–219 was shorter than the one found in most of the other *Bacteriovorax* members (see Fig. Ab in IJSEM Online), suggesting an early branching event in the *Bacteriovorax* clade. Also, helix 198–219 was longer in strain TRA2 (*Bdellovibrio* cluster 9) and in clone Oral CA006 (see Fig. Ab in IJSEM Online), which form the most outlying branches in the *Bdellovibrio* clade.

On the basis of (1) the large internal sequence heterogeneities within the *Bdellovibrio* (>14%) and *Bacteriovorax*–*Peredibacter* (>16%) lineages, (2) the great distance separating these clades (>20%), (3) the fact that they do not form a monophyletic group, (4) the very low DNA–DNA hybridization values seen between sampled members of these groups (Baer *et al.*, 2000), and (5) the differences observed in 16S rRNA secondary structures at positions 198–219, typical of other δ -*Proteobacteria* (see the supplementary data in IJSEM Online; Schwudke *et al.*, 2001), we propose the reclassification of the *Bdellovibrio* and *Bacteriovorax*–*Peredibacter* groups into two families, i.e. the existing '*Bdellovibrionaceae*' and *Bacteriovoracaceae* fam. nov., respectively. The large phylogenetic diversity within these groups suggests that each is composed of additional undefined species and genera for which more data are needed to complete their taxonomic definition. No clear data concerning characteristics unique to these two families are available, but since they are so phylogenetically distant, new information will surely emerge when genomic projects are completed.

Re-evaluation of BALO-targeted oligomers

Only a few 16S rRNA-targeted, BALO-specific oligonucleotides are available (Jurkevitch & Ramati, 2000). Oligomer 842 was designed as a BALO-specific oligonucleotide, under the assumption that BALOs form one clade. This wrong assumption was probably the reason why this oligomer was not 100% specific for BALOs and included non-BALO bacteria (Jurkevitch & Ramati, 2000). A re-evaluation of its sensitivity showed that the sequences from 13 out of the 17 clusters described in this study fully matched, or exhibited only one mismatch with, oligomer 842. In combination with a *Bacteria*-domain-targeted primer, amplification from strains belonging to these 13 clusters was positive under all stringencies used. Amplicons could be obtained with sequences exhibiting two mismatches (clusters 2 and VIII) under low stringency (annealing temperature <52 °C) but were usually impossible to obtain with sequences exhibiting three or more mismatches (clusters II and VI). Oligomers 434R and 483R, which were designed as *Bdellovibrio*- and *Bacteriovorax stolpii*-specific oligonucleotides, respectively (Jurkevitch & Ramati, 2000), excluded all non-BALO sequences. Whilst oligomer 434R exhibited up to two mismatches with *Bdellovibrio* sequences (in clusters 2 and 8), oligomer 483 showed one mismatch, at most, with Uki2^T group members (clusters I and II). However, it exhibited five or more mismatches with all the other members of the *Bacteriovorax* clade. In conclusion, the re-evaluation of published BALO-targeted oligonucleotides showed that they are useful tools for the analysis of isolated strains but that they are not sensitive or specific enough for precise culture-independent analysis of BALO populations in environmental samples. The extensive phylogenetic data obtained provide a robust basis for the design of new oligomers which will enable the implementation of cultivation-independent BALO studies.

Description of *Bacteriovoracaceae* fam. nov.

Bacteriovoracaceae (Bac.te.ri.o.vo'ra.ca'ce.ae. N.L. fem. n. *Bacteriovorax* the genus of Gram-negative, aerobic, predators of Gram-negative bacteria, the type genus of the family; suffix *acaeae* denoting a family; N.L. fem. pl. n. *Bacteriovoracaceae* the *Bacteriovorax* family – the feminine gender was chosen).

The description of *Bacteriovoracaceae* is based on the description of the genus *Bacteriovorax* by Baer *et al.* (2000) and our own data. Gram-negative, comma-shaped rods, 0.5–1.4 µm in length. Wild-type cells, which are obligate predators of Gram-negative bacteria, exhibit a biphasic life cycle, including a periplasmic stage within the prey. Cells in their free-swimming stage are motile by means of a single, polar flagellum. Predator-independent mutants can grow saprophytically in rich nutrient medium. They are pleomorphic, exhibiting a range of cell shapes, from simple rods to long, tightly spirally shaped cells. Oxidase- and catalase-positive. Some predator-independent mutants may demonstrate variable catalase activity as some strains have been

shown to lose this ability upon repeated transfer. Obligately aerobic. The G + C content ranges from 41.0 to 43.5 mol%. Members of the family exhibit no DNA–DNA hybridization (<4%) with the ‘*Bdellovibrionaceae*’, although they were previously considered to belong to that family. Members of the family are found in terrestrial and aquatic environments, including marine habitats, while the ‘*Bdellovibrionaceae*’ are not found in halophilic environments. Some members of the family grow optimally below 20 °C, while the optimal temperature of others is around 30 °C. 16S rRNA gene sequence analysis shows that the members of the genera *Bacteriovorax* and *Peredibacter* are more related one to another (maximum divergence <17%) than to the ‘*Bdellovibrionaceae*’ clade (minimum divergence >20%). Together, the ‘*Bdellovibrionaceae*’ and the *Bacteriovoracaceae* do not form a monophyletic group. Secondary structures of the 16S rRNA (*Escherichia coli* positions 198–219 and 446–488) are typical of the δ -*Proteobacteria* and differ from those found in the ‘*Bdellovibrionaceae*’. Total genomic restriction digest patterns (PFGE) for *Bacteriovorax stolpii* Uki2^T and *Peredibacter starrii* A3.12^T exhibit little similarity to the patterns produced by members of the ‘*Bdellovibrionaceae*’.

The type genus is *Bacteriovorax* Baer *et al.* 2000.

Description of *Peredibacter* gen. nov.

Peredibacter (Pe.re.di.bac'ter. L. v. *peredere* to eat up, to devour; N.L. masc. n. *bacter* rod; N.L. masc. n. *Peredibacter* bacterium-devourer).

Consists of predatory, Gram-negative, bacteriovorous species that require a Gram-negative host as prey to complete a biphasic life cycle. Members of the genus are soil-dwellers. Phylogenetic rRNA gene sequence analysis shows that it forms a separate, monophyletic group within the *Bacteriovoracaceae*.

The morphological description of the genus is the same as that of the type strain and only species in the genus, *Peredibacter starrii*.

Description of *Peredibacter starrii* comb. nov.

Peredibacter starrii (star'ri.i. N.L. gen. masc. n. *starrii* of Starr, named after the American microbiologist Mortimer P. Starr).

Basonym: *Bacteriovorax starrii* (Seidler *et al.* 1972) Baer *et al.* 2000.

The description of the species is based on that by Baer *et al.* (2000), following Seidler *et al.* (1972). All members of the species were isolated from soil. Its major cellular fatty acids are 16:1 ω 9c, 13:0 iso, 13:0 and 15:1 ω 8c. Sensitivity to the antibiotics penicillin, streptomycin, neomycin, kanamycin, gentamicin, methicillin and vancomycin has been shown in A3.12^T, but, in contrast to *Bacteriovorax stolpii* Uki2^T, A3.12^T is resistant to pteridine 0/129 and nalidixic acid. The

optimal temperature range is 20–30 °C. Phylogenetically its closest species is *Bacteriovorax stolpii*, as shown by 16S rRNA gene sequence analysis (divergence >12.5%).

The type strain is A3.12^T (=ATCC 15145^T=NCCB 72004^T). Isolated from soil in California, using *Pseudomonas fluorescens* ATCC 12633 as prey. Its G + C ratio is 43.5 mol% (Seidler *et al.*, 1972).

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