Small eats big: ecology and diversity of Bdellovibrio and like organisms, and their dynamics in predator-prey interactions

Shemesh Yair, Davidov Yaacov, Koval Susan, Edouard Jurkevitch

Agronomie 23 (2003) 433–439
© INRA, EDP Sciences, 2003
DOI: 10.1051/agro:2003026

Review article

Small eats big: ecology and diversity of *Bdellovibrio* and like organisms, and their dynamics in predator-prey interactions

Shemesh YAIR[a], Davidov YAACOV[a], Koval SUSAN[b], Edouard JURKEVITCH[a]*

[a] Department of Plant Pathology and Microbiology, Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, POB 12, Rehovot 76100, Israel
[b] Department of Microbiology and Immunology, University of Western Ontario, London, Ontario N6A 5C1, Canada

(Received 14 October 2002; accepted 7 May 2003)

Abstract – The ecological role of predation is well established in the animal world. Not so in the bacterial realm where the number of known bacterial predators is small and their phylogenetic affiliations largely unknown. The best-characterized bacterial predators belong to the *Bdellovibrio-Bacteriovorax* group (*Bdellovibrio* and like organisms, the BLOs). As predation at this trophic level may be of ecological significance, there is a need to better understand the diversity and the phylogeny of bacterial predators as well as the kinetics of their interactions with their prey. Such studies could also help to develop new approaches for the control of plant and animal Gram negative pathogenic bacteria. Here, we present a short review on the ecology, diversity and the taxonomy of predatory bacteria, with an emphasis on BLOs as well as on the dynamics of the interaction between a selected strain of *Bdellovibrio bacteriovorus* and its *Erwinia carotovora* subsp. *carotovora* prey under high and low predator:prey ratios.

Bdellovibrio / bacteria / predation / BLO

Résumé – Quand le petit mange le grand : écologie et diversité de *Bdellovibrio* et organismes apparentés, et leurs dynamiques dans les interactions prédateur-proie. Le rôle écologique de la prédation est bien établi dans le monde animal. Ce n'est pas le cas des bactéries où le nombre de prédateurs bactériens connus est faible et leurs affiliations phylogénétiques largement inconnues. Les prédateurs bactériens les mieux caractérisés appartiennent au groupe des *Bdellovibrio-Bacteriovorax* (*Bdellovibrio* et organismes apparentés, les BLOs). Comme la prédation à ce niveau trophique peut avoir une incidence écologique, nous avons besoin de mieux comprendre la diversité et la phylogénie des prédateurs bactériens tout comme les cinétiques de leurs interactions avec leur proie. De telles études pourraient aussi aider à développer de nouvelles approches pour le contrôle des bactéries Gram négatif pathogènes pour les plantes et les animaux. Ici, nous présentons une brève synthèse sur l'écologie, la diversité et la taxonomie des bactéries prédatrices, avec une attention particulière portée aux BLOs tout comme sur la dynamique de l'interaction entre une souche sélectionnée de *Bdellovibrio bacteriovorus* et sa proie *Erwinia carotovora* subsp. *carotovora* avec des rapports prédateur/proie élevé et faible.

Bdellovibrio / bactérie / prédation / BLO

1. THE WONDERS OF BACTERIAL PREDATION

Predation is of utmost importance for ecological balance, nutrient acquisition and energy flow, as it is present at every trophic level. It is well studied in the animal kingdom but much less researched at the microbial level, with most of the research on bacterial predation having been performed with phages, protozoan and metazoan bacterial predators. Bacterial predation of bacteria is even much less understood although it may play an important role in bacterial ecology.

Bdellovibrio and like organisms (BLO) as they are now denominated, are Gram negative cells, possessing one sheathed polar flagellum, enabling very rapid swimming at up to one hundred body-length·s^{-1} [32]. This motility confers these organisms the title of the fastest motile bacteria [44]. However, the most striking characteristic of most BLOs is their unique predatory behavior: BLOs are obligate predators of Gram negative cells. Most only grow and replicate within the periplasmic compartment of their hosts. Attachment and penetration of the substrate cell by a BLO, free-swimming attack cell is quickly followed by the inactivation of the substrate cell's metabolism and by a loss of prey viability and the formation of a bdelloplast, as the BLO-invaded cell-BLO is called. The bdelloplast offers BLO protection against

Communicated by Philippe Lemanceau (Dijon, France)

* Correspondence and reprints
jurkevi@agri.huji.ac.il

photooxidation damage [8], phage attack [39] and increased resistance to pollutants [22, 37]. Filamentous growth and DNA replication of the invading BLO occurs within the bdelloplast. Exhaustion of the cytoplasmic content of the prey leads the long, intraperiplasmic BLO cell to division by multiple fission into progeny attack cells which grow a flagellum, lyse the ghost remnant, and burst outside [26, 33].

The overall volume of research on these fascinating organisms is rather small and has little expanded since the 1990s. The reasons for that may essentially be technical: Isolation of BLOs is not always successful, and demands a dedicated isolation procedure, including differential centrifugation, filtrations and a double layered growth medium in Petri dishes. This is often an addition too cumbersome and heavy to the design of experiments not necessarily focused on bacterial predation and results in the fact that BLOs are seldom looked for. Moreover, as these are not dominant populations, they are almost never detected in rDNA clone libraries obtained from the environment (about 25 environmental clones clustering with BLOs can be found in the Gene Bank). Therefore, because they do not form colonies on standard growth media, and because they are not represented in clone libraries, they have been neglected by microbiologists.

1.1. Survival of bacterial predators

The two-membered BLO-substrate cell system can be described in terms of a parasite-host as well as in terms of a predator-prey relationship, as it exhibits features relevant to both definitions: the substrate cell is invaded, a prerequisite for replication, (parasite) but its cell machinery is not used by the BLO while its contents constitutes its food base and the substrate is killed in the process (predation). Moreover, this model, as well as other microbial models, has been used for modeling predator-prey interactions, as it is convenient and accurate to measure. The BLO-prey interaction has been described as an oscillating system with inconsistent periodicity [1, 36]. Although the Lotke-Volterra model has been applied to describe the oscillations of the system and its maintenance [38], in the natural world a "decoy" effect can be expected to occur as most of the cells surrounding the predator may not be potential prey, leading to ineffective predator-prey encounters. Such a decoy effect would damp the oscillations and would likely reduce the probability of prey extinction [42].

Under laboratory conditions, a high density of prey is necessary for BLO survival. Various authors have reported that minimal prey concentrations of 10^5 to 10^6 CFU·g^{-1} soil or mL^{-1} are required [18, 40]. Using the Lotka-Volterra model, Varon and Zeigler [38] calculated that in order to give BLOs a 50% chance of survival, at least 3×10^6 prey cells were needed. Therefore, it was generally concluded that BLOs only survive in special ecological niches. However, these calculations were performed based on two-membered cultures serving as models. Since BLOs are usually not stringently specific in their host range, the concentration of substrate cells in natural settings may well be high enough to sustain predatory populations. It is now accepted that only a fraction (ranging from less than 1 to a few percent) of the bacterial cells contained in environmental samples is amenable to cultivation [2]. Rice et al. [25], who quantified the number of BLO-susceptible bacteria in an estuarine environment found that 70 to 85% of the recovered bacteria were preyed upon by BLOs isolated from the same sampling sites. Assuming 10% cultivability for the bacteria retrieved in the samples, it was calculated that the level of susceptible populations was sufficient to ensure survival of the predators.

As explained below, biofilms can potentially provide a habitat fit for predation by BLOs in low microbial density biotas, the predator expending beyond that realm during bacterial population surges.

The cell composition of BLOs is rapidly altered and viability quickly reduced in starved BLO bacterial suspensions kept without a prey [13, 21]. However, BLOs were shown to survive long periods in nutrient-poor environments [4, 9], and have been retrieved from long-term stored dry soils [10]. It was suggested that population heterogeneity [37], higher resilience of bdelloplasts [27] and the formation of bdellocysts [37], although the number of strains able to develop this morphology seems to be rather limited, could explain survival. No knowledge on molecular responses to starvation is available.

1.2. Environmental niches

BLOs are quite ubiquitous in natural and manmade habitats. They are commonly retrieved from soil, are associated with the rhizosphere of plant roots, are found in water of various qualities – in rivers, in the brackish environment of estuaries, in the open sea, at the various stages of treatment in water treatment plants – and associated with biotic and abiotic surfaces [16]. BLOs have been retrieved from the gills of crabs [17], from oyster shells [16], and more recently from hen and mammals feces [30]. The number of BLOs detected in environmental samples using the double-layer isolation procedure – as for the isolation of phages, a suspension of potential prey cells is poured as a soft agar layer on top of bottom agar, to form a layer of cells in which plaques will develop – is usually low, ranging from tens to tens of thousands of plaque forming units per gram or milliliter of sample. Also, BLO strains exhibit different prey ranges. Although most are able to use a number of prey, BLOs have been isolated that can only utilize one type of substrate cell (see below). In other words, BLOs do not represent dominant populations, a fact that is not unexpected, as predators generally do not numerically dominate ecosystems.

Although BLOs are aerobic, and oxic conditions appear to best sustain their multiplication, it was shown that halotolerant strains are able to grown under microaerobic conditions, and that – at least – marine BLOs are also able to survive anoxic periods as attack phase cells or as bdelloplasts [29]. Spells of low oxygen tension occur in soils and in water and the BLOs seem to be adapted to these conditions. Morever, BLOs have been isolated from the feces of humans, horses and hens [30]. Stable colonization or transient passage through the gut implies that at the very least, these BLOs are able to cope with anaerobic conditions. The range of possible niches that can support growth and survival of BLOs may therefore be larger than solely permanent aerobic biotas.

BLOs are also found associated with surfaces and biofilms. In the continuous space between the solid phases of biofilms,

dissolved chemicals, suspended particles and cells move freely [41]. Biofilms may provide a sustainable habitat for BLO multiplication and survival: whereas planktonic BLO cells were not systematically recovered from tested seawater samples, biofilms-associated BLOs were detected at a much higher rate [16, 43]. Biofilms may offer BLOs improved conditions for growth and survival, especially in oligotrophic habitats as the gel-matrix can sustain a higher concentration of potential prey along with physical protection: surface-associated BLOs were shown to survive various environmental insults whereas free-living cells died rapidly [22]. It is hypothesized that the small size of BLOs, their high motility and their mode of multiplication can play a role in shaping the structure of biofilms, which are naturally composed of consortia of microorganisms. Framatico and Cooke, [7] reported that a BLO isolate effectively reduced the level of biofilm *E. coli* cells on stainless steel.

1.3. BLO diversity

BLOs are part of the class delta-*Proteobacteria*. Only recently, have the natural diversity of BLOs been addressed systematically and their phylogeny revisited. Phylogeny based on the analysis of the 16S rRNA gene lead to the definition of two genera, *Bdellovibrio* and *Bacteriovorax* [3]. To date, *Bdellovibrio* comprises only one species, *B. bacteriovorus*, while the *Bacteriovorax* genus is composed of two species, *B. starrii* and *B. stolpii*. Marine BLOs exhibit a different GC content than the terrestrial strains [34] and require sodium, potassium and calcium for growth. They recently were shown to form a separate cluster in *Bacteriovorax* [31]. Other studies reveal that new species of both *Bdellovibrio* and *Bacteriovorax* should be defined (unpublished data). A study using a combined analysis of the 16S rRNA gene and prey range of soil and rhizosphere isolates showed that BLOs belonging to both these genera include various heterogeneous sub-groups that can be found co-existing in the same environment [15]. Moreover, prey range and phylogenetic affiliation appear not to be linked. Culture-independent analysis of BLOs can now be envisaged, as BLO-targeted oligonucleotides have been designed [14].

A variation on the theme of "classical" intracellular predation has been reported by Koval and Hynes [19], with the isolation of a BLO that has no periplasmic stage in its life cycle. This predator was isolated from raw sewage on lawns of *Caulobacter crescentus* cells that do not form an S-layer. This strain (named JSS) did not enter the periplasmic space of the prey cell, but it remained attached at its surface and utilized the cytoplasmic contents of the prey. No bdelloplast was formed, and the empty prey cell retained its original shape. Growth was by binary fission at the prey cell surface. Interestingly, of the potential prey cells tested, *C. crescentus* was the only prey organism suitable for predation by this strain (unpublished data). Recently, more BLOs were isolated on lawns of *C. crescentus* from garden soil, compost and again from raw sewage. These BLOs resembled strain JSS in that they remained extracellular during predation on caulobacters, and could not use *E. coli* as a prey cell (Fig. 1e). Thus, other predatory bacteria resembling JSS may be found in other ecological niches.

Also, a number of other bacteria have been described as "micropredators". Most are extracellular (*Ensifer, Vampirovibrio, Vampirococcus* [6]). Interestingly, a Gram negative bacterium invading and dividing within the cytoplasm of its prey – *Daptobacter* – has been described [12]. Moreover, gliding bacteria such as *Myxobacteria, Cytophaga* or *Herpetsiphon* are endowed with the capacity to lyse and utilize living bacterial cells as food substrate. The different strategies exhibited by these predators were recently summarized by Martin [23]: wolfpack, or group predation, describes predation by a number of predatory cells excreting hydrolytic enzymes, e.g. predation by *Myxococcus*; Epiobiotic, fits predation by *Vampirococcus* and by *Bdellovibrio* strains JSS and KL8 (Fig. 1e), when a predatory cell attaches to the prey, degrades and assimilates prey components; direct invasion, occurs when a predator invades the prey's cytoplasm, (*Daptobacter*), and; Periplasmic describes predation for almost all BLOs. Only BLOs appear to be obligate predators, the other bacteria being able to grow heterotrophically and multiply in the absence of prey. The phylogenetic affiliations of most of these bacteria – and therefore their evolutionary relationships – are unknown.

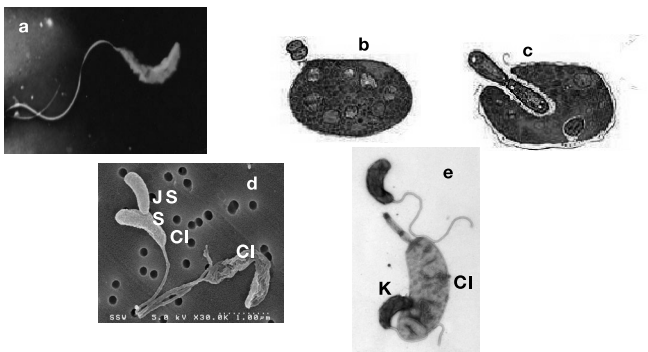

Figure 1. (a) *Bdellovibrio bacteriovorus* strain SNE. (b) *Vampirovibrio.* (c) *Daptobacter*. (d) A *Bdellovibrio* strain JSS cell (JSS) attached to a *Caulobacter cresentus* prey (Cl). To the right of the attacked cell an emptied Cl cell. (e) Isolate KL8 (K), isolated from compost and preying on *C. crescentus*. Similarly to strain JSS, the predator does not penetrate the substrate cell (Cl) (a, b) Electron microscopy (b , c, drawings, [12]).

Electron micrographs of various predatory bacteria are shown in Figure 1. A phylogenetic tree based on 16S rDNA analysis of the BLOs is presented in Figure 2 [from 31].

2.DANCING WITH THE WOLVES: DYNAMICS OF PREY-PREDATOR INTERACTIONS

2.1. BLOs as biocontrol agents of phytopathogens

As seen above, the dynamics of predation by BLOs has been the subject of a number of studies [1, 36, 38, 42]. Few of the studies performed have compared the dynamic behavior of different BLO strains. Also, there is a lack of knowledge on the kinetics of the various changes occurring in a developing lysate, i.e. release of attack cells, bdelloplast formation and prey reduction.

A limited number of studies have been published on the potential of BLOs as biocontrol agents. The most comprehensive

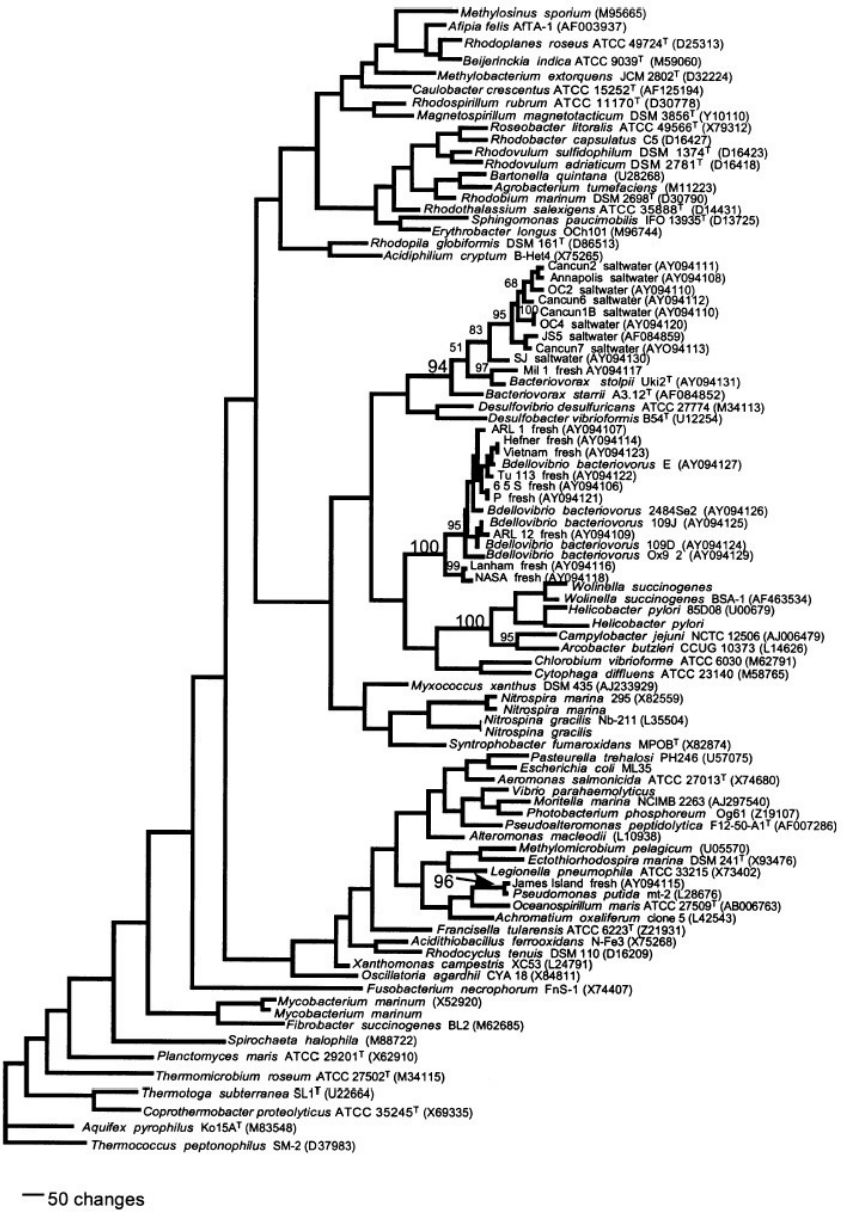

Figure 2. Neighbour-joining tree of BLO isolates. A neighbour-joining tree was constructed for the 17 salt-water and nine freshwater isolates by aligning these sequences with other selected members from the prokaryotic domain. Listed beside each organism or strain name is the GenBank accession number (in parentheses). Numbers at branch-points represent confidence values obtained after bootstrap analysis of the neighbour-joining tree using 1000 replicates [31, by permission].

work on using BLOs to control phytopathogenic bacteria was performed by Uematsu [40] who showed that BLOs efficiently reduced *Xanthomonas oryzae* populations from rice paddy field water but obtained mixed results against *E. carotovora* subsp. *carotovora* in soil. Soybean rhizophere BLO isolates were used to control bacterial blight caused by *Pseudomonas glycinea* [28], and a significant reduction in disease severity and in systemic symptoms were observed. The possibility of a deleterious impact of BLOs on plant growth-promoting rhizobacteria was brought forward by Germida [10], who isolated BLOs parasitic to *Azospirillum brasilense* from soils. Another study showed an increase in rhizosphere BLOs preying upon fluorescent pseudomonads in Chinese cabbage inoculated with a beneficial strain of *Pseudomonas fluorescens* [5].

Erwinia carotovora subsp. *carotovora* (Ecc) is the cause of soft rot diseases in many crops, including vegetables, flowers and tubers, resulting in large scale losses. BLOs are potential biocontrol agents to control these diseases, but knowledge on predator-prey interactions is needed for a judicious application of such systems.

Table I. Efficiency of plaque formation of soil and river water BLOs on different prey cells. Efficiency relates to the relative number of plaque formed in comparison to the number of plaques formed on *Erwinia carotovora* subsp. *carotovora*. X = no plaque growth.

Strains	*B. megaterium*	*A. brasilense*	*A. tumefaciens*	*P. syringae*	*E. coli*	*E. carotovora*	Origin	Original prey
SNE	X	1	1	1	1	1	Soil, Israel	*E. carotovora*
CHI	X	1	1	1	10	1	River, Spain	*E. coli*
FCE	X	X	X	X	1	1	River, Spain	*E. coli*
SRE 11	X	1	1	1	1	1	Soil, Israel	*E. carotovora*
SRE13	X	1	1	1	1	1	Soil, Israel	*E. carotovora*
DPE	X	1	1	1	1	1	Soil, Israel	*E. carotovora*
PRE	X	1	1	1	1	1	Soil, Israel	*E. carotovora*
SJE	X	1	1	1	10	1	Soil, Israel	*E. carotovora*

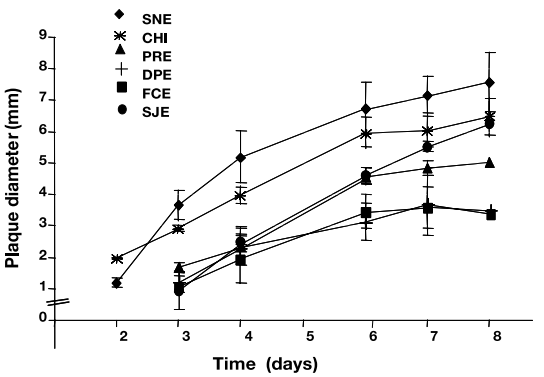

Figure 3. Kinetics of plaque formation of BLO isolates growing on *Erwinia carotovora* subsp. *carotovora*. A single plaque of the tested predator was suspended in diluted nutrient broth with about 10^8 cfu·ml^{-1} Ecc prey. A lysate was obtained (usually overnight) yielding 2 to 5×10^8 cells·ml^{-1} of predators. Lysates were filtered (0.45 μm) to remove remaining prey cells, mixed with the tested prey in soft agar, and then poured on a diluted nutrient agar Petri dish. Plaque growth was examined daily for eight days. Bars represent standard error when larger than the signs.

2.2. Preying behavior at high and low predator prey ratios

A number of BLO strains able to prey on Ecc were isolated from soil and water and their ability to utilize various preys was analyzed on double agar plates (Tab. I). Although most isolates behaved similarly, preying on all proposed prey, the efficiency of plaque formation differed. For example, strain CHI – isolated from a river in Spain – and strain SJE, originating from a soil in Israel, preyed most efficiently on *E. coli*, while strain FCE, isolated from the rhizosphere of strawberry, could only use *E. coli* and Ecc. As expected, none of the isolates could prey upon *Bacillus megaterium*, a Gram positive bacterium. The kinetics of plaque formation and growth also differed between the strains, using Ecc as a prey. Plaques became visible after two (strains SNE, CHI) or three (strains PRE, DPE, FCE and SJE) days. The final sizes of the plaques varied by up to 130%, with strain SNE forming very large (> 7 mm in diameter) plaques while plaques of strains FCE and DPE only reached two to three mm in diameter (Fig. 3). It was also observed that after 8 days, plaques from certain strains were still expanding (strains SNE, SJE) while others seemed to have reached their maximal size (the remaining strains). This shows that remarkable differences occur between BLO isolates in their abilities to use similar prey. Efficiency of plaque formation may be linked to the ability of the predator to irreversibly attach to prey cells, as this capacity appears to be the first step for successful predation [11].

Strain SNE, which exhibited a more rapid and sustained growth as plaques than the other isolates, was identified as *B. bacteriovorus*, based on amplified ribosomal DNA restriction analysis and 16S rDNA sequencing (not shown). This strain was grown in liquid culture and formed lysates with Ecc. As BLOs grow in liquid culture with concomitant exploitation of the prey population, the cell suspension clears. Therefore, the development of a lysate in liquid culture can be tracked by simple spectrophotometric readings. However, this type of measurement, as well as the plating of prey cells and BLOs in double agar standard growth media can only yield the concentrations of the remaining prey population and of the plaque-forming attack cells, respectively, while bdelloplast formation and the dynamics of progeny cell release remain undetected. Round bdelloplasts, larger prey cells and small predatory attack cells can be differentiated after DAPI staining and counted under epifluorescence microscopy (Fig. 4a), enabling the tracking of each of these populations. This was used to follow the dynamics of two-membered cultures in suspensions containing predator and prey at different ratios. At a 10:1 predator to prey ratio, the viable prey population decreased by three to four orders of magnitude within less than one hour, followed by a much slower decline. At a 1:1 ratio, no change in prey concentration could be detected during the first two hours, which was followed by a second phase of rapid decline (Fig. 4b).

In the former case, the rapid loss in prey viability was probably due to rapid infection of the substrate cells by attack cells, while in the latter case, infection was not as efficient. This is clearly seen in Figure 4c, which depicts the kinetics of bdelloplast formation while also tracking the attack cells' population. At a 10:1 predator:prey ratio, the almost entire prey population was transformed into bdelloplasts in a quasi-synchronous manner [24] with progeny bursting from these bdelloplasts after about two and a half hours. Under these conditions, multiple infections occur (as seen by video microscopy, not shown), with more than one predator penetrating the substrate cell, resulting in an undetectable increase in total attack cell

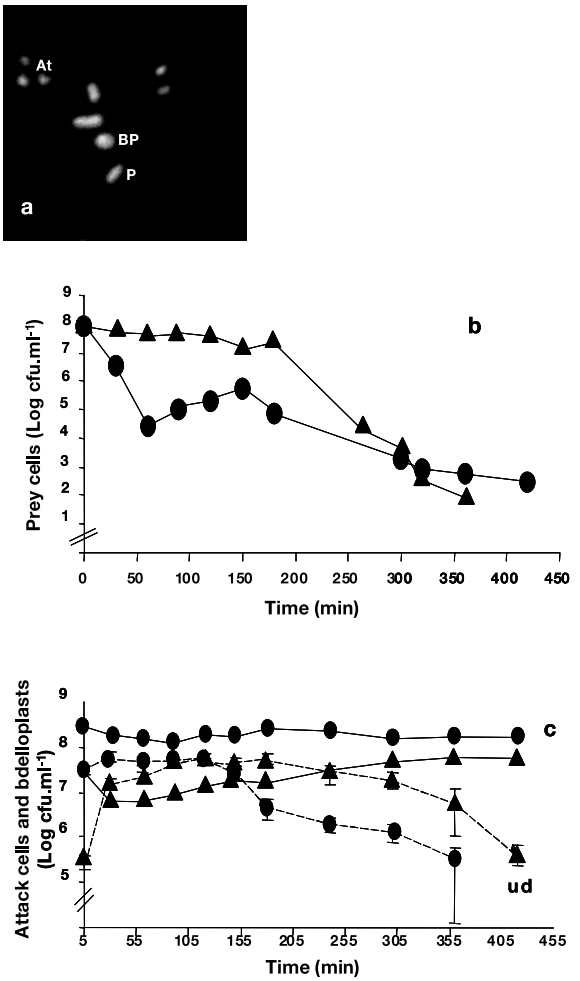

Figure 4. Dynamics of prey consumption, bdelloplast formation and *Bdellovibrio* attack cell release at two different predator: prey concentrations as measured by DAPI staining, cell tracking by epifluorescence microscopy and colony counting. Predator and prey were mixed at 1:1 and 10:1 ratios and samples taken during the following seven hours. One µl per sample was spotted in a well on a gelatin-treated Teflon-covered glass slide, dried, dehydrated and stained with 0.7 $mg \cdot ml^{-1}$ of 4,6-diamidino –2-phenylindole (DAPI) for 10 minutes, followed by washing with ice-cool water. After air-drying, cells were counted under an epifluorescence microscope. Twenty fields were counted or at least 400 of each bdelloplasts and attack phase cells. The concentration of prey was measured by dilution plating on nutrient agar. In all cases, triplicate samples were used. a. DAPI staining of *Erwinia carotovora* subsp. *carotovora* prey (P), bdelloplast (BP) and *Bdellovibrio bacteriovorus* SNE attack cells (At). b. Survival of *Erwinia carotovora* subsp. *carotovora* prey cells (2×10^8 $cfu \cdot ml^{-1}$ at the start of the experiment) after exposure to 2×10^8 (▲, 1:1 ratio) or 2×10^9 (●, 10:1 ratio) $pfu \cdot ml^{-1}$ of *B. bacteriovorus* strain SNE. c. Dynamics of bdelloplast formation (----) and attack cells (——). Ratio key is as in (b). u.d. under minimal detection level. Experiments were performed three times, and one representative experiment is shown. Bars represent standard error when larger than the signs.

population after progeny release (about five progeny cells are made per infected prey). At the start of the experiment with the lower ratio, the concentration of attack cells decreased by about 50% within 30 minutes, while a similar corresponding level of bdelloplasts was formed. The remaining prey cells kept dividing, with few of these prey showing attack cells attached onto them. Progeny were released gradually, starting two and a half hours after mixing the prey and the predator, leading to an increase in the concentration of attack-phase predators. Under these conditions, the level of bdelloplasts remained constant for a longer period than at the high predator: prey ratio, due to continuous bdelloplast formation, and the release of new, attack phase cells was more gradual than at the high predator:prey ratio.

At a 10:1 predator:prey ratio, attack cells are always more numerous than at a 1:1 ratio. However, and after one round of cell replication (in the present case about 150 min), bdelloplasts and "freshly released" attack cells are more numerous at a 1:1 ratio. It appears that predation is more efficient at the 1:1 ratio, requiring 200 min to bring prey population to a level that took 350 min to reach at a 10:1 ratio.

Although no such regulation can be seen in this type of experiments, some kind of bdelloplast population density control mechanism may be at play, as was reported with a strain of marine BLO that exhibited growth arrest upon rapid dilution of bdelloplasts [35]. Growth was rescued by the addition of polyamines, which have been shown to increase growth in prey-independent BLOs [11]. Another factor that may influence the dynamics of predator:prey interactions is the recent finding that the mutation of methyl-accepting chemoreceptors in *B. bacteriovorus* leads to a reduction of predation, suggesting that chemotaxis is involved in finding the prey [20].

Newly formed BLOs appear to be more active than older cells [13] and these experiments show that slow release of attack cells may be a better strategy for the control of target populations than massive input of predators during a short period. But, if one is to apply BLOs in the real world, we think that the cause of the non-eradication of prey cells in lysate cultures should be studied. Also, the influence of predator:prey ratio on the survival of the a targeted prey (such as a pathogen) should be studied in more natural settings in which other prey and non-prey species are present.

Acknowledgment: The authors would like to thank Krista Lyle for excellent technical support, and the Natural Sciences and Engineering Research Council of Canada and the Israel Science Foundation (No. 132/99-16.2) for financial support.

REFERENCES

[1] Afigenova A.V., Ratner E.N., Lambina V.A., Variations in the numbers of *Bdellovibrio bacteriovorus* and the bacterial host in a two-component system, Mikrobiologiya 2 (1978) 265–269.

[2] Amann R., Ludwig W., Schleifer K.H., Phylogenetic identification and in situ detection of individual cells without cultivation, Microbiol. Rev. 59 (1995) 143–169.

[3] Baer M.L., Ravel J., Chun J., Hill R.T., Williams W.N., A proposal for the reclassification of *Bdellovibrio stolpii* and *Bdellovibrio starrii* into a new genus, *Bacteriovorax stolpii* comb. nov. and *Bacteriovorax starrii* comb. nov., respectively, Int. J. System. Evol. Microbiol. 50 (2000) 219–224.

[4] Daniel S., Étude de l'influence de *Bdellovibrio bacteriovorus* dans l'auto-épuration marine, Rev. Int. Ocean. Med. 15-16 (1969) 61–102.

[5] Elsherif M., Grossmann F., Role of biotic factors in the control of soil-borne fungi by fluorescent pseudomonads, Microbiol. Res. 151 (1996) 351–357.

[6] Esteve I., Gaju N., Bacterial symbioses. Predation and mutually beneficial associations, Int. Microbiol. 2 (1999) 81–86.

[7] Fratamico P.M., Cooke P.H., Isolation of bdellovibrios that prey of *Escherichia coli* 0157:H7 and *Salmonella* species and application for removal of prey from stainless steel surfaces, J. Food Saf. 16 (1996) 161–173.

[8] Friedberg D., Effect of light on *Bdellovibrio bacteriovorus*, J. Bacteriol. 131 (1977) 399–404.

[9] Fry J.C., Staples D.G., Distribution of *Bdellovibrio bacteriovorus* in sewage works, river water, and sediments, Appl. Environ. Microbiol. 31 (1976) 469–474.

[10] Germida J.J., Isolation of *Bdellovibrio* spp. that prey on Azospirillum brasilense in soil, Can. J. Microbiol. 33 (1987) 459–461.

[11] Gray K.M., Ruby E.G., Intercellular signalling in the bdellovibrio developmental growth cycle, in: Dworkin M. (Ed.), Microbial Cell-Cell Interactions, ASM.Washington DC, 1991, pp. 333–366.

[12] Guerrero R., Pedros-Alio C., Esteve I., Mas J., Chase D., Margulis L., Predatory prokaryotes: Predation and primary consumption evolved in bacteria, Proc. Natl. Acad. Sci. 82 (1986) 2138–2142.

[13] Hespell R.B., Thomashow M.F., Rittenberg S.C., Changes in cell composition and viability of Bdellovibrio bacteriovorus during starvation, Arch. Microbiol. 97 (1974) 313–327.

[14] Jurkevitch E., Ramati B., Design and uses of *Bdellovibrio* 16S rRNA-targeted oligonucleotides, FEMS Microbiol. Lett. 184 (2000) 265–271.

[15] Jurkevitch E., Minz D., Ramati B., Barel G., Prey range characterization, ribotyping, and diversity of soil and rhizosphere *Bdellovibrio* spp. isolated on phytopathogenic bacteria, Appl. Environ. Microbiol. 66 (2000) 2365–2371.

[16] Kelley J.I., Turng B.F., Williams H.N., Baer M.L., Effects of temperature, salinity, and substrate on the colonization of surfaces in situ by aquatic bdellovibrios, Appl. Environ. Microbiol. 63 (1997) 84–90.

[17] Kelley J.I., Williams H.N., Bdellovibrios in *Callinectus sapidus*, the blue crab, Appl. Environ. Microbiol. 58 (1992) 1408–1410.

[18] Keya S.O., Alexander M., Regulation of parasitism by host density: the *Bdellovibrio-Rhizobium* interrelationship, Soil Biol. Biochem. 7 (1975) 231–237.

[19] Koval S.F., Hynes S.H., Effect of paracrystalline protein surfaces layers on predation by *Bdellovibrio bacteriovorus*, J. Bacteriol. 173 (1991) 2244–2249.

[20] Lambert C., Smith M.C.M., Sockett R.E., A novel assy to monitor predator-prey interactions for Bdellovibrio bacteriovorus 109J reveals a role for methyl-accepting chemotaxis proteins in predation, Environ. Microbiol. 5 (2003) 127–132.

[21] Marbach A., Varon M., Shilo M., Properties of marine bdellovibrio, Microbiol. Ecol. 2 (1976) 284–295.

[22] Markelova N.Y., Effect of toxic pollutants on *Bdellovibrio*, Proc. Biochem. 37 (2002) 1177–1181.

[23] Martin M.O., Predatory prokaryotes: an emerging research opportunity, J. Mol. Biotechnol. 4 (2002) 467–477.

[24] McCann M.P., Solimeo H.T., Cusick Jr., Panunti F., McCullen B.C., Developmentally regulated protein synthesis during intraperiplasmic growth of *Bdellovibrio bacteriovorus* 109J, Can. J. Microbiol. 44 (1998) 50–55.

[25] Rice T.D., Williams H.N., Turng B.F., Susceptibility of bacteria in estuarine environments to autochtonous bdellovibrios, Microbiol. Ecol. 35 (1998) 256–264.

[26] Ruby E.G., The genus *Bdellovibrio*, in: Balows A., Truper H.G., Dworkin M., Harder W., Schleifer K.H. (Eds.), The Prokaryotes, 2nd ed., Springer Verlag, NY, 1991, pp. 3400–3415.

[27] Sanchez Amat A., Torrella F., Formation of stable bdelloplasts as a starvation-survival strategy of marine bdellovibrios, Appl. Environ. Microbiol. 56 (1990) 2717–2725.

[28] Scherff R.H., Control of bacterial blight of soybean by *Bdellovibrio bacteriovorus*, Phytopatholy 63 (1973) 400–402.

[29] Schoeffield A.J., Williams H.N., Turng B.F., Falkler W.A. Jr., A comparison of the survival of intraperiplasmic and atack phase bdellovibrios with reduced oxygen, Microbiol. Ecol. 32 (1996) 35–46.

[30] Schwudke D., Strauch E., Krueger M., Appel B., Taxonomic studies of predatory bdellovibrios based on 16S rRNA analysis, ribotyping and the *hit* locus and characterization of isolates from the gut of animals, Syst. Appl. Microbiol. (2001) 385–394.

[31] Snyder A.R., Williams H.N., Baer M.L., Walker K.E., Colin Stine O., 16S rDNA sequence analysis of environmental Bdellovibrio and like organisms (BALO) reveals extensive diversity, Int. J. System. Evol. Microbiol. 52 (2002) 2089–2094.

[32] Stolp H., Film: Lysis von bacterien durch den parasiten *Bdellovibrio bacteriovorus,* I.W.F., Gottingen, 1967.

[33] Stolp H., Starr M.P., *Bdellovibrio bacteriovorus* gen. et sp., a predatory, ectoparasitic, and bacteriolytic microorganism, Anton. van Leeuw. 29 (1963) 217–248.

[34] Taylor V.I., Baumann P., Reichelt J.L., Allen R.D., Isolation, enumeration, and host range of marine bdellovibrios, Arch. Microbiol. 98 (1974) 101–114.

[35] Varon M., Fine M., Stein A., Effects of polyamines on the intraperiplasmic growth of *Bdellovibrio* at low cell densities, Arch. Microbiol. 136 (1983) 158–169.

[36] Varon M., Selection of predation-resistant bacteria in continuous culture, Nature 277 (1979) 386–388.

[37] Varon M., Shilo M., Ecology of aquatic bdellovibrios, in: Droop M.R., Jannash H.W. (Eds.), Advances in microbial ecology, Academic Press, London, 1978, pp. 1–48.

[38] Varon M., Zeigler B., Bacterial predator-prey interaction at low prey density, Appl. Environ. Microbiol. 36 (1978) 11–17.

[39] Varon M., Seijffers J., Symbiosis-indepedent and symbiosis-incompetent mutants of *Bdellovibrio bacteriovorus* 109J, J. Bacteriol. 124 (1975) 1191–1197.

[40] Uematsu T., Ecology of Bdellovibrio parasitic to rice bacterial leaf blight pathogen, *Xanthomonas oryzae*, Rev. Plant Protec. Res. 13 (1980) 12–26.

[41] Wanner O., Modeling of population dynamics, in: Characklis W.G., Wilderer P.A. (Eds.), Structure and function of biofilms, Wiley & Sons, New York, 1989, pp. 91–110.

[42] Wilkinson M., Predation in the presence of decoy: an inhibitory factor on pathogen control by bacteriophages or bdellovibrios in dense and diverse ecosystems, J. Theor. Biol. 208 (2001) 27–36.

[43] Williams H.N., Schoeffied A.J., Guether D., Kelley J., Shah D., Falkler W.A., Recovery of bdellovibrios from submerged surfaces and other aquatic habitats, Microbiol. Ecol. 29 (1995) 39–48.

[44] Young M.C., Guinness World Records 2002, in: Cunningham A. (Ed.), Mass Market Paperback, 2002.