Special Instructions

Cultivation of Bdellovibrios

Bdellovibrios are unique microorganisms that prey upon a wide variety of susceptible Gram-negative bacteria. Their **predatory life style** is characterized by two distinct phases, a free-living attack phase and an intraperiplasmic growth phase. Although members of the order *Bdellovibrionales* are phenotypically quite similar, they do not form a coherent phylogenetic group. Currently, they are classified into five different genera, *Bdellovibrio*, *Bacteriovorax, Peredibacter, Halobacteriovorax and Pseudobacteriovorax.* Bdellovibrios can be found in a wide variety of habitats, ranging from soil to sewage, provided these environments are densely populated with bacteria.

Some strains represent **prey-independent mutants** that have lost their requirement for prey cells and hence are facultatively predacious (e.g., *Bacteriovorax stolpii* DSM 12778) or adapted to an obligate saprophytic life-style (e.g., *Bdellovibrio bacteriovorus* DSM 12732).

Prey-dependent bdellovibrios are usually sensitive to lyophilization and consequently delivered as **actively growing cultures** from the DSMZ. Fresh samples of attack phase bdellovibrios are shipped either on double-layered agar plates or in liquid broth culture. Presumably, due to the high endogenous respiration rates of bdellovibrios, their viability rapidly decreases after complete lysis of prey cells. Therefore, it is important to transfer the obtained cultures immediately upon receipt into freshly prepared media containing suspensions of susceptible prey cells. An axenic culture of prey cells is shipped along with prey-dependent strains of bdellovibrios.

A detailed description of the cultivation of ***Bdellovibrio bacteriovorus*** **DSM 50701**^{T} follows below to exemplify the recommended handling of prey-dependent strains.

You will receive from the DSMZ a double layer agar plate of DSMZ medium 257 containing predator and prey cells in the top layer and a tube of slant agar (DSMZ medium 54) with an axenic culture of the prey bacterium *Pseudomonas* sp. DSM 50906. The top agar has a **linear, plaque-like clearing** across the center of the plate. This is the zone of lysed cells in the lawn of prey bacteria. It can be easily recognized by viewing the plate against a dark background. In Fig. 1A the clearing is marked by a black stippled line. Plaques of bdellovibrios have sharp, distinct boundaries and increase in size with prolonged incubation. Therefore, it is important to check the agar plate immediately upon receipt of the culture. For the microscopical observation of attack-phase bdellovibrios with their characteristic swift motility remove a sample from the cleared zone of the top agar that is adjacent to the boundary of intact prey cells (area is marked by a red box in Fig. 1A). In this area the predators are most active, because prey cells are abundant in contrast to the center of the plaque, where most prey cells already have been lysed. Cells of *B. bacteriovorus* DSM 50701^{T} are Gram-negative, small, curved rods that are **highly motile** by a single, polar, sheathed flagellum. Under the phase-contrast microscope bdellovibrios can be easily distinguished from prey cells by their small diameter (0.25-0.4 µm) and swift motility. However, in older cultures most cells appear immobile. A phase-contrast micrograph of two cells of DSM 50701^{T} attacking a cell of *Pseudomonas sp.* DSM 50906 is shown in Fig. 1B.

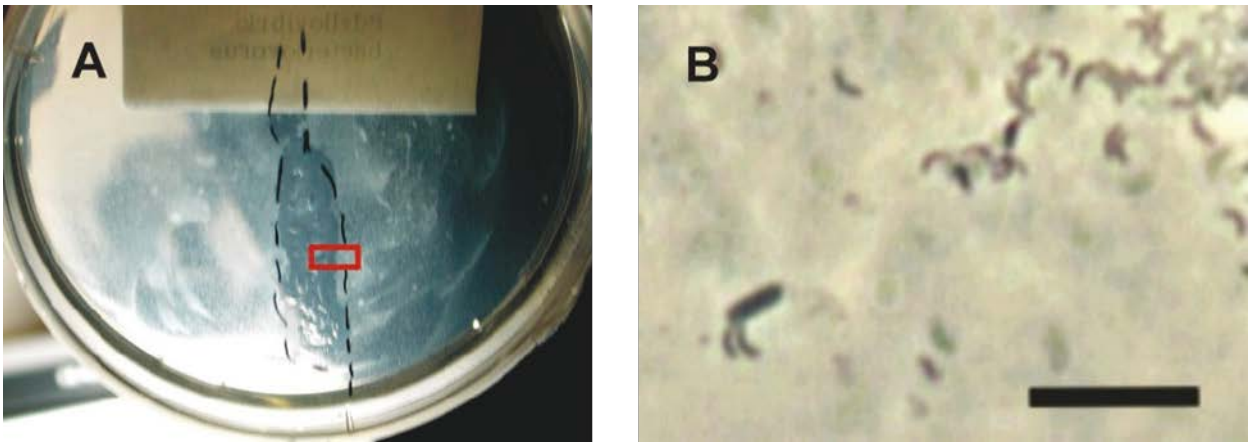

Fig 1 Growth of *Bdellovibrio bacteriovorus* DSM 50701^T in agar culture. (A) *Bdellovibrio* plaque in the top agar of DSMZ medium 257. (B) Phasecontrast micrograph of a single host cell along with several *Bdellovibrio* cells. Bar = 5 µm.

The propagation of prey-dependent bdellovibrios can be achieved by two alternative approaches: The double-layer agar technique is suitable for the maintenance and purification of strains, whereas cultivation in liquid suspensions of prey cells is more appropriate for the large-scale culture.

We recommend as first step of the propagation procedure the transfer in DSMZ medium 1012 (DN broth). Excise with a small, sterile metal spatula or inoculation loop agar from the clearing zone together with a piece of the surrounding unlysed prey cell lawn (the target area is enclosed with a red box in Fig. 1A). The removed piece of agar is resuspended in approx. 15 ml DN broth and shaken at 30 °C for 24 to 48 h. Like all other bdellovibrios *B. bacteriovorus* DSM 50701^T is **strictly aerobic** and needs aeration in liquid culture, e.g. by agitation of flasks on a rotary shaker with approx. 200 rpm. The prey cells first multiply, creating a suspension dense enough to enable a high yield of bdellovibrios. This first lysate can then be subcultured.

For the propagation on **double layer agar plates** use medium 257 as indicated on the DSMZ web site http://www.dsmz.de/microorganisms/medium/pdf/DSMZ_Medium257.pdf using a suspension of *Pseudomnas sp.* DSM 50906 as prey cells. To obtain single plaques inoculate the top agar with a serial dilution of the bdellovibrio lysate. Plaques will develop in the turbid lawn of prey cells within 2-4 days. Bdellovibrios can be harvested from the plaques by emulsifying pieces of agar in sterile buffer. By low speed centrifugation (approx. 700g for 10 min.) the agar sediments and the supernatant can be used to inoculate fresh plates.

For the **cultivation in liquid medium** prepare a suspension of prey cells in 30–40 ml DN broth using a 250 ml flask. Usually the resuspension of two overnight cultures of DSM 50906 on agar slants of DSMZ medium 54 will yield a final concentration of 10^8 to 10^9 cells per ml DN broth. This suspension is inoculated with 100 µl of a fresh bdellovibrio lysate and incubated for 24 to 48 h to lyse all the prey cells. The lysis of most prey cells becomes visible by a decreased turbidity of the culture and can be confirmed by phase-contrast microscopy. For some purposes it may be necessary to remove unlysed prey cells from the lysate. This can be achieved by a **filtration of the bdellovibrio suspension** using 0.8 µm membrane filters followed by a second filtration step using 0.45 µm filters.

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When maintaining bdellovibrio cultures in broth, serial transfers should be done at least weekly. According to Jurkevitch (2000) supplementation of the fresh bdellovibrio suspension with 5 mM glutamate and storage at 4 °C increases the viability of the predators considerably.

Literature

Jurkevitch, E. 2000. The genus *Bdellovibrio*. In M. Dworkin et al. (eds.), The Prokaryotes: An Evolving Electronic Resource for the Microbiological Community, 3rd ed., release 3.1, Springer-Verlag, New York, http://link.springer-ny.com/link/service/books/10125/.

Notes

1. Abbreviations (excl. chemicals, reagents and measuring units):

   approx.= approximately

   fig= figure

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