Bacterial capsules: no barrier against *Bdellovibrio*

Susan F. Koval¹ and Manfred E. Bayer²

Author for correspondence: Susan F. Koval. Tel: +1 519 661 3439. Fax: +1 519 661 3499. e-mail: skoval@julian.uwo.ca

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**INTRODUCTION**

*Bdellovibrio bacteriovorus* is a small, rapidly motile Gram-negative bacterium that uses another Gram-negative bacterium as its sole source of nutrition. The most significant taxonomic characteristic of the genus *Bdellovibrio* is the presence of an intracellular growth phase within the periplasmic space of an invaded bacterium (Burnham & Conti, 1984). The life cycle of wild-type *bdellovibrios* alternates between an extracellular, flagellated, but non-growing phase and an intracellular, non-flagellated, periplasmic growth phase. The process of recognition and attachment to prey cells by *B. bacteriovorus* is complex and not completely understood. The range of susceptible prey cells varies with the *Bdellovibrio* strain but is restricted to Gram-negative bacteria. *B. bacteriovorus* probably encounters its prey cells solely by random collision, because no signalling mechanisms have been identified in the location of specific prey (Gray & Ruby, 1991).

The mechanism of attachment and penetration appears to be a two-step procedure (Dunn *et al.*, 1974). The initial, reversible stage of attachment following collision is characterized by a loose association in which the *bdellovibrio* cell rotates about its long axis from the point of attachment to the prey cell surface. It may stop rotating, then start again, or suddenly detach and swim away. Reversible attachment can be non-specific and involve inert surfaces such as microscope slides or cover slips, or non-suitable prey cells (i.e. Gram-positive bacteria). To form an irreversible attachment, the *bdellovibrio* cell forms a tight junction with the prey cell, which itself begins to rotate about the long axis of the *bdellovibrio* cell. The two cells then spin as a unit, with the *bdellovibrio* cell pushing the prey cell through the suspending medium. This irreversible stage of attachment is quickly followed by detachment of the flagellum and penetration into the prey cell. The involvement of specific receptor interactions in prey recognition has been investigated (Gray & Ruby, 1991), but a specific receptor, even for one strain of *Bdellovibrio*, has not been identified. The question of cell-surface barriers to attachment and penetration of *bdellovibrios* has rarely been addressed but does arise when the function of surface structures of bacteria is considered. Koval & Hynes (1991) have shown that paracrystalline protein surface layers (S-layers) protect Gram-negative cells against predation by *B. bacteriovorus*.

This study examines the effect of capsules of *Escherichia coli* on predation by *B. bacteriovorus*. *E. coli* K29 was used since its capsule has been well-characterized biochemically (Fehmel *et al.*, 1975), crystallographically (Moorehouse *et al.*, 1977) and morphologically (Bayer *et al.*, 1985). As controls, two non-capsulated strains were used: *E. coli* K29⁺ (a stable revertant of K29 to a non-capsulated form) and *E. coli* ML35 (the *E. coli* strain upon which *B. bacteriovorus* 109J is maintained). *B. bacteriovorus* strain 109J was selected because it is one of the best-studied strains of *B. bacteriovorus* (Ruby, 1992; Gray & Ruby, 1991).

**METHODS**

**Culture conditions.** All *E. coli* strains were maintained on slants of trypticase soy agar at 4 °C. *B. bacteriovorus* was maintained on prey cells of *E. coli* ML35 as described by Koval & Hynes (1991). Lysates containing *B. bacteriovorus* were stored at 4 °C in screw-capped test tubes.
To assay predation, two-membered culture systems were set up, using a modification of the procedure of Koval & Hynes (1991) described here. E. coli K29 and K29⁺ were grown in L-broth (1% tryptone, 0.5% yeast extract, 0.5% NaCl, pH 7.0) containing 0.5% glucose for 24 h at 30 °C with shaking. E. coli ML35 was grown in L-broth (without glucose) under the same conditions. After 24 h growth, E. coli K29 cells had a large, well-defined capsule, as visualized by India ink and other stains (Bayer, 1990). The production of capsules was not as good on cells grown on agar medium. Thirty-five millilitres of each culture was centrifuged aseptically at 12000 g for 20 min and each pellet resuspended in 10 ml 25 mM HEPES plus 2 mM CaCl₂ buffer at pH 7.8 (HEPES plus Ca²⁺). The E. coli K29 cells retained their capsule after centrifugation and resuspension in buffer. To 35 ml of HEPES plus Ca²⁺ buffer in a 250 ml side-arm flask with baffles, 5 ml of a prey cell suspension and 5 ml of a fresh B. bacteriovorus 109J lysate were added. Control cultures of prey cells in buffer alone were set up in side-arm flasks without baffles. To these flasks, 5 ml buffer was added instead of B. bacteriovorus. All flasks were incubated at 30 °C with shaking and predation monitored by phase-contrast microscopy. Turbidity measurements were taken on a Klett-Summerson photoelectric colorimeter fitted with a green filter (no. 54). For reference, the approximate equivalence in OD₅₄₈ for the initial and final turbidities of the E. coli K29 two-membered culture are: 150 Klett units = 0.9 OD₅₄₈ units; 105 Klett units = 0.5 OD₅₄₈ units.

**Electron microscopy.** Samples (2.5 ml) of E. coli K29 cells were exposed to 1 ml B. bacteriovorus and the culture shaken at 30 °C. After 45 min (when many B. bacteriovorus cells had attached to E. coli rods), the cells were centrifuged for 5 min at 3700 g, resuspended in 1.5 ml 0.1 M sodium phosphate buffer (pH 7) and 0.5 ml anti-capsule K29 serum (rabbit) added to stabilize the capsule and prevent further penetration of the capsule. The cells agglutinated within 10–20 s. After 10 min at 4 °C, the cells were centrifuged (2 min at 2000 g) and the pellet resuspended in 2 ml 0.1 M cacodylate buffer containing 2% glutaraldehyde and 1% formaldehyde, both diluted from 25% (v/v) and 16% (v/v) solutions, respectively. After 60 min at 4 °C and centrifugation at 2000 g, the pellet was overlaid with cacodylate buffer for 15 min. After removal of the buffer, the cells were post-fixed in 1% (w/v) OsO₄ for 40 min in cacodylate buffer, washed in buffer, dehydrated in an alcohol series and embedded in Spurr resin. Sections were post-stained with uranyl acetate and lead citrate and examined in a Philips 420 electron microscope.

**RESULTS AND DISCUSSION**

E. coli strains K29 and K29⁺ (Fig. 1) and ML35 (Fig. 2) were all susceptible to predation by B. bacteriovorus 109J. After 1–3 h, bdellovibrios were attached to E. coli cells both with (Fig. 3b) and without capsules (data not shown). After 5 h, many bdelloplasts and free-swimming bdellovibrios were seen in all cultures, but very few rod-shaped E. coli. By 7 h, the E. coli K29 and K29⁺ two-membered cultures contained mostly free-swimming bdellovibrios. Those bdelloplasts present often had many (10–15) bdellovibrios attached on the outside. At 7 h, the E. coli ML35 plus B. bacteriovorus culture had more bdelloplasts present than the K29 culture, but then the initial turbidity (i.e. the number of E. coli cells) was higher than for the E. coli K29 and K29⁺ cultures. By 9 h, all lysates contained only free-swimming bdellovibrios. The turbidity of the control cultures of each E. coli strain in buffer did not decrease significantly. There was no difference in the time it took for the turbidity of the two-membered E. coli K29 and K29⁺ cultures to decrease (Fig. 1). Thus the capsule of E. coli K29 was not an effective barrier against attachment and penetration of B. bacteriovorus 109J.

The capsule of E. coli K29 comprises polysaccharide fibres which exhibit strong negative charges due to the glucuronic acid and pyruvate residues of their subunits (Fehmel et al., 1975; Bayer & Sloyer, 1990). Individual fibres extend through the capsule's entire thickness of several hundred nanometres (Bayer, 1990). Although the
Fig. 3. Thin sections of capsulated *E. coli* K29 showing (a) partial penetration of the thick capsule by *B. bacteriovorus* and (b) attachment of *B. bacteriovorus* 109 to the cell envelope. Bar, 200 nm.
capsule consists of more than 95% water (Sutherland, 1977), it provides a highly effective protection against macromolecular and viral attack. During collision with a capsulated cell, the bdellovibrio encounters the gel-like capsular matrix which is flexible and whose fibres are apparently not cross-linked. Bdellovibrios penetrated the thick capsule without disintegrating the neighbouring capsular matrix (Fig. 3). We did not attempt to determine the time required for penetration of the capsule. However, it cannot be very rapid, since many bdellovibrio cells were immobilized mid-way through the capsule (Fig. 3a).

In contrast to the diffusion-driven collision events between virus particles and host cell, B. bacteriovorus cells are actively propelled by flagellar activity and achieve surprisingly high speeds. The successful attachment of the bdellovibrio to capsulated prey cells most likely results from an increase in the area of contact during the deepening penetration, rather than from enzymic attack of the polysaccharide, as has been shown for capsule-specific bacteriophages (Fehmel et al., 1975; Bayer et al., 1979).

Another observation from our study was that more than one bdellovibrio could penetrate the capsule and that the resulting bdelloplast of E. coli K29 retained its capsule. Capsulated bdelloplasts could clearly be seen in India ink preparations for light microscopy (data not shown) and by electron microscopy (Fig. 4). This is the
first report of a capsulated bdelloplast and demonstrates the stability of the anchoring of the capsular elements during bdelloplast formation.

Many freshwater and terrestrial Gram-positive bacteria possess capsules (e.g. *Bacillus* spp.) but are not suitable prey cells for *B. bacteriovorus*. The question of a protective anti-predation barrier for these organisms does not arise, whereas we demonstrate here that capsules of Gram-negative bacteria seem to fail as a protective barrier against predation by *B. bacteriovorus*.

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