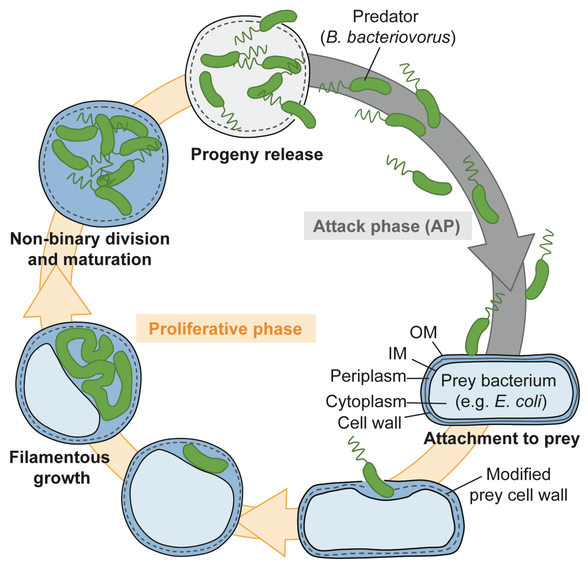
jovana.kaljevic@uclouvain.be
www.lalouxlab.weebly.com

Uncovering the choreography of the chromosome during the non-binary cell cycle of the predatory bacterium *Bdellovibrio bacteriovorus*

Jovana Kaljevic, Geraldine Laloux



Bdellovibrio bacteriovorus is a predatory delta-proteobacterium that invades the periplasm of other Gram-negative bacteria. Once the *Bdellovibrio* cells are inside, the prey bacterium forms a structure termed "bdelloplast" whereas the predator grows as a filament before dividing synchronously into a variable number of monoploid cells by a non-binary division process. **Molecular understanding of how this micro-predator proliferates inside the envelope of other bacteria is still missing.**

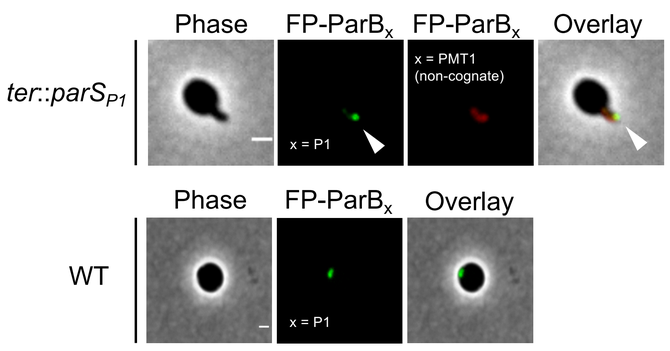
Main questions:

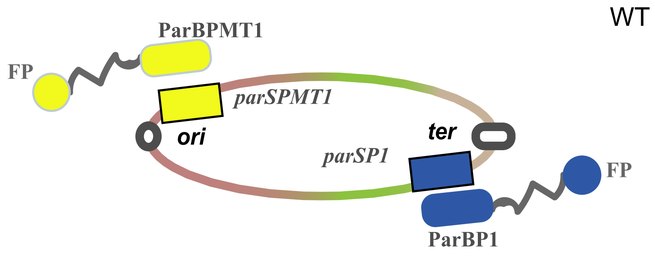
1. How are chromosomal regions positioned throughout the cell cycle of *Bdellovibrio*?

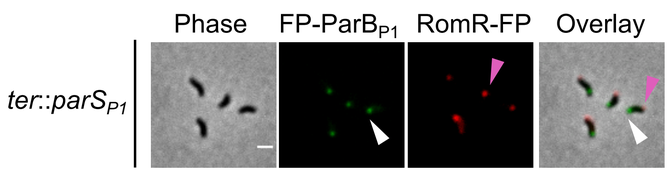
2. When and where are the chromosomal copies segregated during filamentous growth?

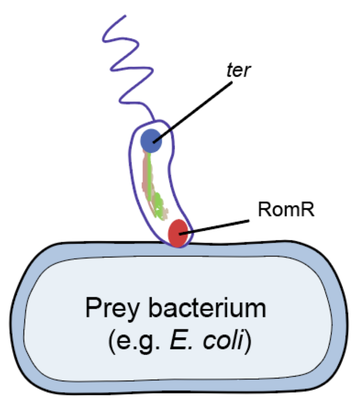
1. Spatial arrangement of the *Bdellovibrio* chromosome

The spatio-temporal arrangement of the *Bdellovibrio* chromosome remains unknown. **We are investigating this feature using the orthogonal ParB-*parS* system.**





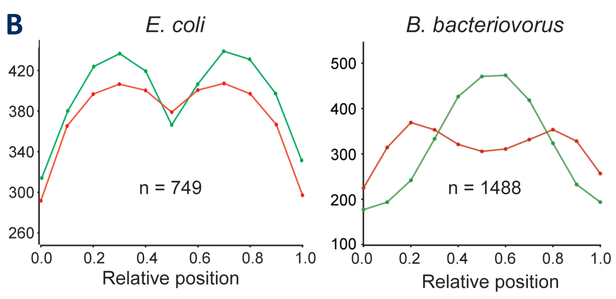


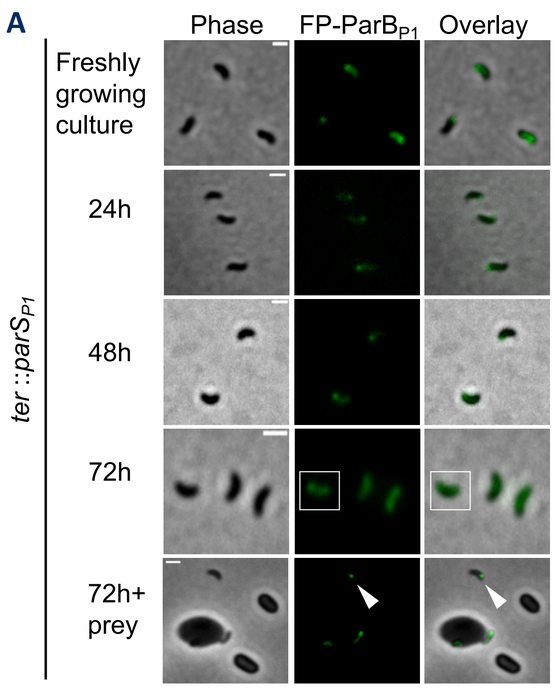


The *ter* locus is positioned at the flagellated pole in the attack phase of the cell cycle (*arrowheads*), in contrast with previously reported *ter* localization in other flagellated species. Colocalization with RomR-TdTomato (*pink arrowheads*) confirms that *ter* is at the non-invasive pole. *Scale bar: 1 µm.* Representative cells are shown.

2. Dynamics of the *Bdellovibrio* chromosome

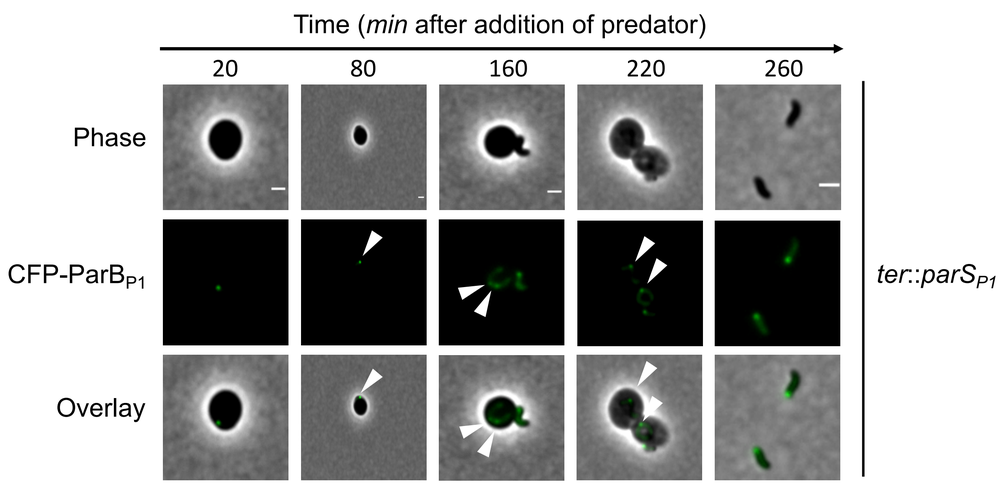
A) Our observations also reveal intriguing dynamics of the level of nucleoid compaction in *Bdellovibrio.* After a certain period without the prey, polar localization of FP-ParB$_{P1}$ was lost (*72h, inset*). We hypothesize that this is due to DNA condensation preventing ParB binding to *parS* at *ter*. When mixed with prey, signal was again visible (*bottom panel, arrowheads*)



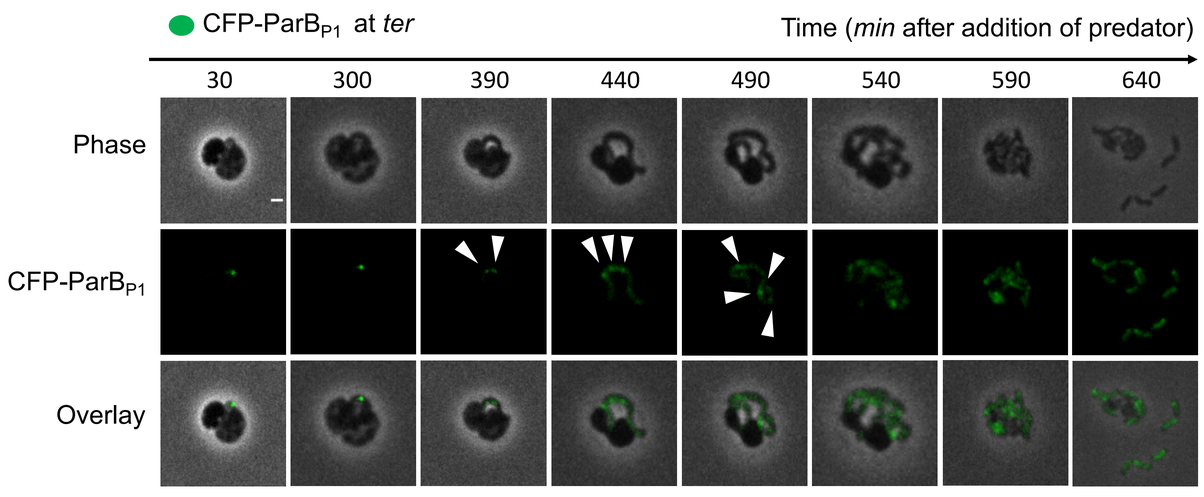


B) Average pole-to-pole profiles from *Bdellovibrio* attacking phase cells, showing nucleoid occlusion of tdTomato in *B. bacteriovorus.*

These intriguing data suggest that *Bdellovibrio* cells can "sense" the prey via an unknown mechanism that modifies nucleoid compaction. *Scale bar: 1 µm.* Representative cells are shown.



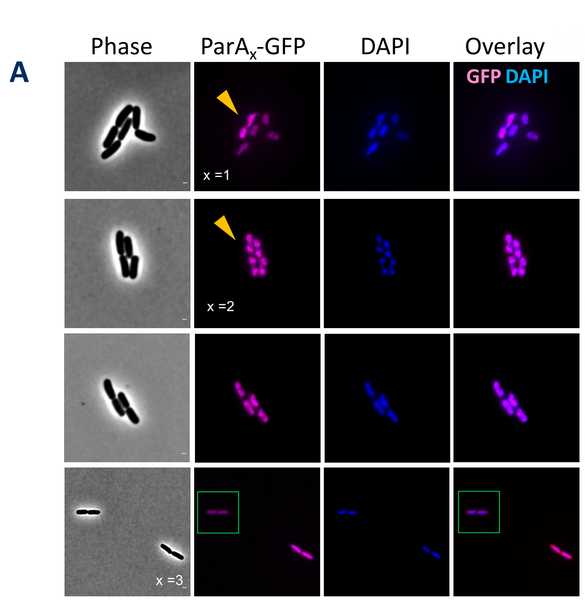
Synchronous predation of *E. coli* by *B. bacteriovorus* HD100 *ter:parS$_{P1}$* with constitutive expression of CFP-ParB$_{P1}$, used as a proxy for the visualization of terminus of replication (*arrowheads*).

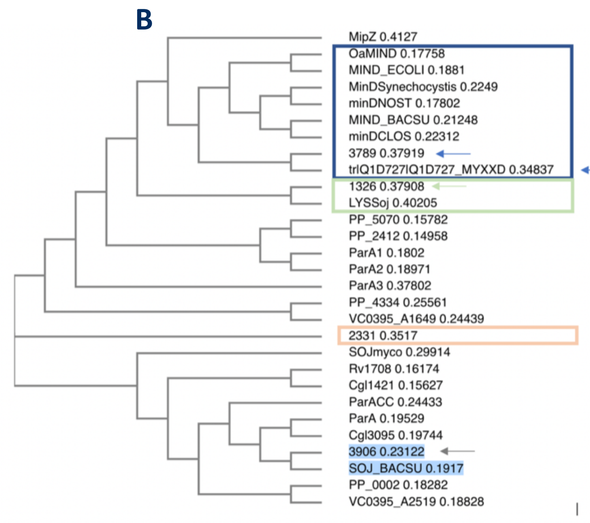


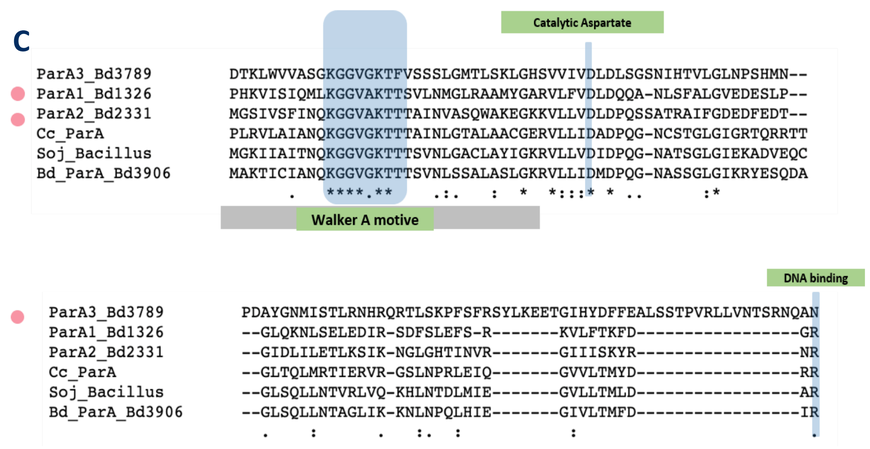
The *ter* locus is polar at early stages after invasion, then *ter* moves towards the midcell point, where multiplication of *ter* takes place (*arrowheads*). *Scale bar: 1 µm.* Representative cells are shown.

3. Segregation of the *Bdellovibrio* chromosome

In most species, chromosome segregation is driven by the ParABs system. Interestingly, in addition to the canonical ParA, we found **three additional ParA-like proteins** encoded in the *Bdellovibrio* genome.







Analyses of *Bdellovibrio* ParA homologs (Figure B). Alignment of ParAs sequences (Figure C). Key motifs are highlighted in blue. Corresponding names are in green.

Walker A motifs in ParA1 and ParA2 are different from known ParA's (Figure C). ParA3 does not harbor the typical DNA binding residue (Figure A and C).

ParA1 and ParA2 colocalize and affect chromosome segregation/compaction when expressed in *E. coli (yellow arrowheads).* ParA3 has a diffused pattern when produced in *E. coli (green inset),* consistent with our prediction from sequence analysis.

All figures on this poster are unpublished data. This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program under grant agreement No 802331.