FD111 TEM Update 160512

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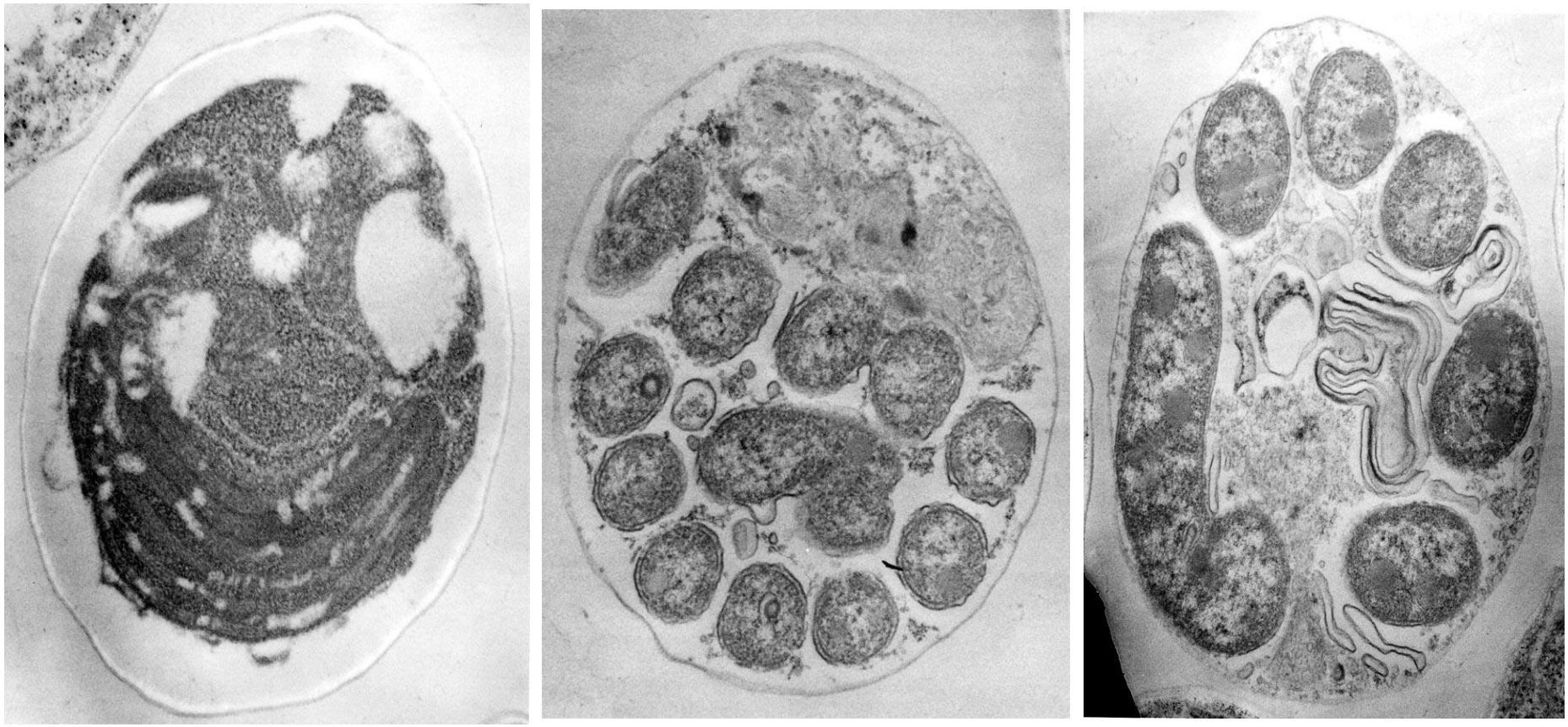
Progression of infection

- D1: ~99% of the host cells appeared healthy and uninfected; only a few host cells had evidence of bacterial infection.
- D2 AM: ~99% of the host cells show evidence of bacterial infection; bacteria are mostly attached to or inside host; few appear free in the medium.
- D2 PM: much the same as D2 AM; also, evidence of bacteria division inside host cell, and release of bacteria across host cell wall.
- D3: about all that remain of host cells is the cell wall; a lot of free bacteria in the medium and outside of host cells; bacterium may exit the same way it got in.

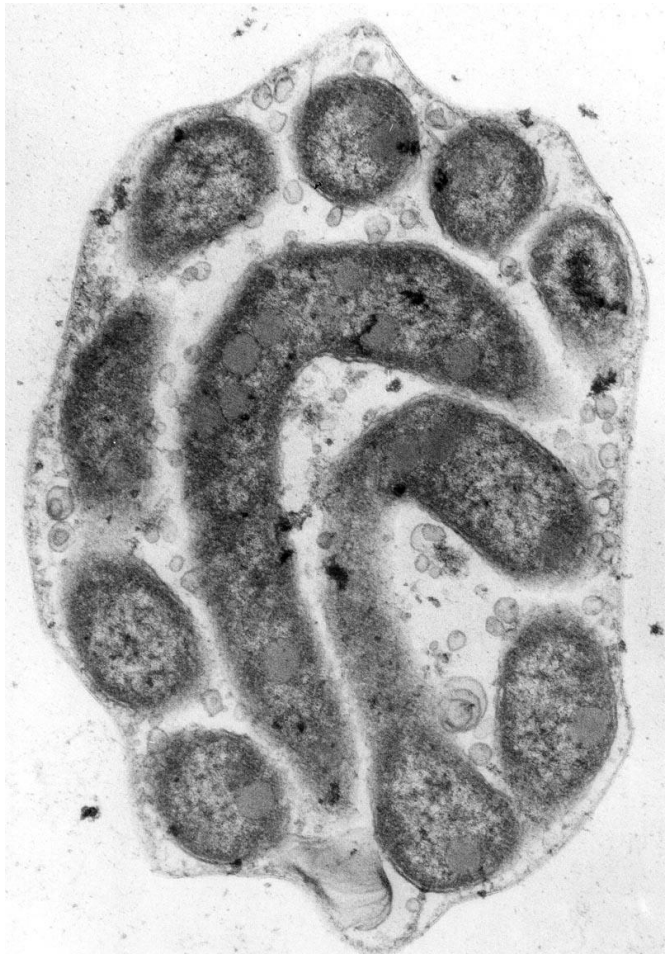
Things to note about infection

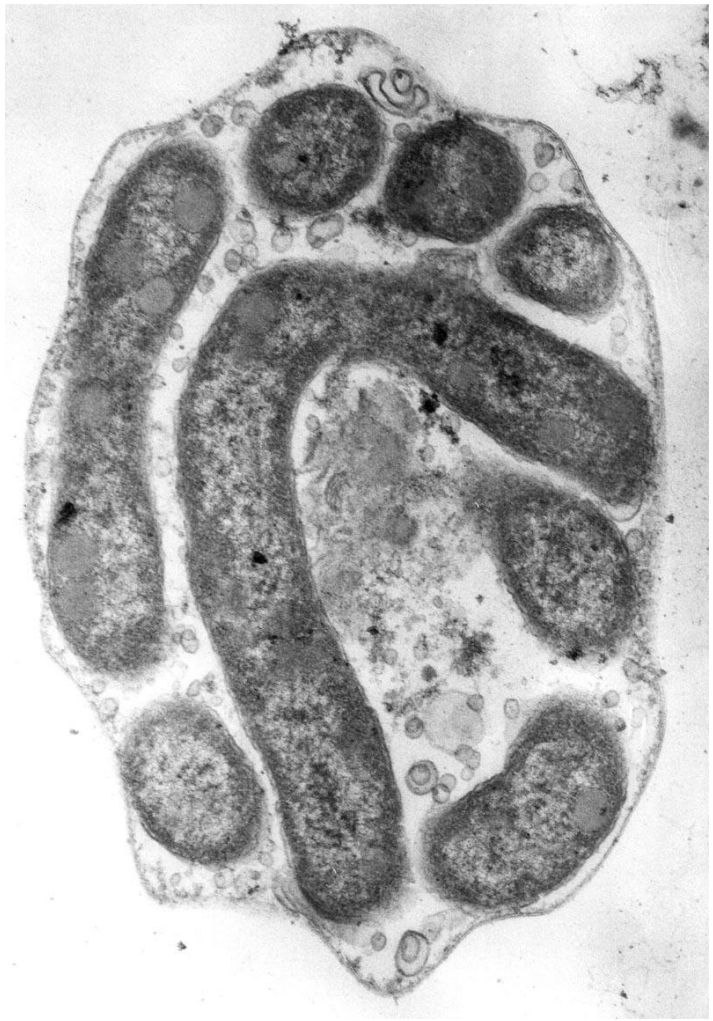
- Bacterium seems to attach to host cell wall by a distinct plate-like structure that is separate/independent from the bacterium cell wall.
- The plate may be involved in breaching the host cell wall. It is evident that the bacteria seen outside the cell are the same as the bacteria inside
- I suspect that after total degradation of host contents, the host cell wall breaks down to allow release of bacteria

D1: ~99% of the cells were uninfected (L), while perhaps 1% were infected (C, R)

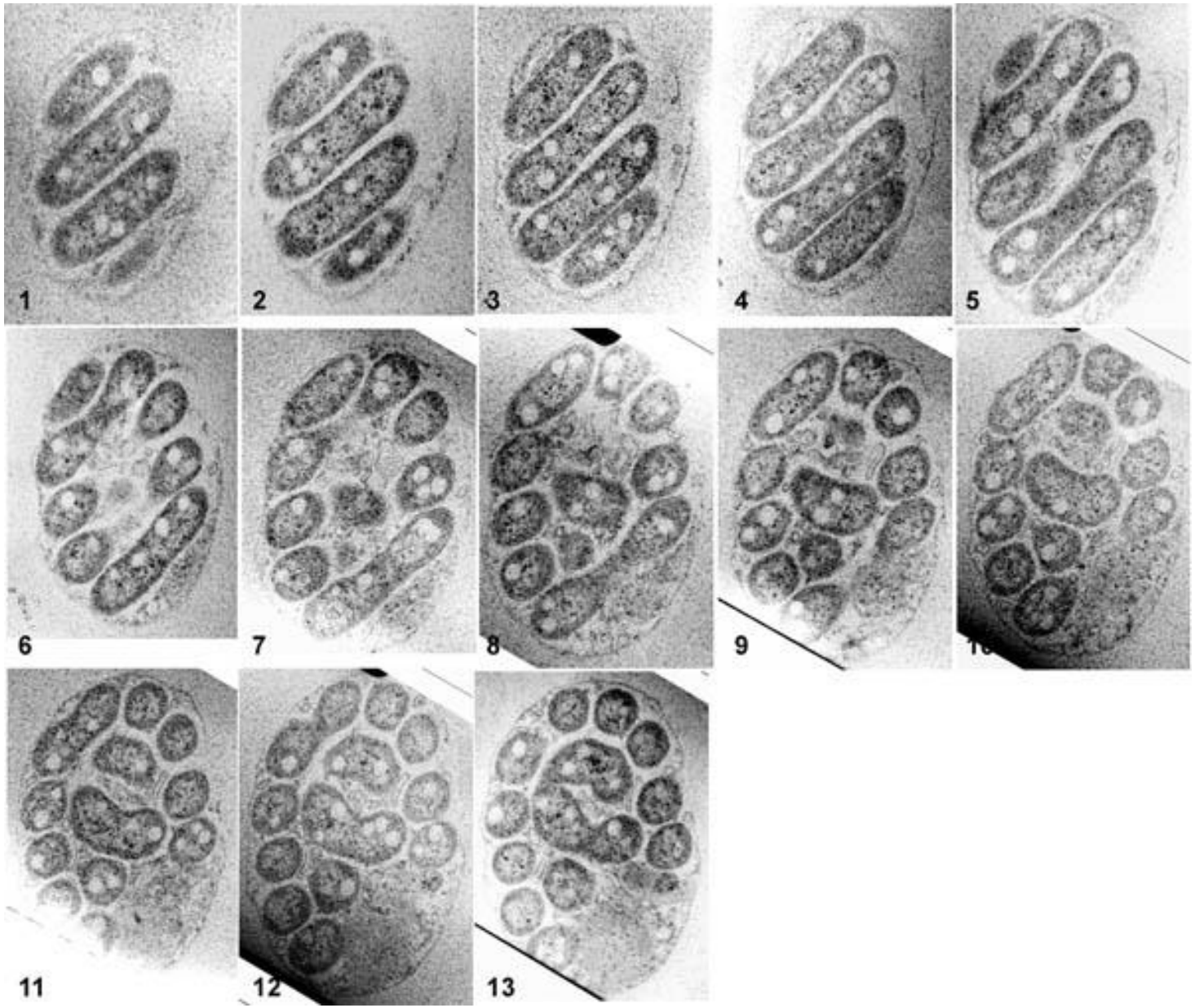


These serial section images are from previous work, but illustrate that “round” sections are simply cross sections through an elongate, coiled bacterium inside the host

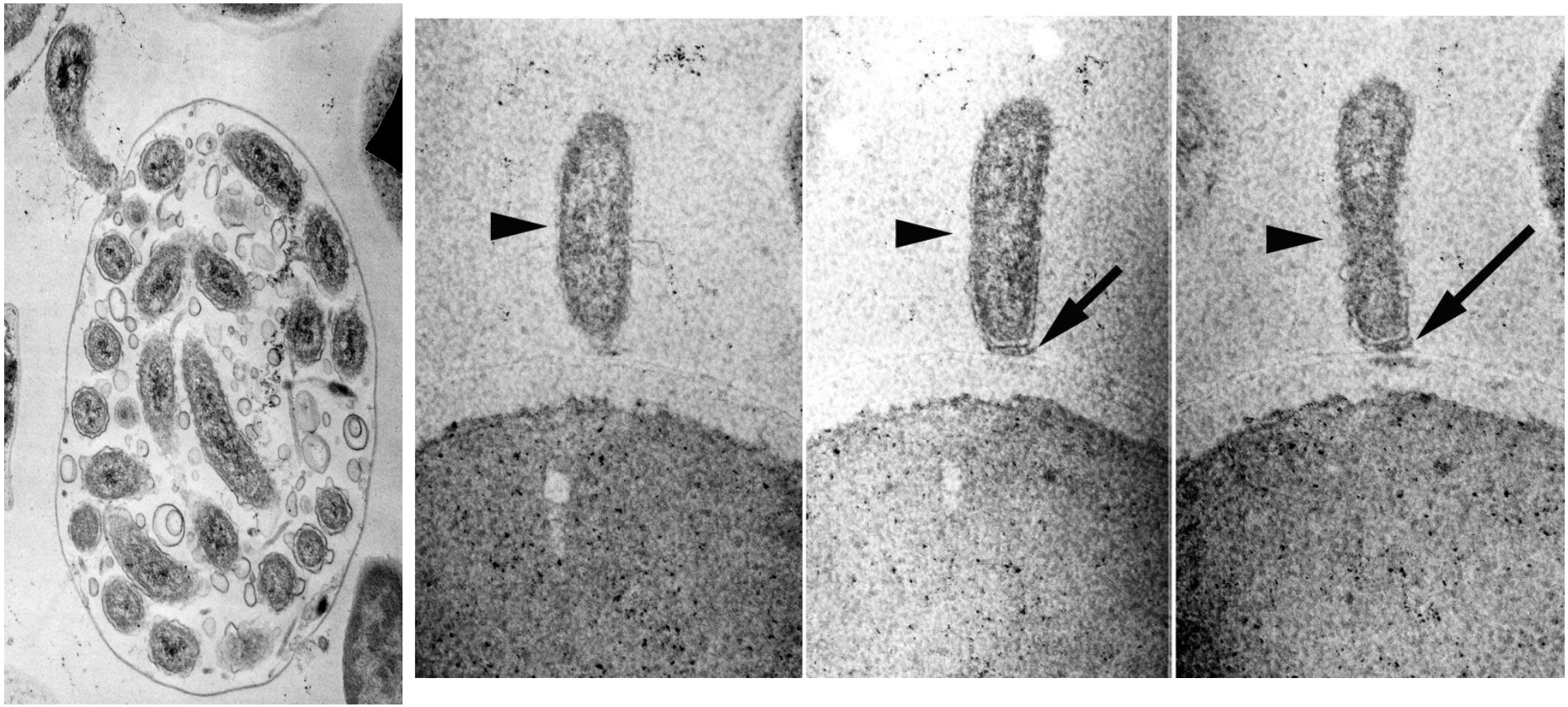




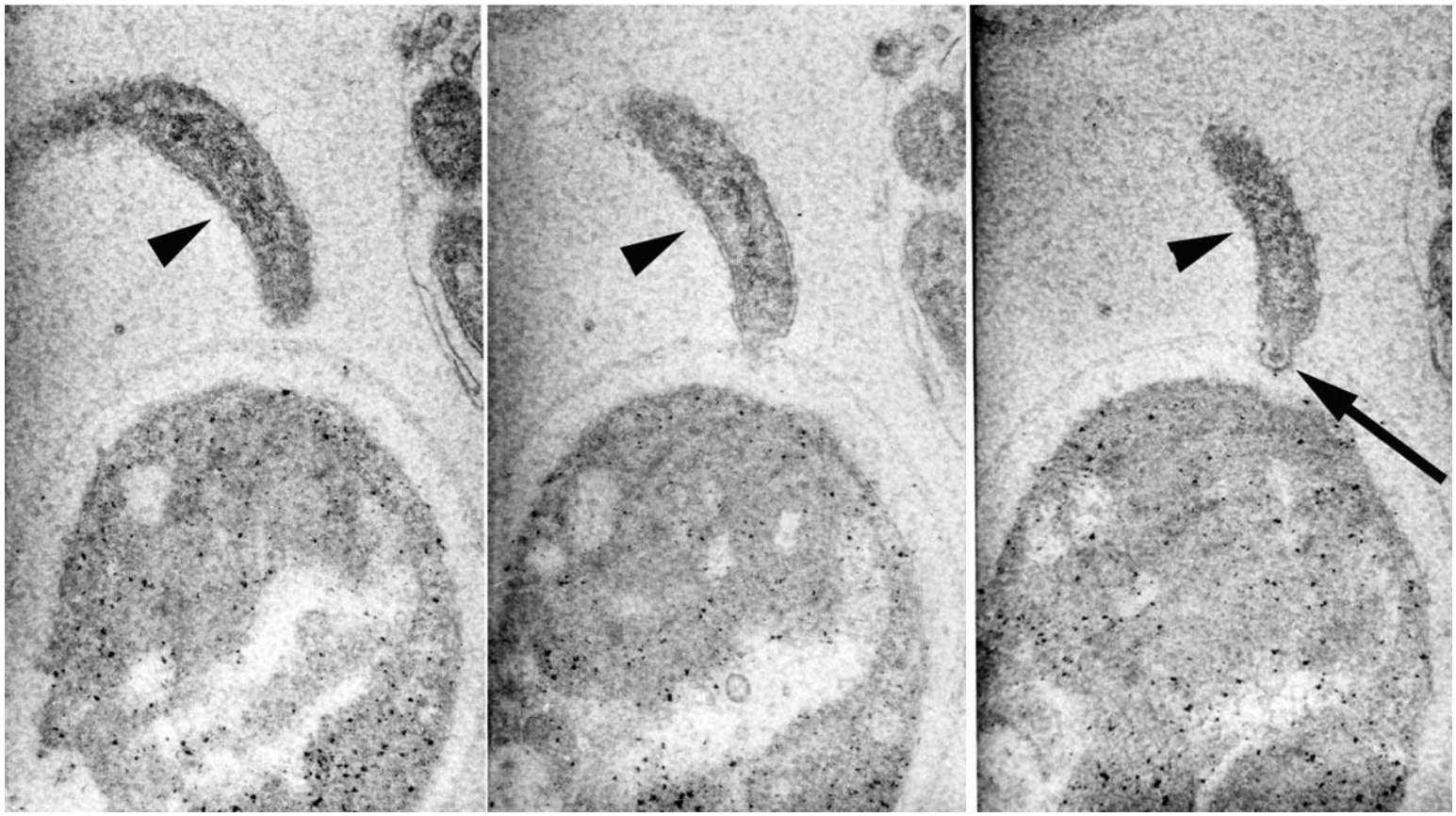
NEW: D2 AM SS1-13: initial infection a single bacterium: no evidence of septa that would indicate multiple entities



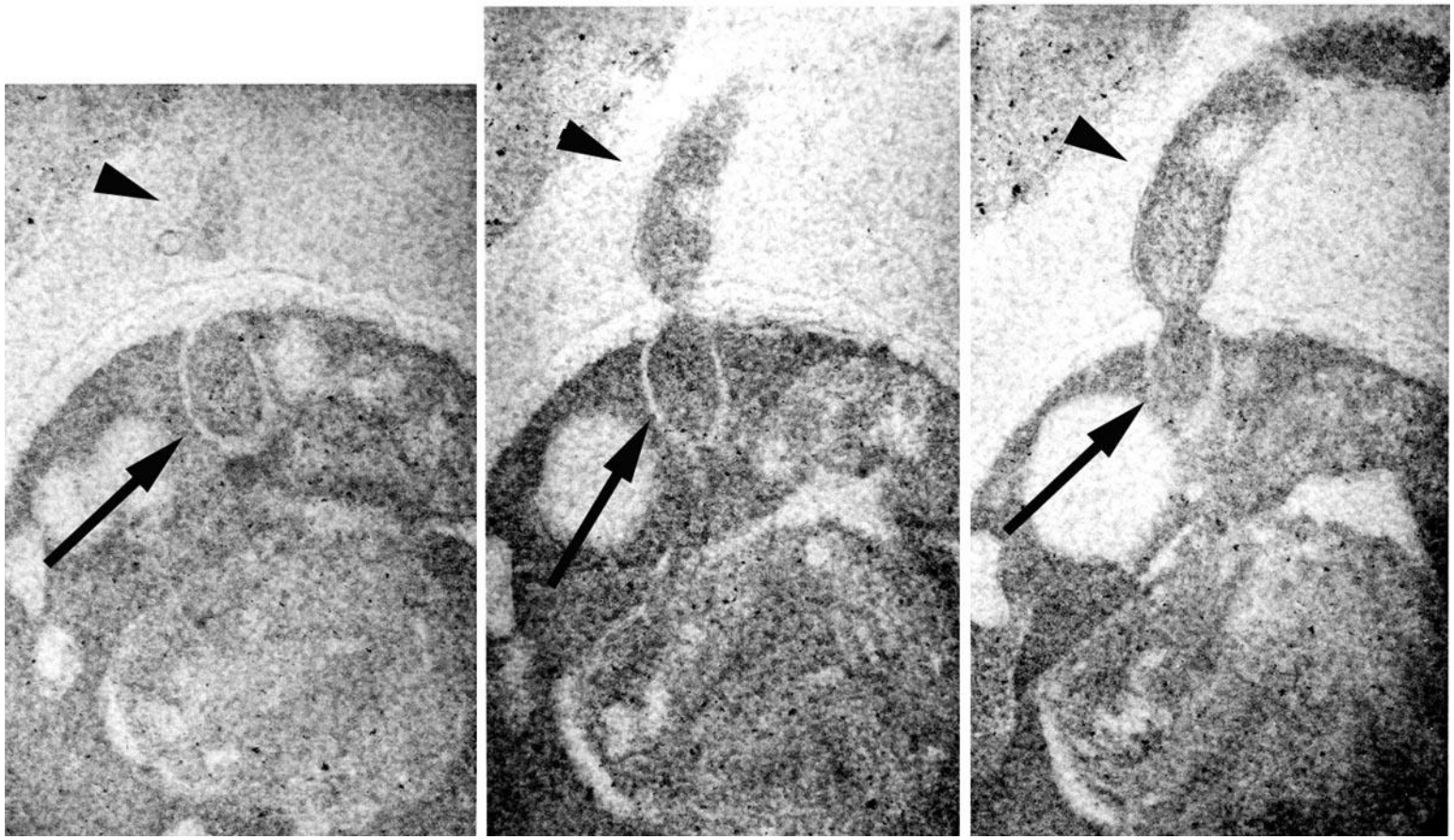
D2 AM:~99% of the cells were infected (L image); serial sections (R image):bacterium (arrowheads) appeared to be attached to the host by a structure perhaps analagous to an appressorium or holdfast (C, R, arrow)



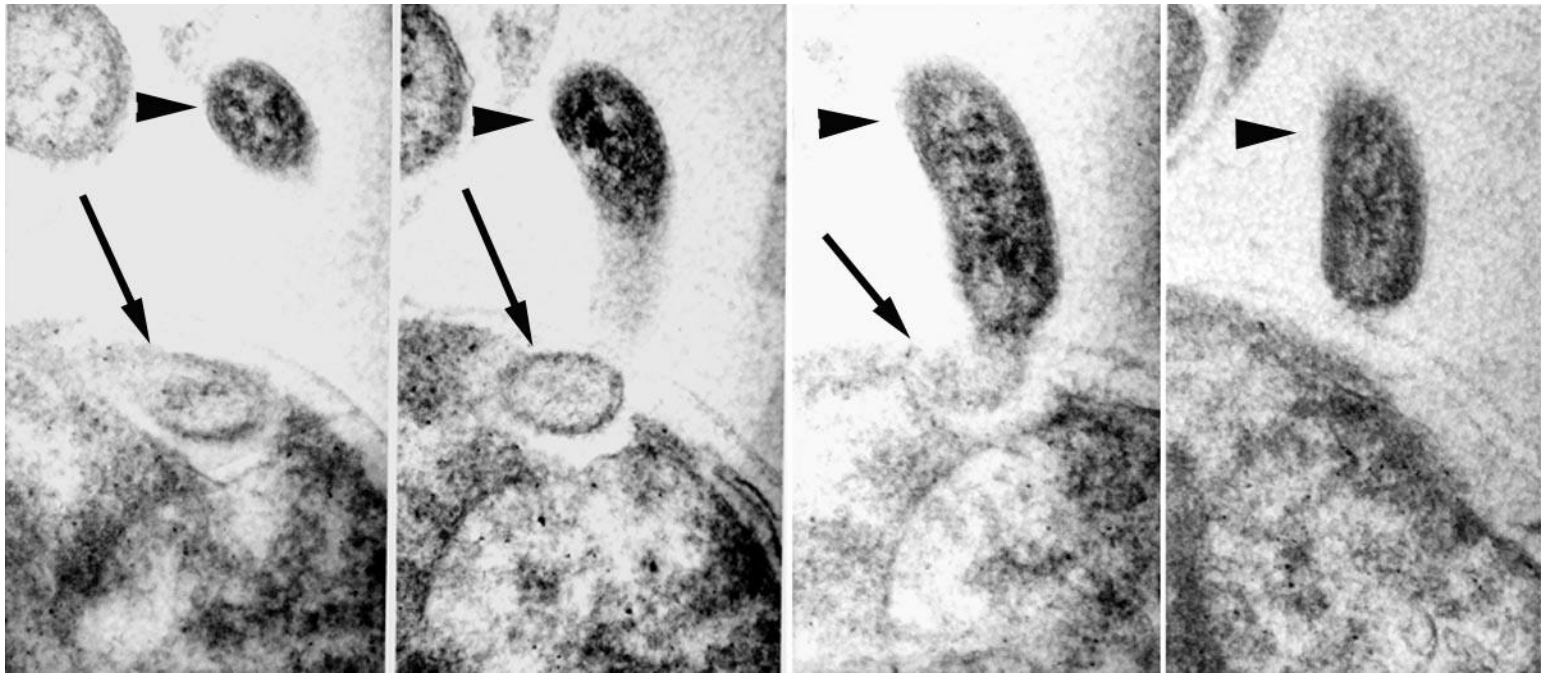
D2 AM: Serial sections: bacterium (arrowhead) appears to penetrate the cell wall (arrow) with a bulb-like structure



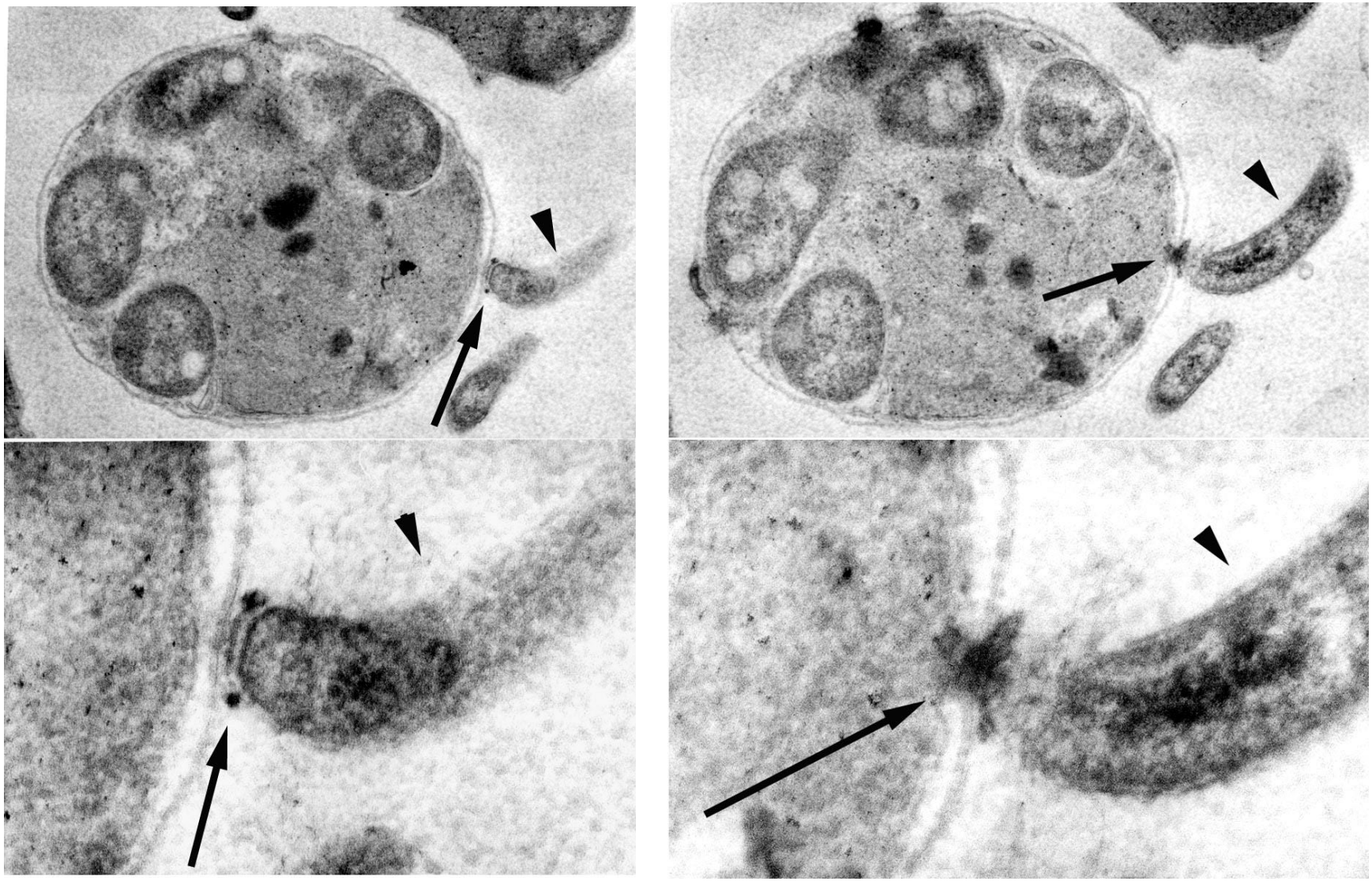
D2 AM: serial sections: bacterium (arrowhead) has penetrated into the host cytoplasm (arrow), and the bulb-like structure enlarges



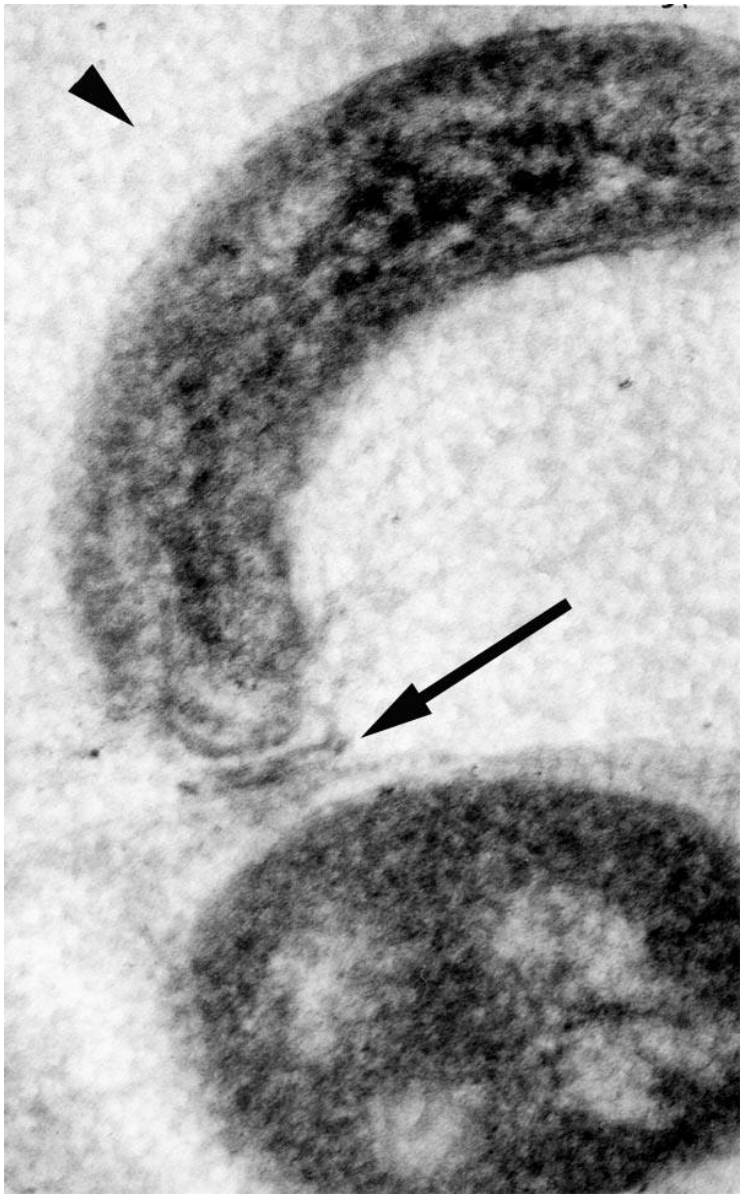
D2 PM: ~100% mortality of host; serial sections: obviously, bacterium (arrowhead) has penetrated and is inside host (arrow)



D2 PM: serial sections and enlargement of bacterium (arrowheads) and attachment plate upon and penetrating host cell wall (arrows)



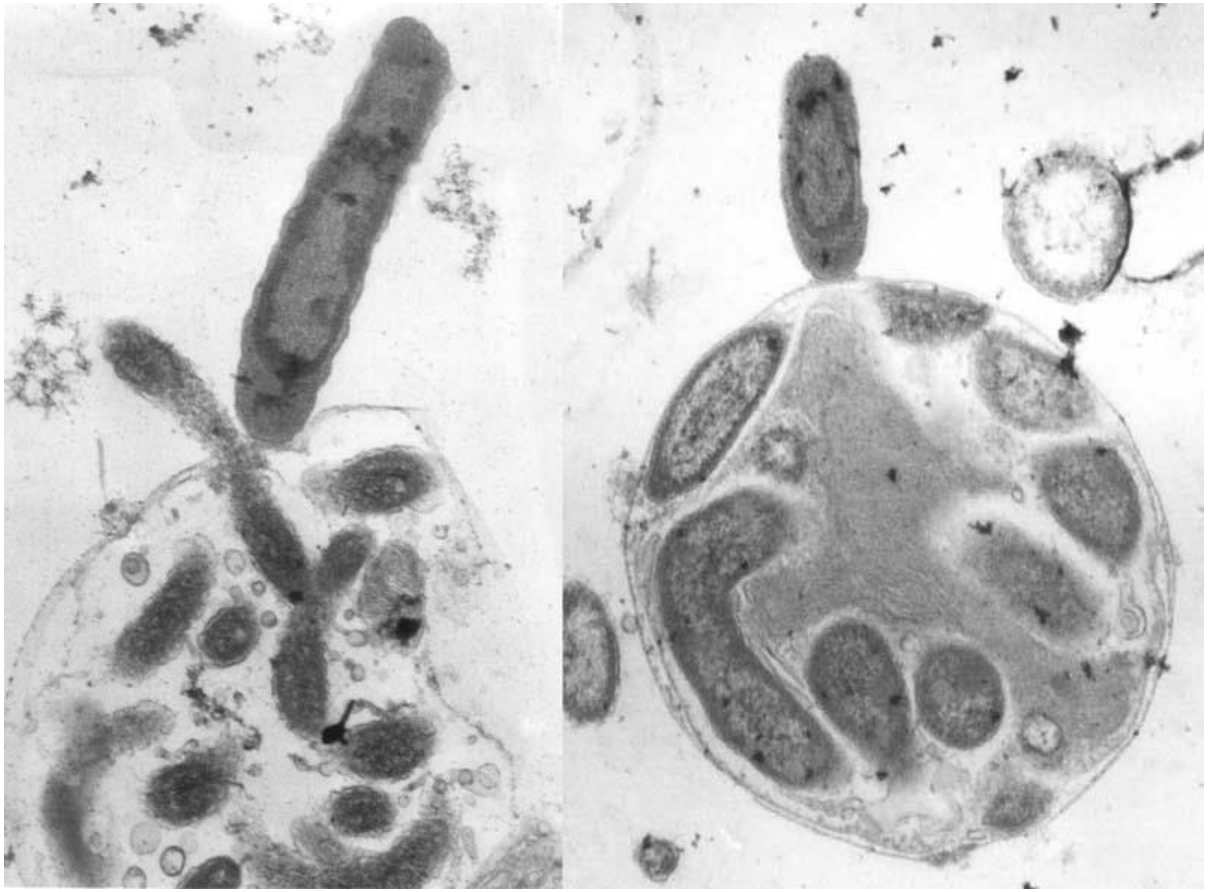
D2 PM: Bacterium (arrowhead) and attachment plate (arrow)



*Unanswered question:

- We now know that the bacterium attaches to the host wall with a plate-like structure;
- The bacterium penetrates the host wall with a bulb-like structure that enlarges in host cytoplasm;
- The bacterium ends up inside the host. Since bacteria divide by binary fission, we can *assume a product of division is in the bulb-like structure, and that product grows as it consumes the contents of the host. This seems to me a very good (parsimonious) way for the bacterium to gain access to the food source.
- Further indication of this is that we often see, on partially and fully consumed host cells, mature bacteria still attached to the outside of the host cell (next slide). But, I don't know if these are part of what is inside, or simply a wandering, extraneous bacterium that has attached to an infected cell.

Mature bacteria attached to cell wall of infected (R) and degraded (L) host cells.



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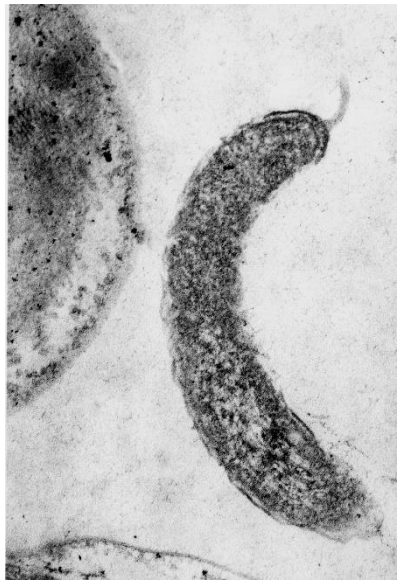
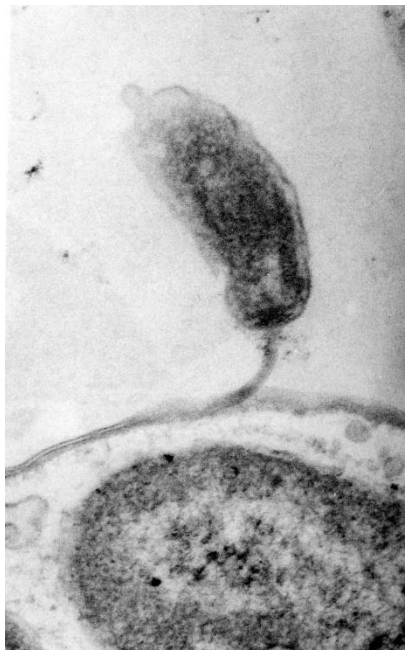
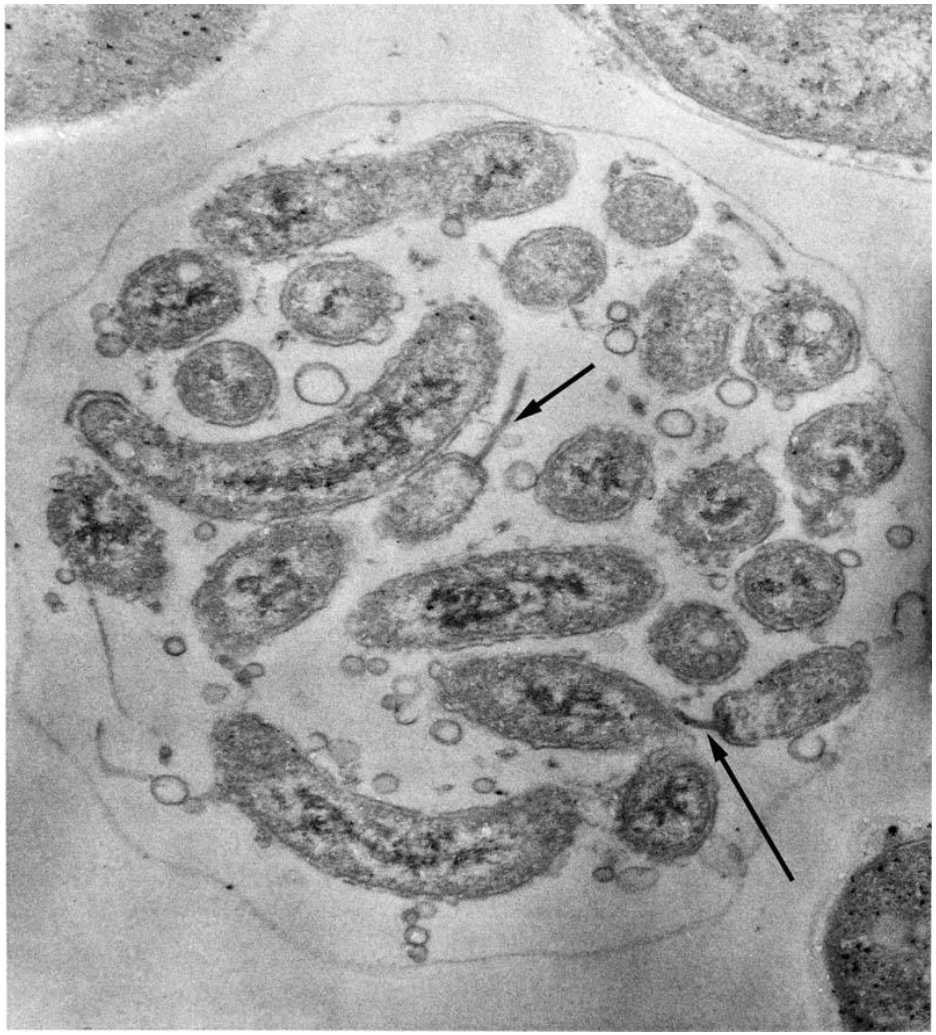
- We also know that the bacterium inside is, at least initially, a single entity. However, I think it does eventually divide INSIDE:

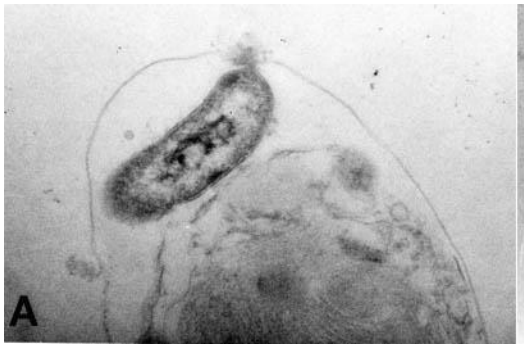
Remember the bacterium with the “tail”? Well, here’s a consumed host, and INSIDE are 2 bacterial profiles with tails (arrows), which is evidence of cell division inside the host. So, what starts out as a single entity inside, becomes multiple entities inside. And, we also now know that the bacterium with the tail and the bacterium inside are the same.

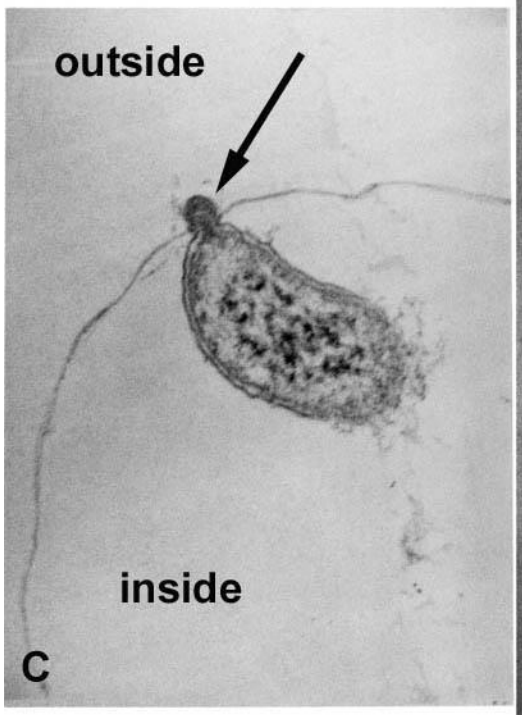


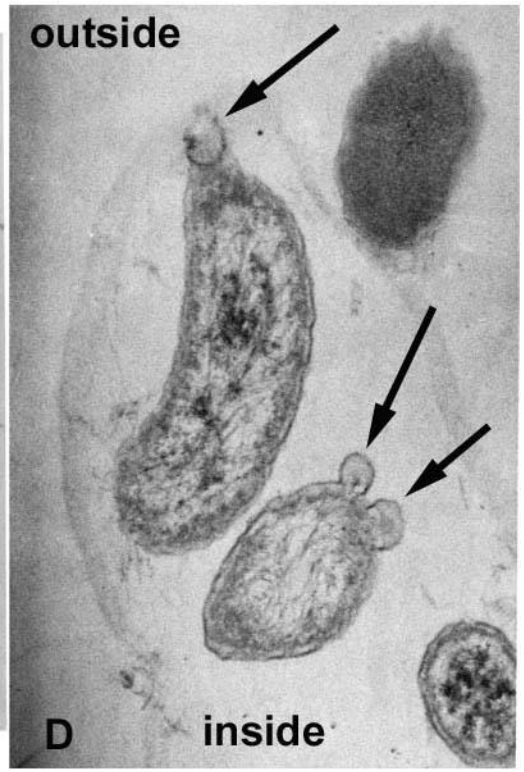


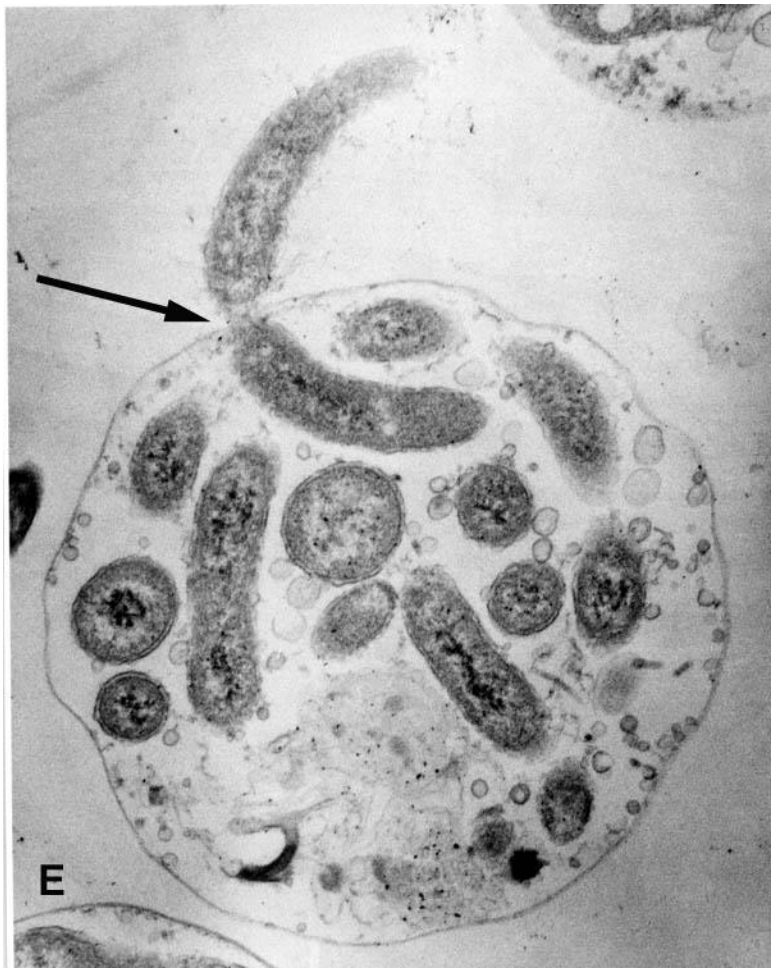


Okay. Now, how does the bacterium get out? I think it gets out the same way it gets in: inside bacterium forms a bulb at the host wall (A, C, D), divides into the bulb (B), and progeny is outside (E). These are random sections, and thus, circumstantial….

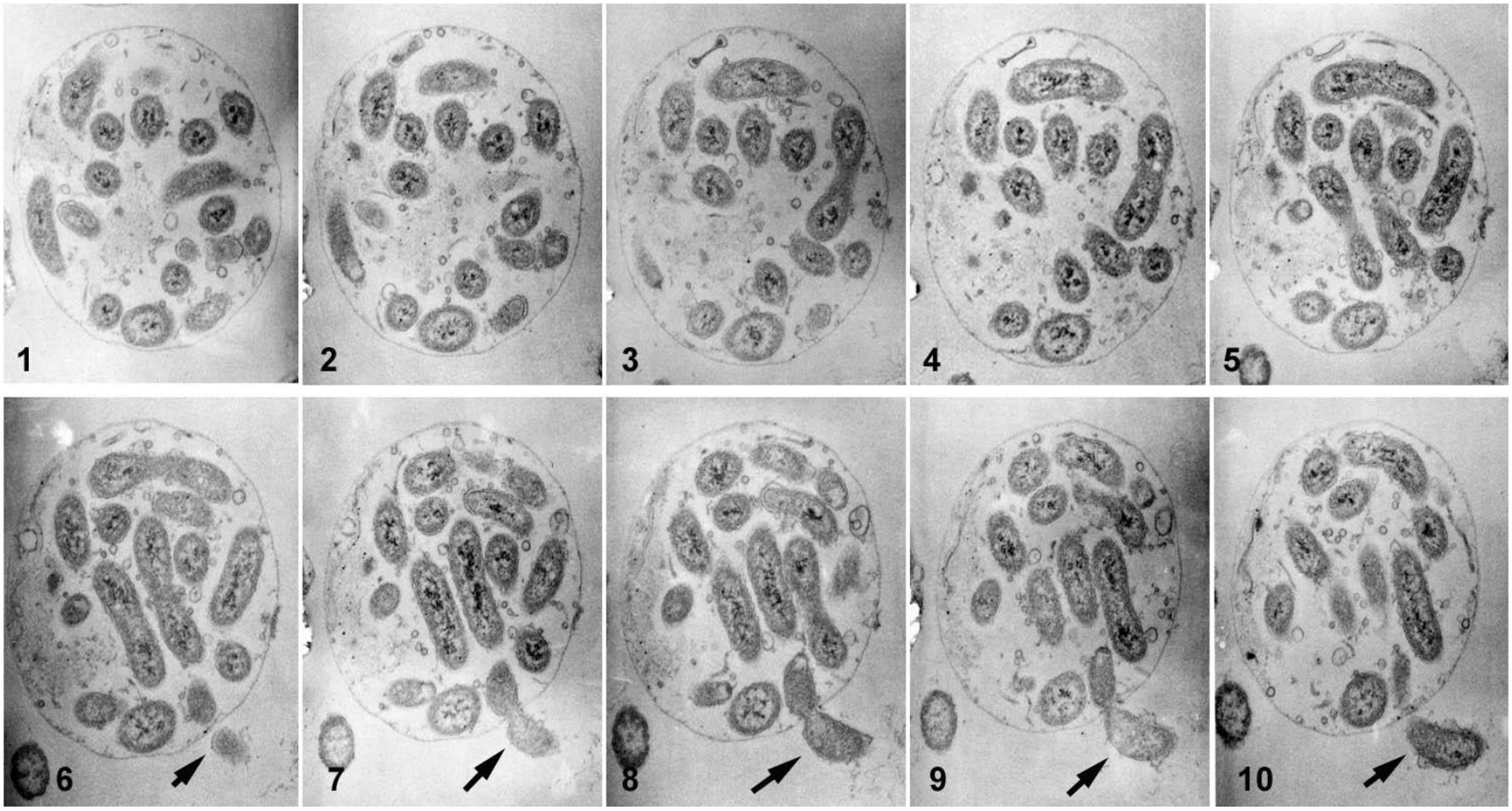




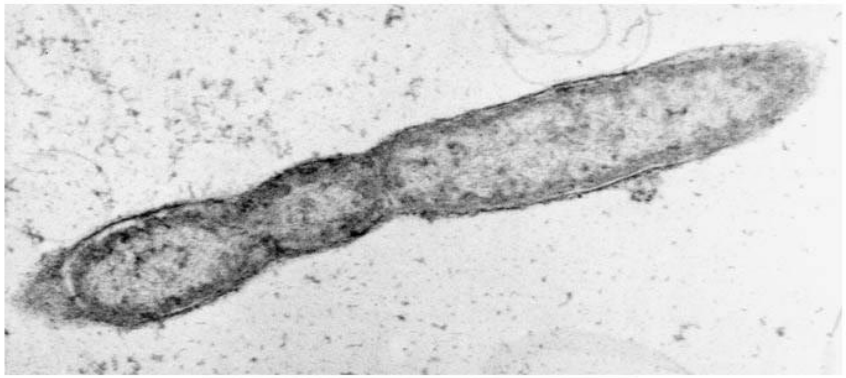


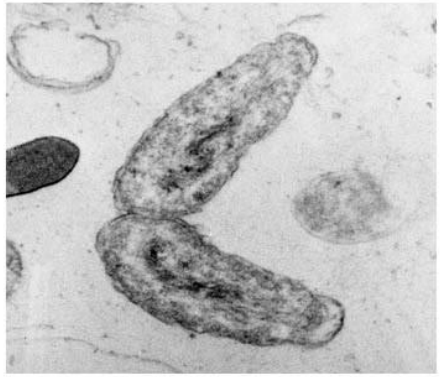


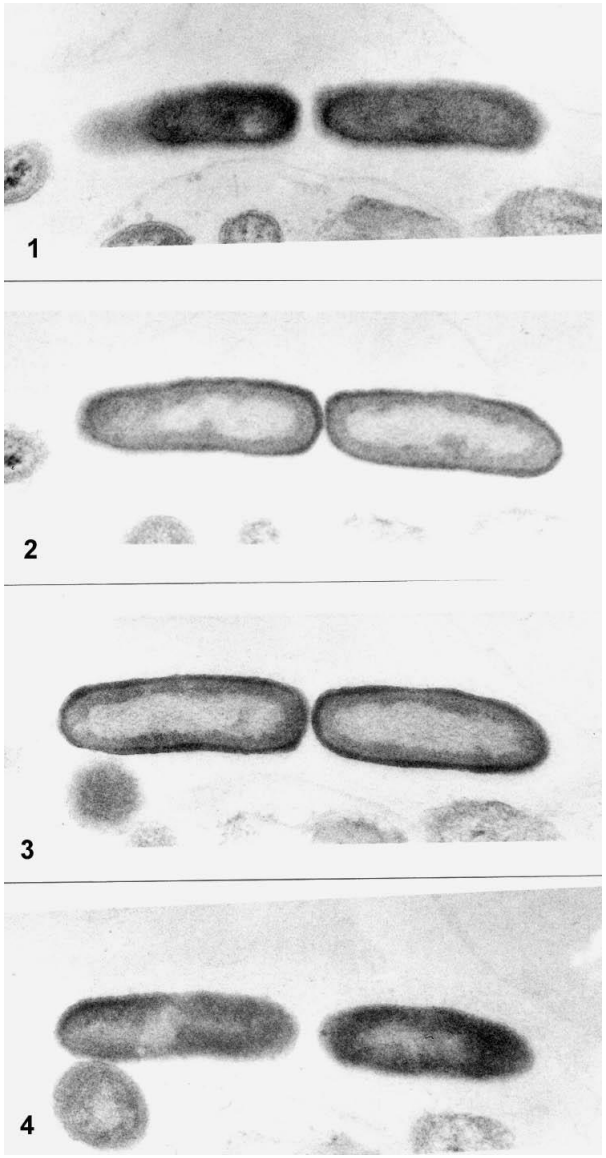
Inside to outside: these are serial sections, and thus NOT circumstantial: images 6-10 show a portion of the bacterium crossing (arrows) from inside to outside.



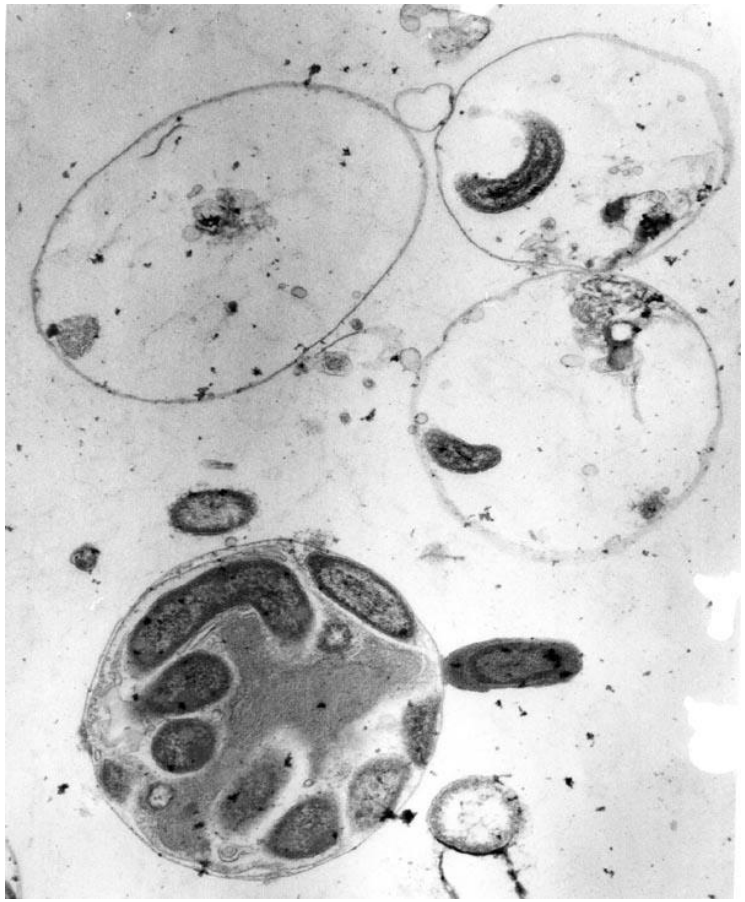
And, division occurs OUTSIDE, as well. Here are (L) 3 bacteria outside of host cells, in various stages of cell division , and (R) serial sections of a recently divided bacterium.





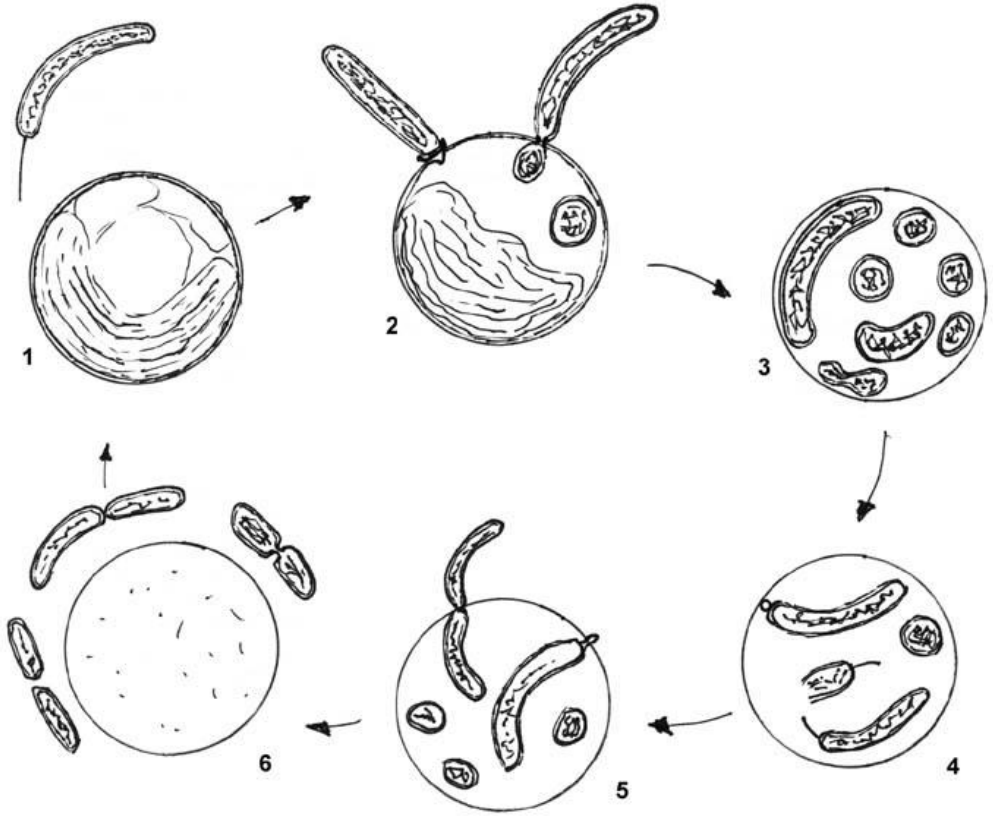


D3 (although this is from D4 of my previous look at this infection, it is what I am seeing with D3 of what you sent this time); algal remnants: many algal cells empty; only cell wall remains; cell wall intact; bacterium that contributed to release still inside; many free bacteria in medium, external to host cells.

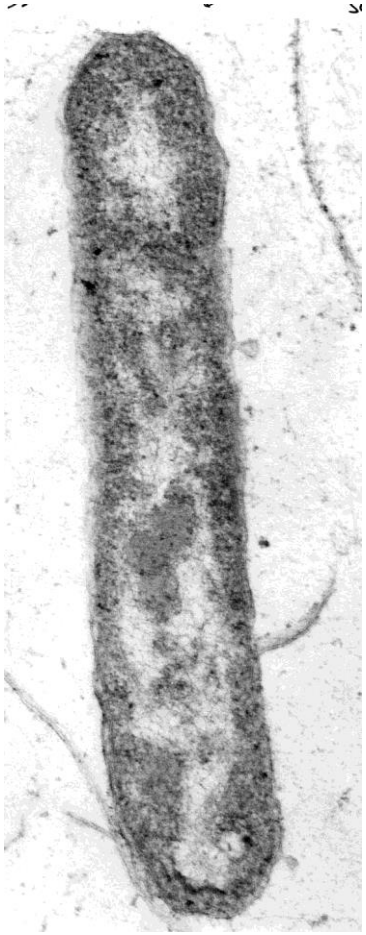


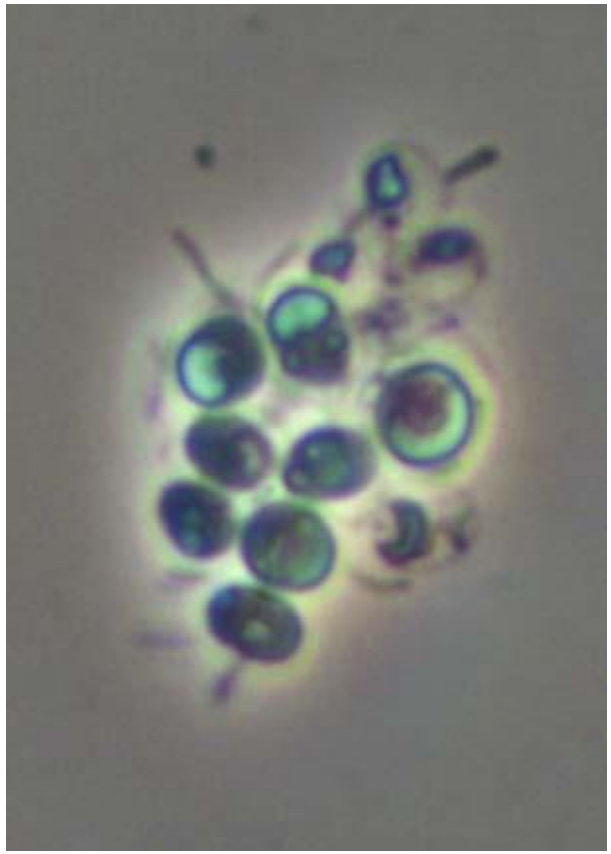
Presumptive, possible life cycle:

1. Motile bacterium and healthy algal cell.
2. Bacterium attaches to outer surface of host cell wall via a plate-like structure, breaches host cell wall, forms bulb-like structure in host cytoplasm, divides into the bulb.
3. Bacterium consumes host contents, develops as a single entity coiled inside host.
4. Bacterium divides into multiple progeny.
5. A progeny attaches to inner surface of host cell wall, breaches wall, forms bulb-like structure outside host wall, divides into bulb.
6. Bacteria external to empty host cell divide.

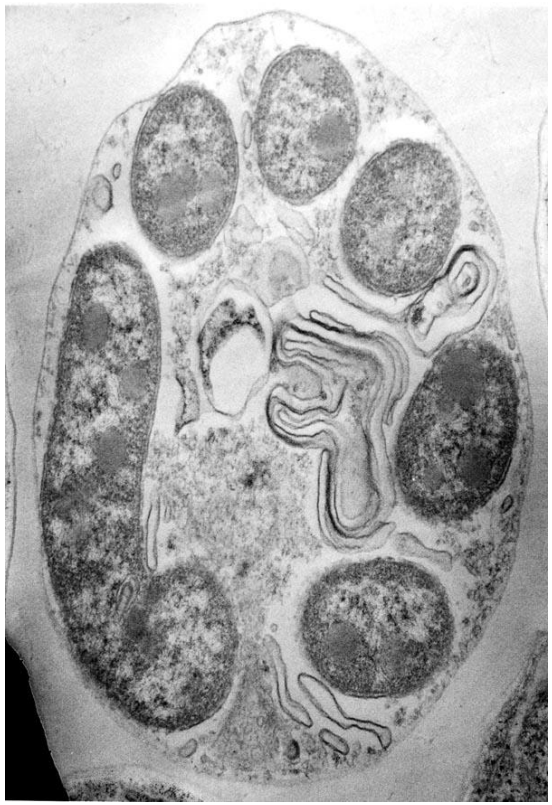


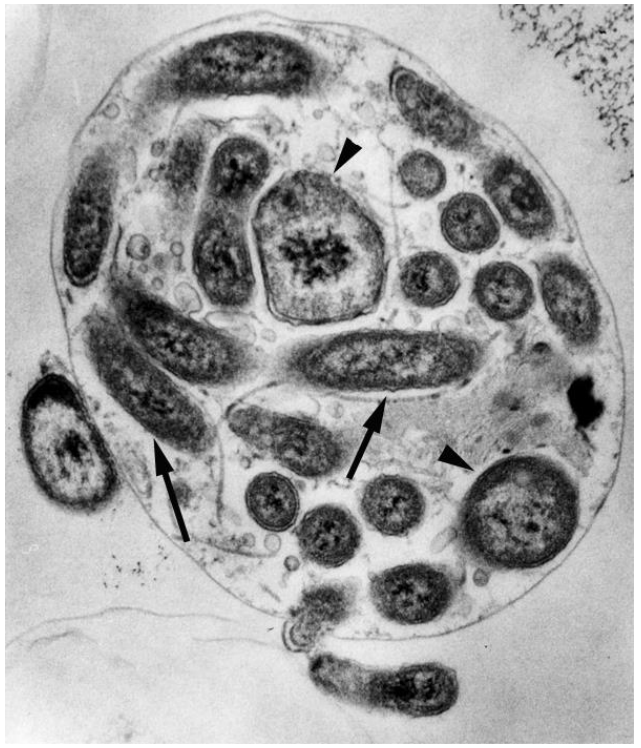
I have a slight problem: does size (and morphology) matter? All three of these bacteria are ~ 360 nm diameter, and I think they correspond to the rod-shaped bacterium we have seen with light microscopy.



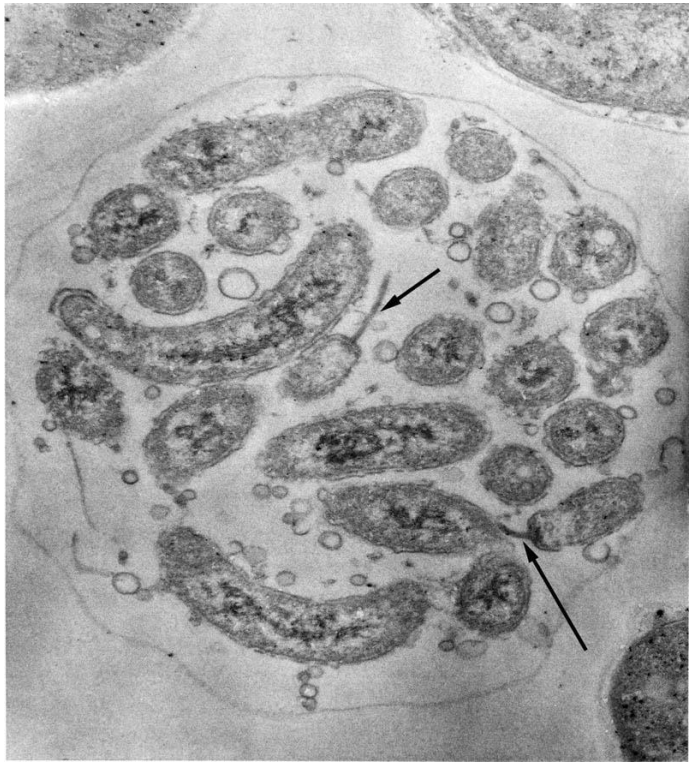


Does size matter? LEFT: profiles are ~450-625 nm diameter. CENTER: round profiles (arrowheads) are ~ 470 nm diam and elongate profiles (arrows) are ~ 260 nm; RIGHT: round profiles (arrowheads) are 570-640 nm diam and elongate profiles (arrows) are ~315 nm diam.





Does size matter? LEFT (disregard arrow): Curved bacterium with possible flagellum, ~250 nm diam; CENTER: profiles 205-250 nm diam; RIGHT: LM of curved bacteria.



So, I just showed this ppt to, and talked with, a colleague here who knows bacteria. She is inclined toward Caulobacter, which is a Gram-negative, oligotrophic bacterium widely distributed in fresh water lakes and streams. (Google it). It has an interesting morphology composed of two cells, one being a flagellated swarmer that perhaps locates a host via chemotaxis, and a non-motile cell for reproduction. The vibrio-shaped (crescent- or hooked-shaped) cell with a flagellum may be the former, while the rod-shaped cell may be the latter. Thus, the two morphologies we are seeing may be of the same organism.

- It is suggested to stain this bacterium for gram-negative;
- It is suggested to obtain 16S with bacterial primers;
- We need sequences to reliably place this organism.
- Molecular placement will let us assess morphology as being similar or dissimilar to whatever is sister to this organism, to determine whether or not it is novel, as an algal parasite.
- This link goes to a page that links Caulobacter to algae:

https://books.google.com/books?id=sTsC65kCJbUC&pg=PA86&lpg=PA86&dq=caulobacter+crescentus+in+algae&source=bl&ots=9r9-qnrXjm&sig=AWM_6pFwaNU2eOi1tmpriQMwqZY&hl=en&sa=X&ved=0ahUKEwjRhI-U7t7MAhUMOyYKHcDYCbEQ6AEIQDAD#v=onepage&q=caulobacter%20crescentus%20in%20algae&f=false