FD111 TEM 160411

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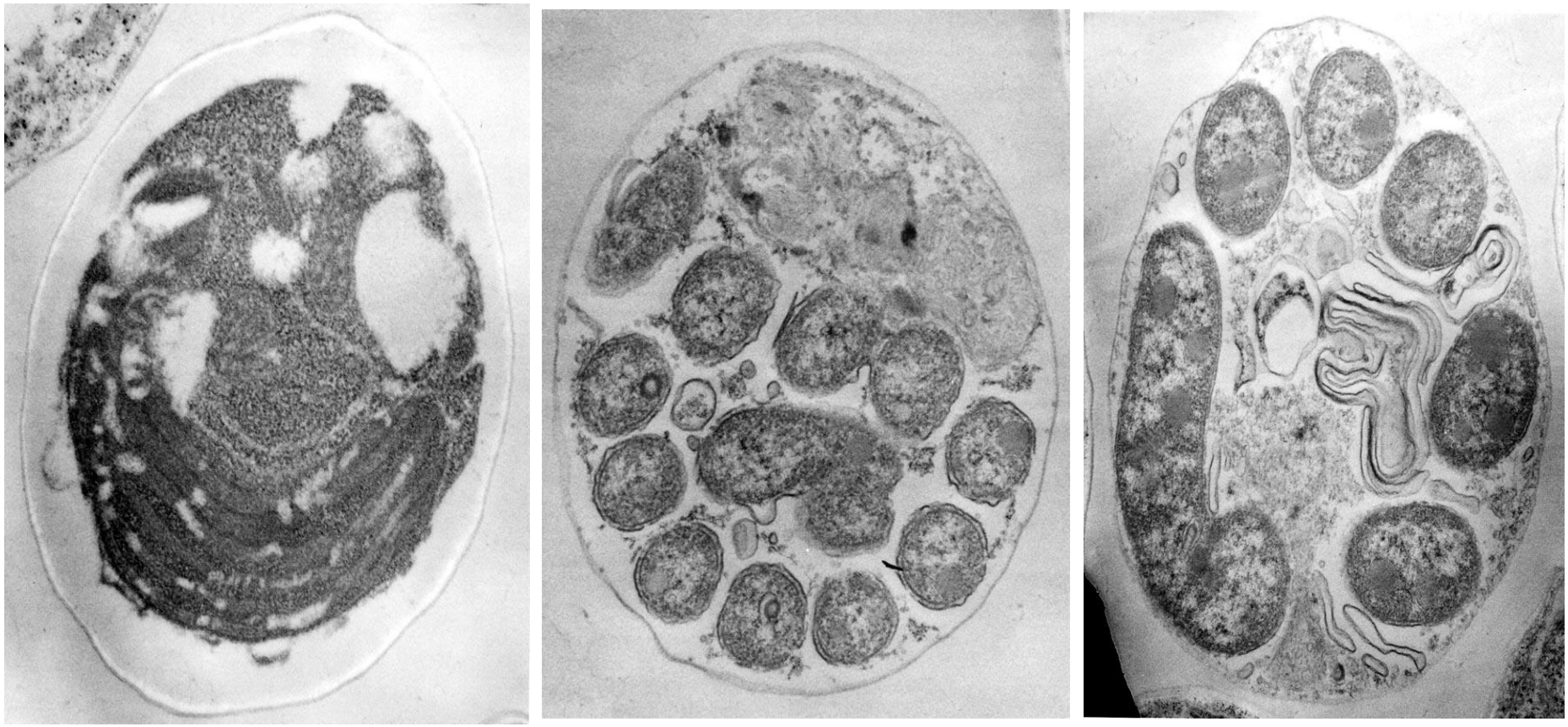
Progression of infection

- D1: [slide 4] ~99% of the host cells appeared healthy and uninfected; only a few host cells had evidence of bacterial infection.
- D2 AM: [slides 6-8] holy mackerel, reverse those stats: ~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: [slides 9-11] much the same as D2 AM
- D3: [slide 12] about all that remain of host cells is the cell wall; a lot of free bacteria in the medium and outside of host cells; not sure how the bacteria get out after degrading the host.

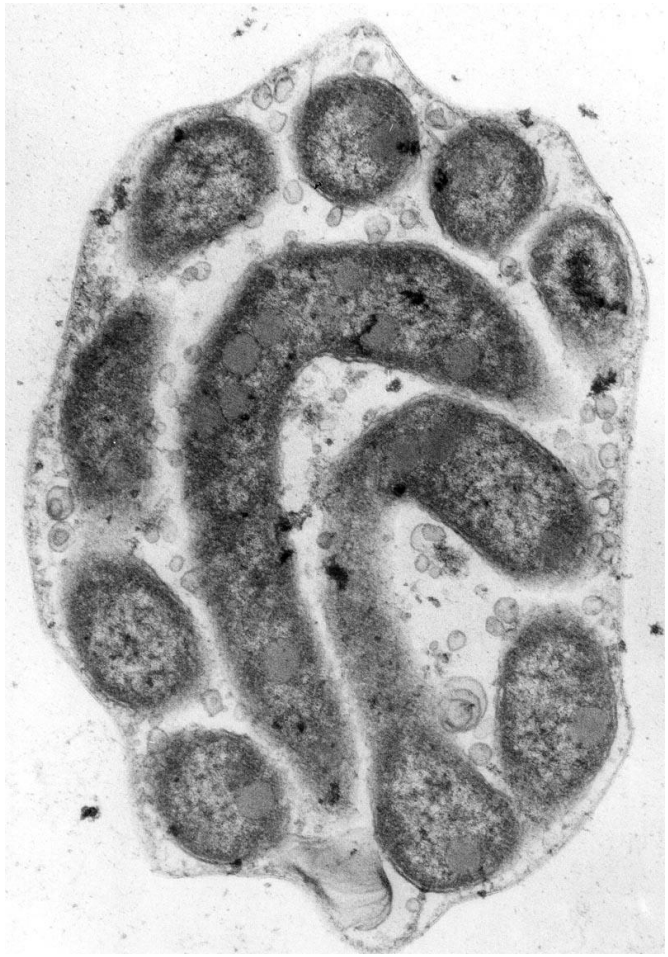
Things to note about infection

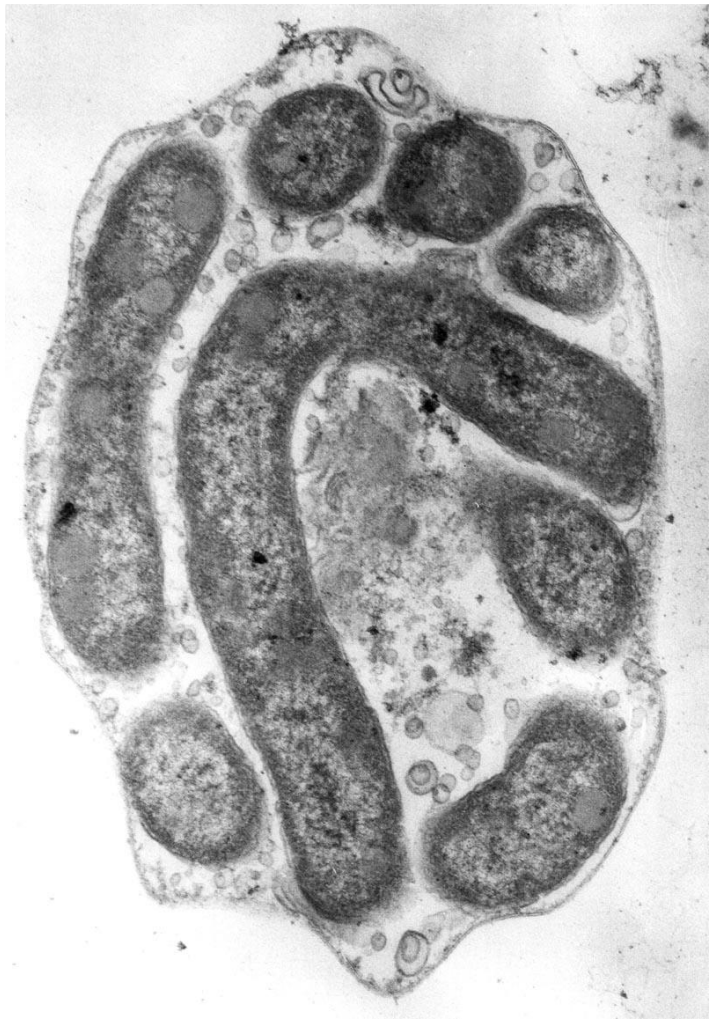
- Bacterium seems to attach to host cell wall by a distinct plate-like structure (slides 6, 10L, 11) that is separate/independent from the bacterium cell wall.
- The plate may be involved in breaching the host cell wall (slides 7, 10R)
- It is evident that the bacteria seen outside the cell are the same as the bacteria inside (slides 8, 9)
- 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)

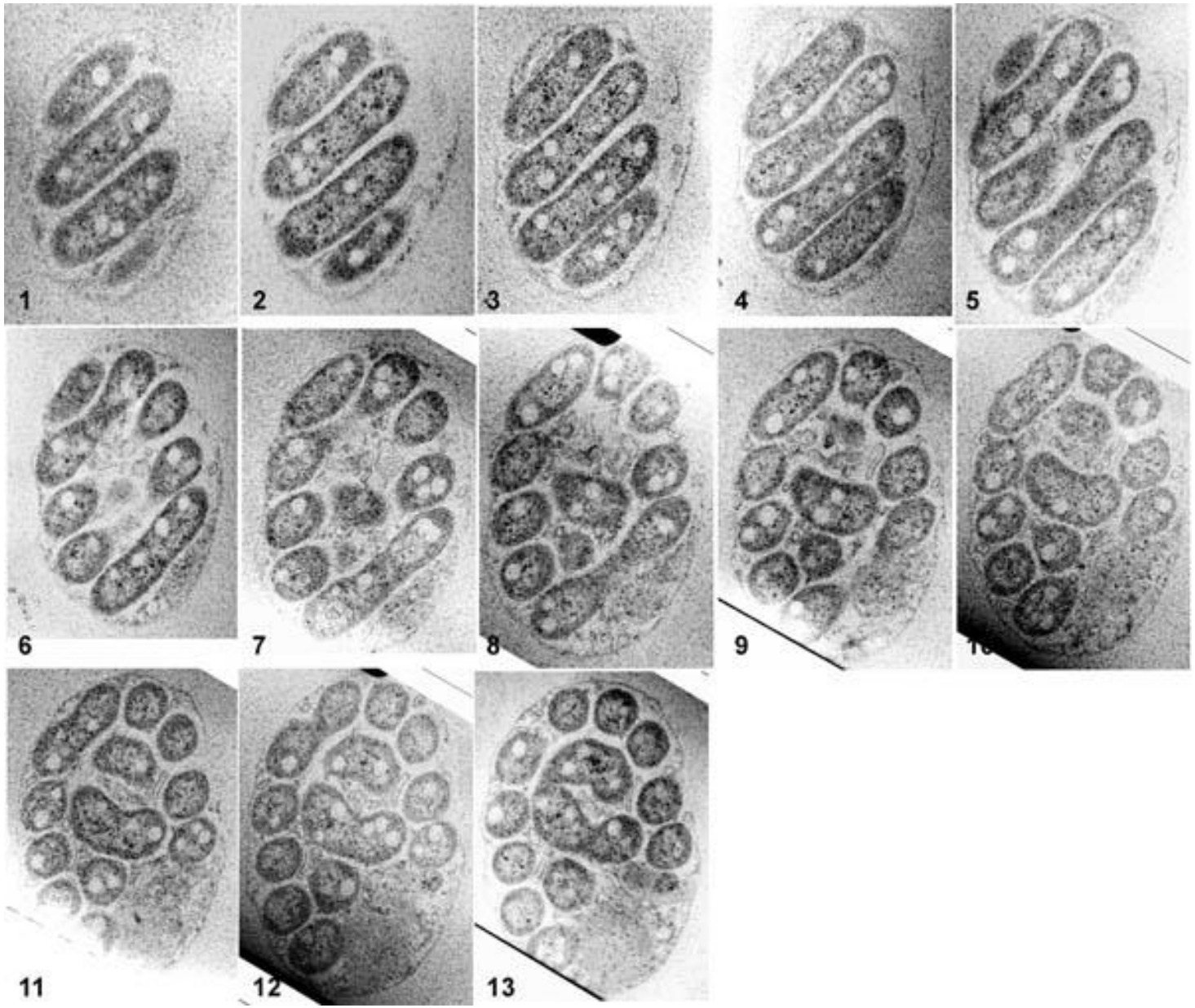


These serial section images are from previous work, but illustrate that “round” sections are simply cross sections through an elongate, coiled bacterium inside the host

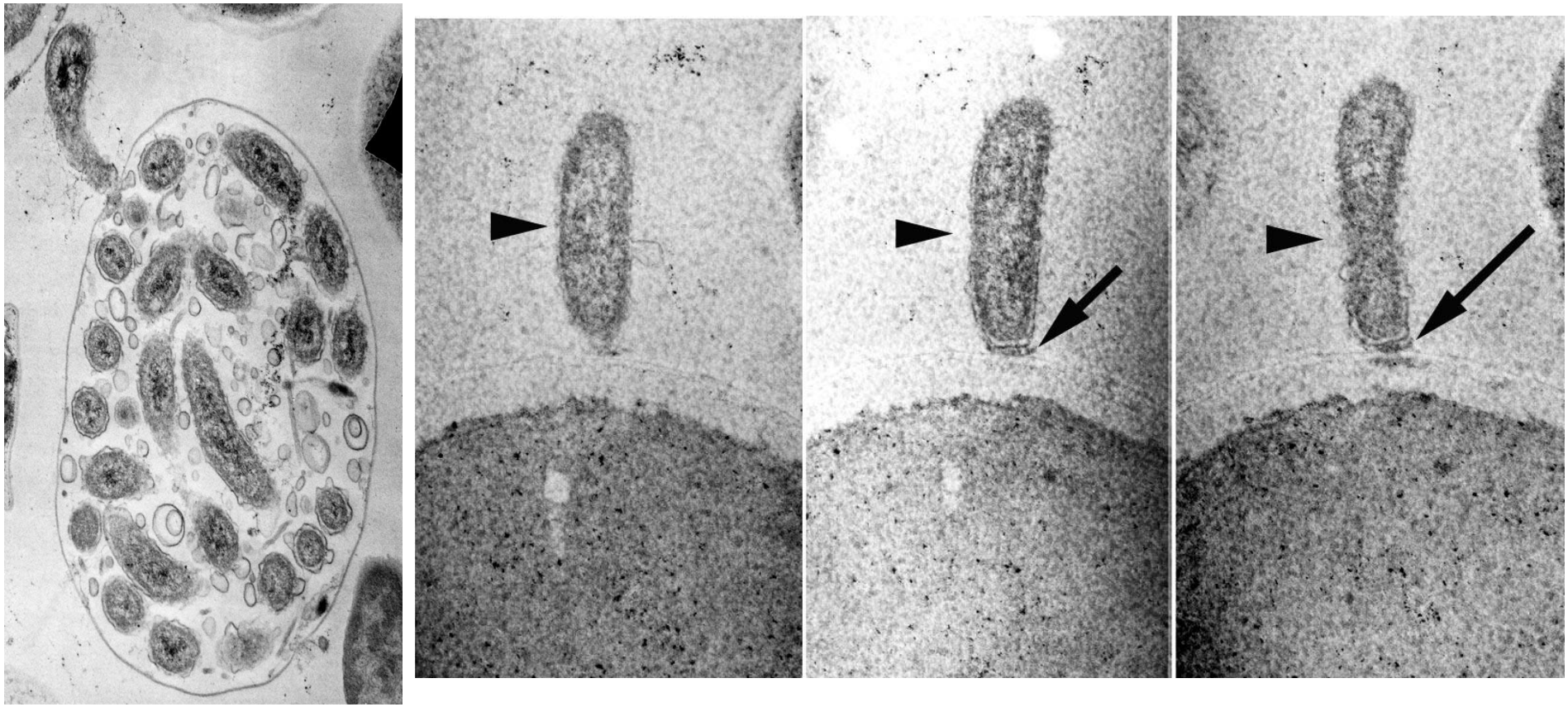




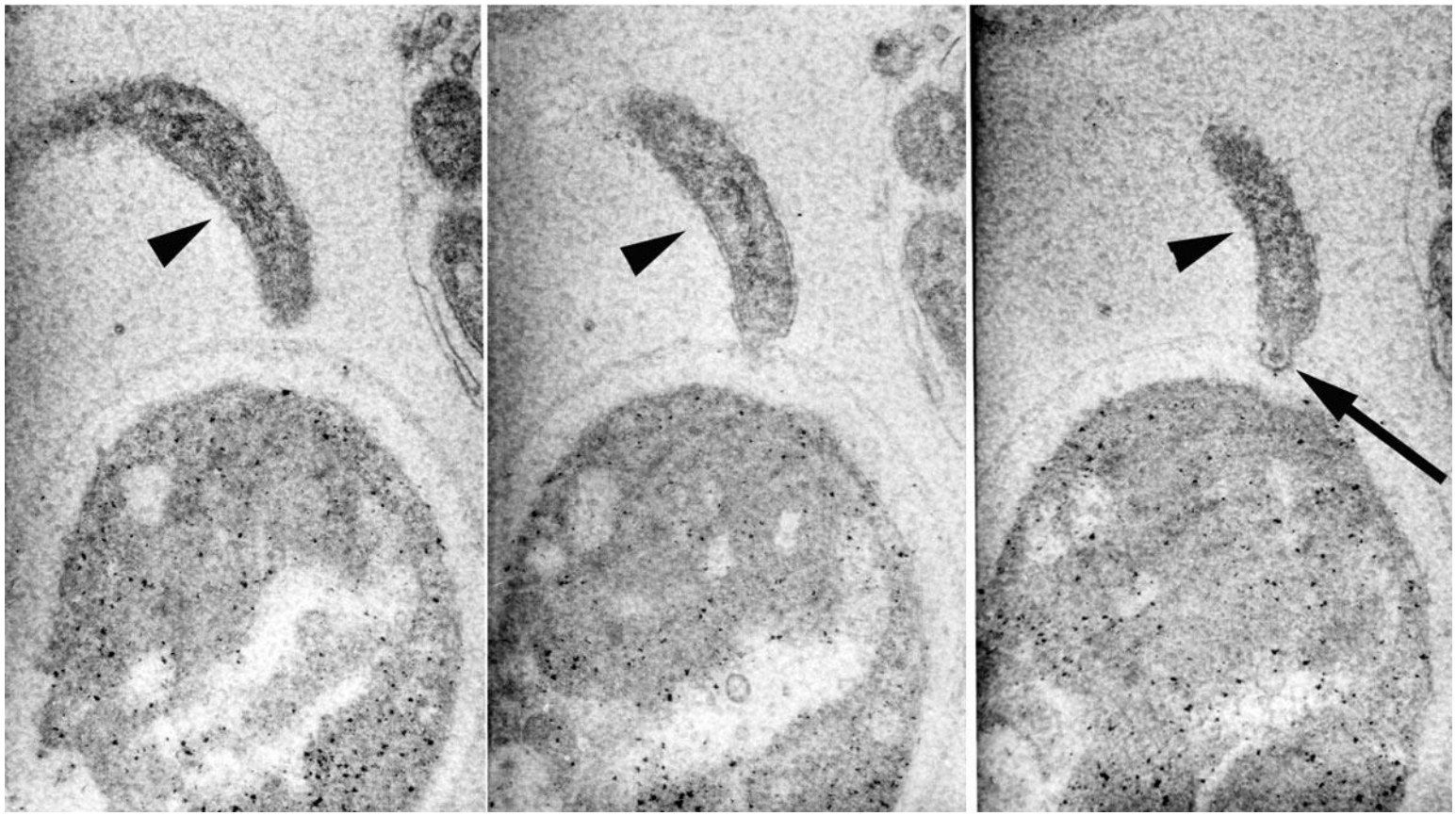
D2 AM SS1-13: initial infection a single bacterium



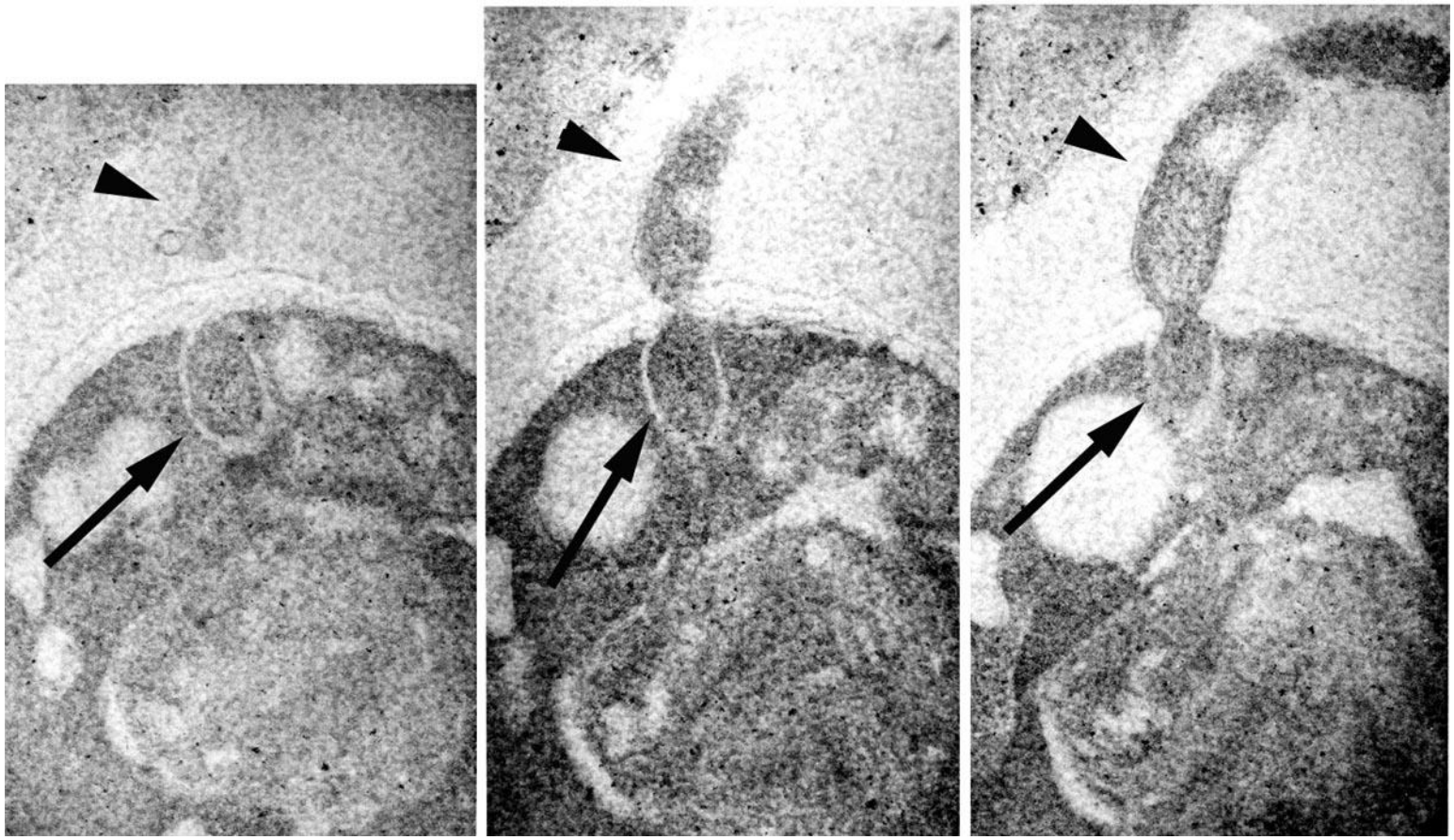
D2 AM:~99% of the cells were infected (L image); serial sections (R image):bacterium (arrowheads) appeared to be attached to the host by a structure perhaps analagous to an appressorium or holdfast (C, R, arrow)



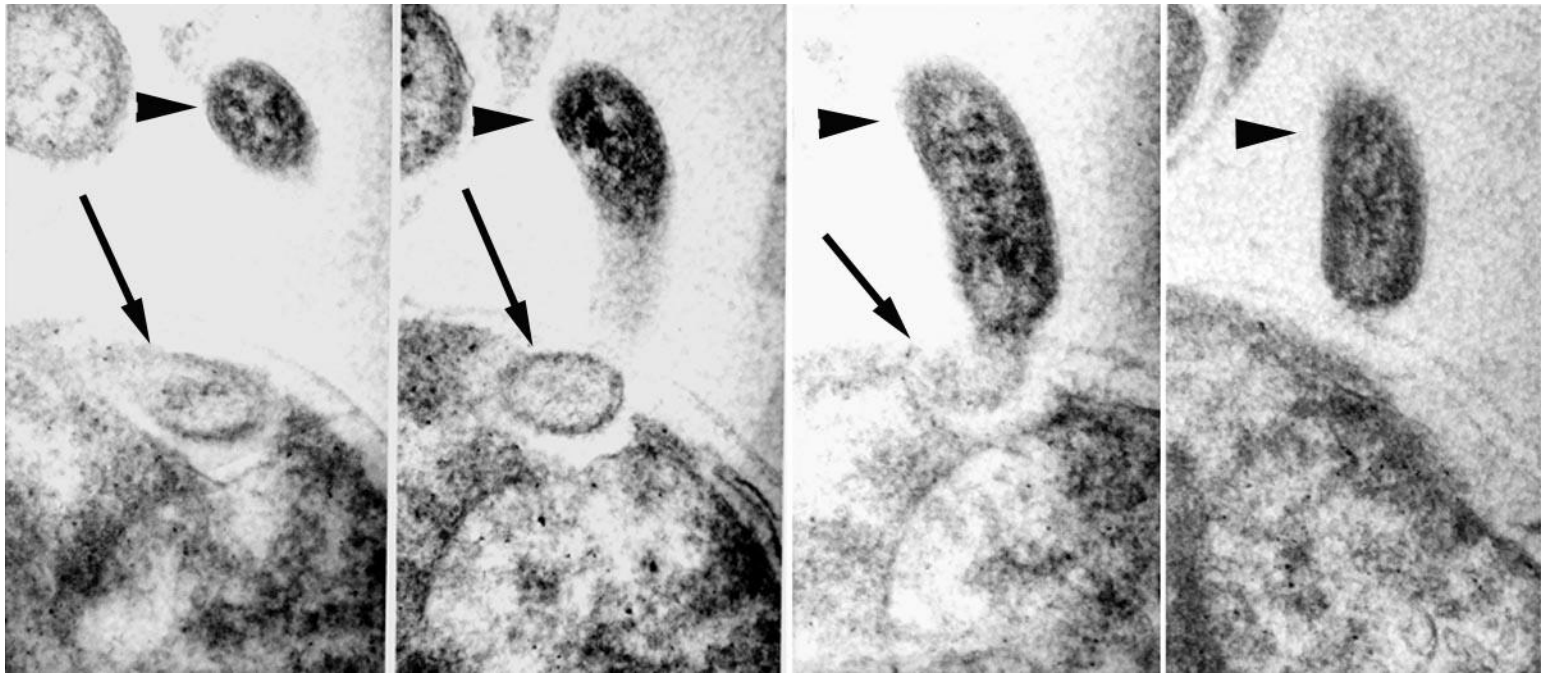
D2 AM: Serial sections: bacterium (arrowhead) appears to penetrate the cell wall (arrow) with a bulb-like structure



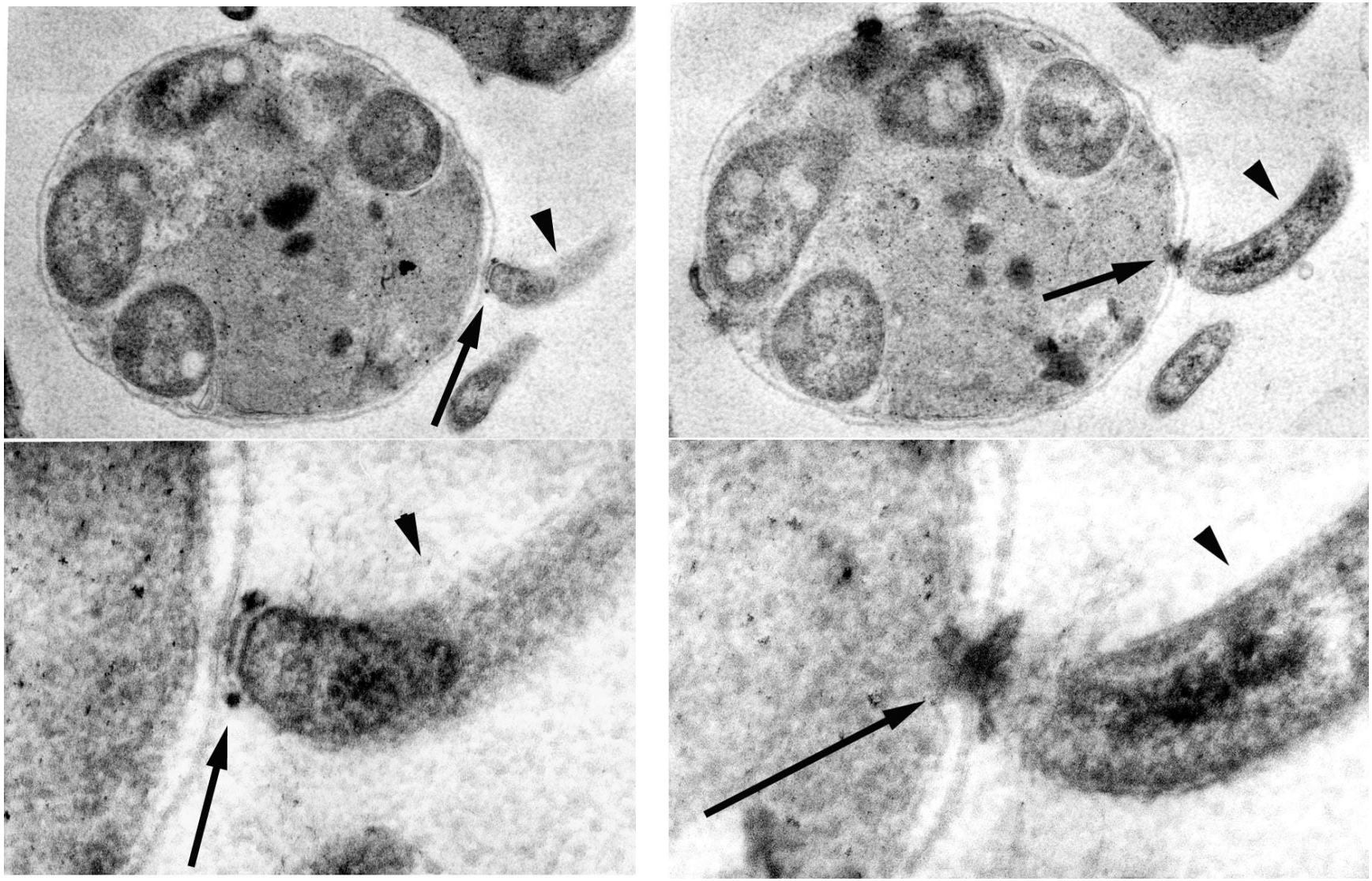
D2 AM: serial sections: bacterium (arrowhead) has penetrated into the host cytoplasm (arrow), and the bulb-like structure enlarges



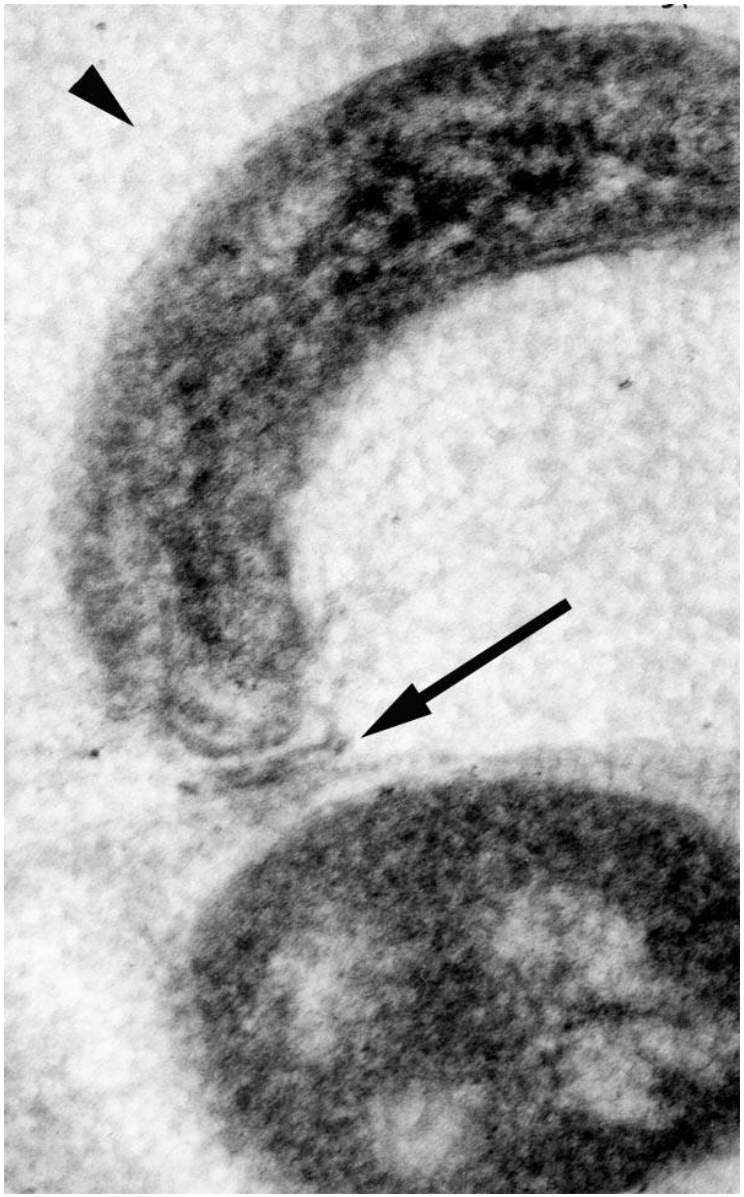
D2 PM: ~100% mortality of host; serial sections: obviously, bacterium (arrowhead) has penetrated and is inside host (arrow)



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

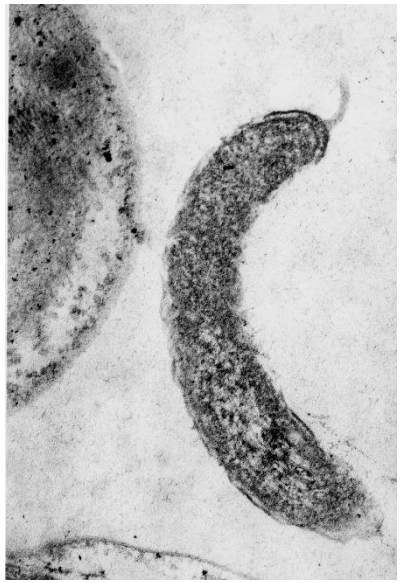


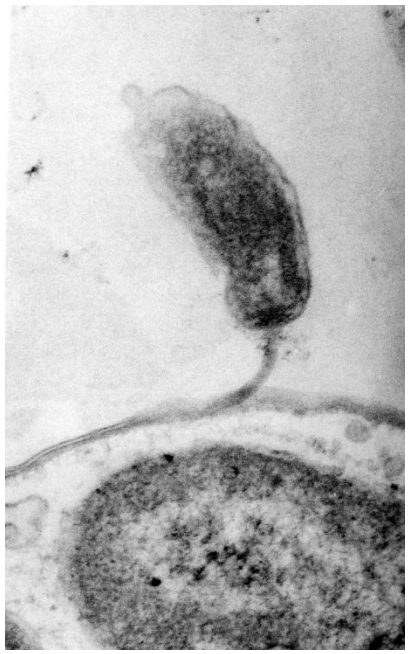
D2 PM: Bacterium (arrowhead) and attachment plate (arrow)



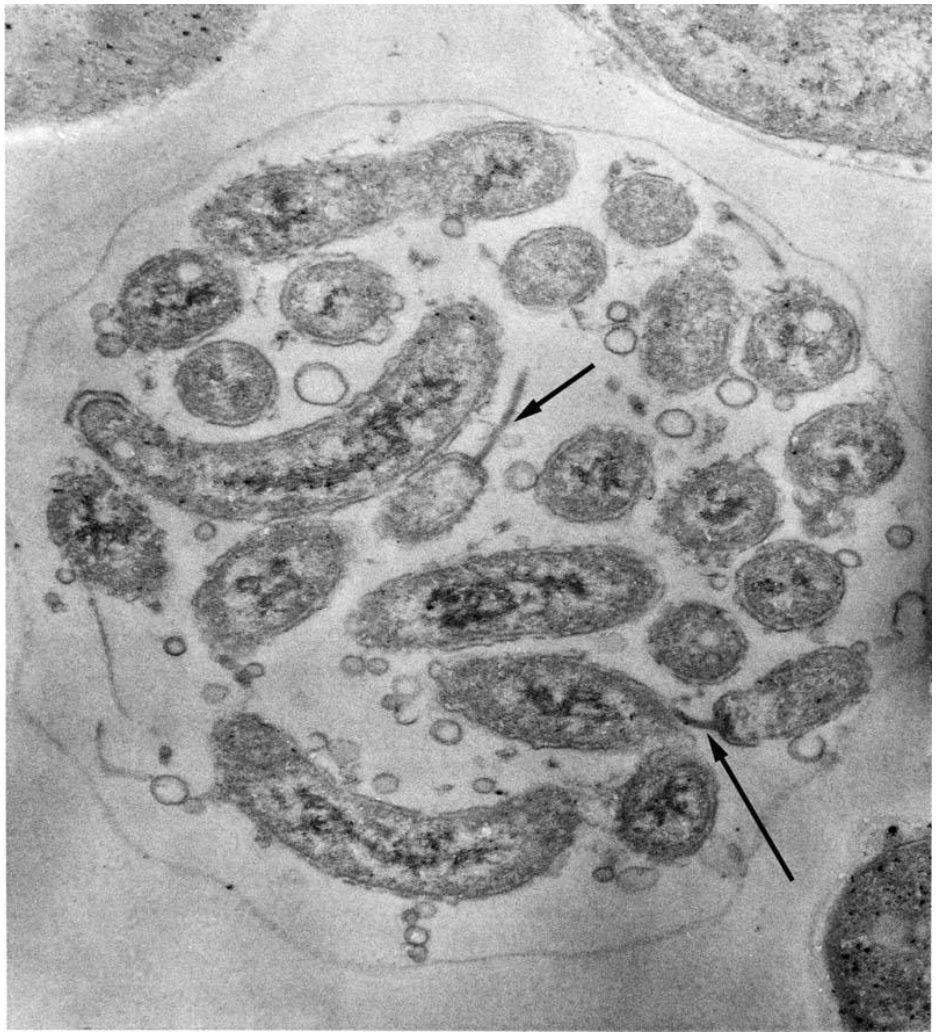
*Unanswered question:

- 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;
- 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 evidence of this is that we often see, on fully consumed host cells, “gravid” (mature) bacteria still attached to the outside of the host cell.
- We also know that the bacterium inside is, at least initially, a single entity. However, it does eventually divide INSIDE:

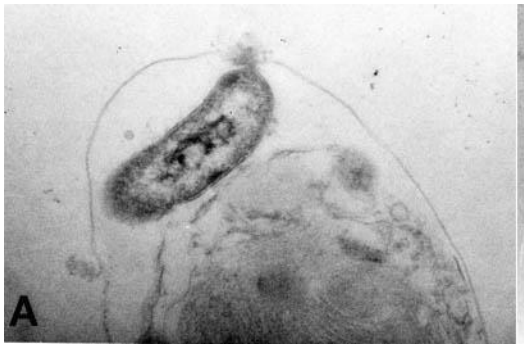


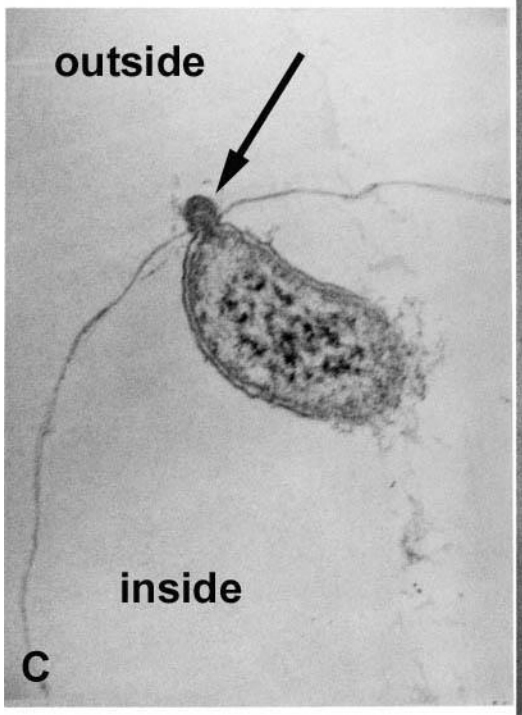


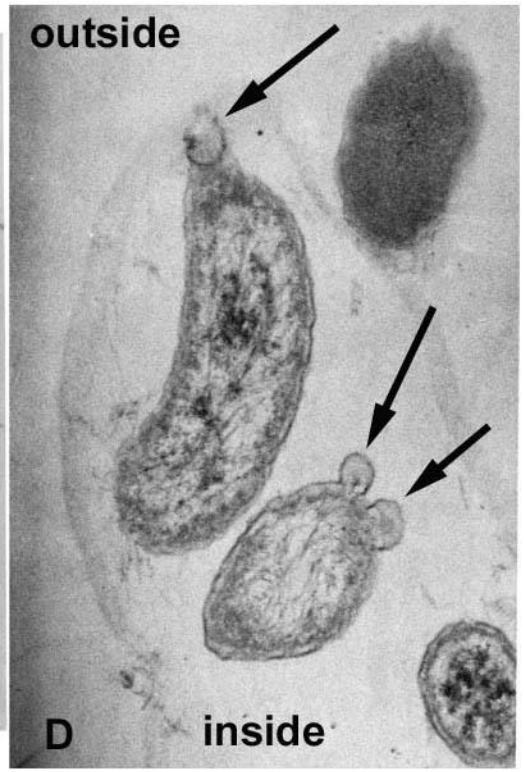
Remember the bacterium with the “tail”? Well, here’s a consumed host, and inside are 2 bacterial profiles with tails (arrows), which is evidence of cell division inside the host. So, what starts out as a single entity inside, becomes multiple entities inside.

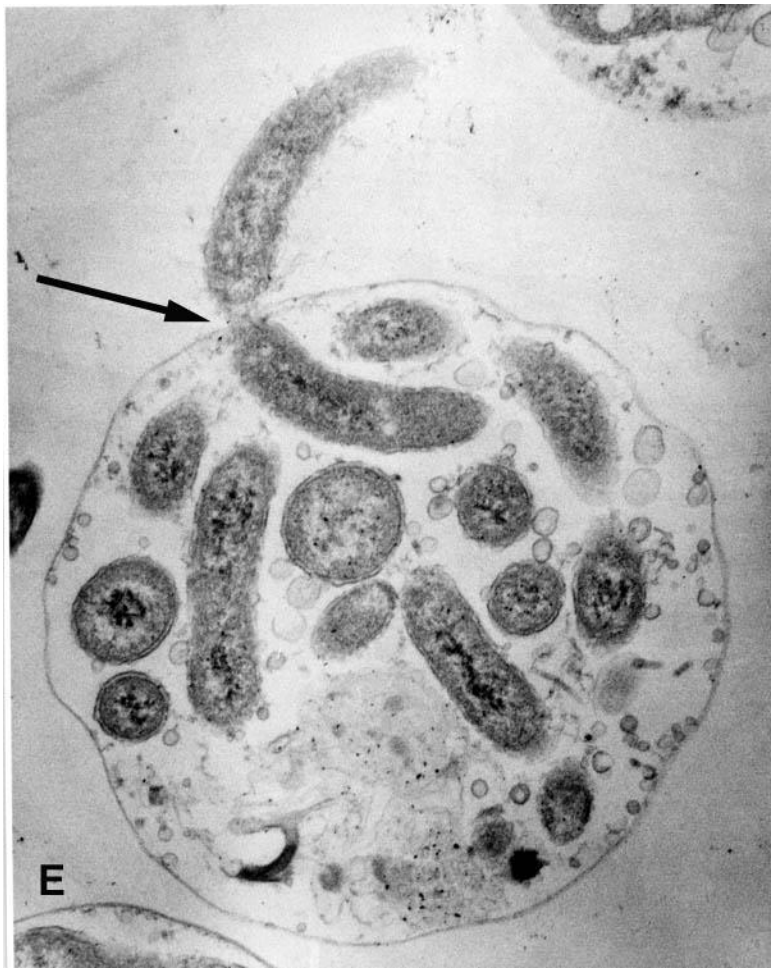


Okay. Now, how does the bacterium get out? I think it gets out the same way it gets in: inside bacterium forms a bulb at the host wall (A, C, D), divides into the bulb (B), and progeny is outside (E). These are random sections, and thus, circumstantial….

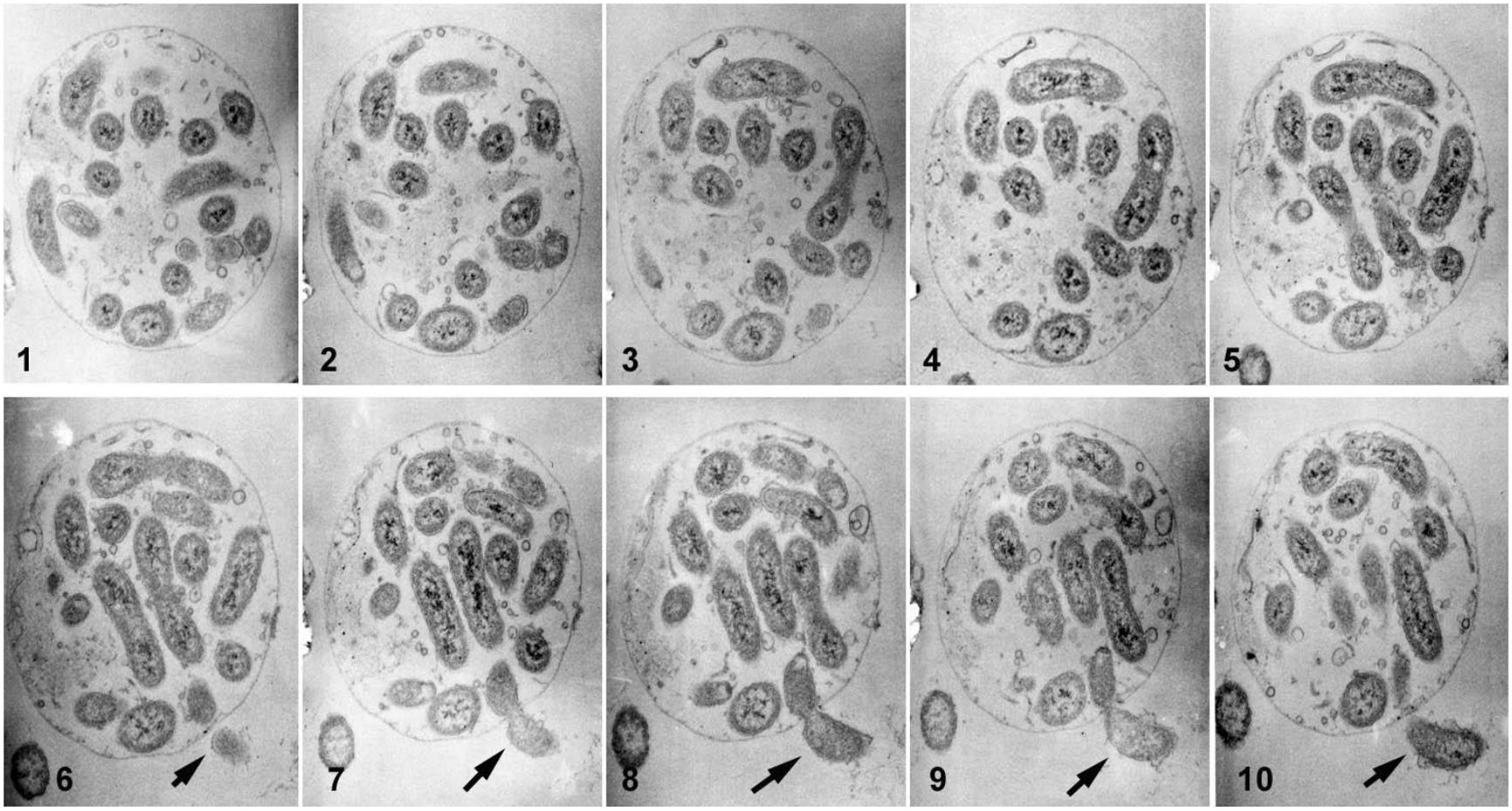




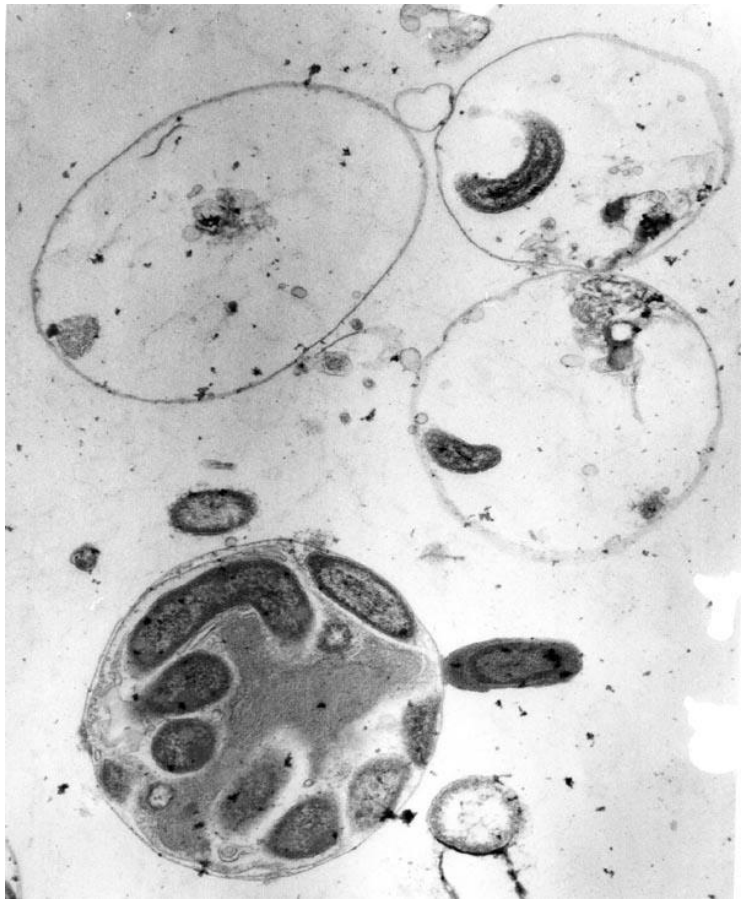




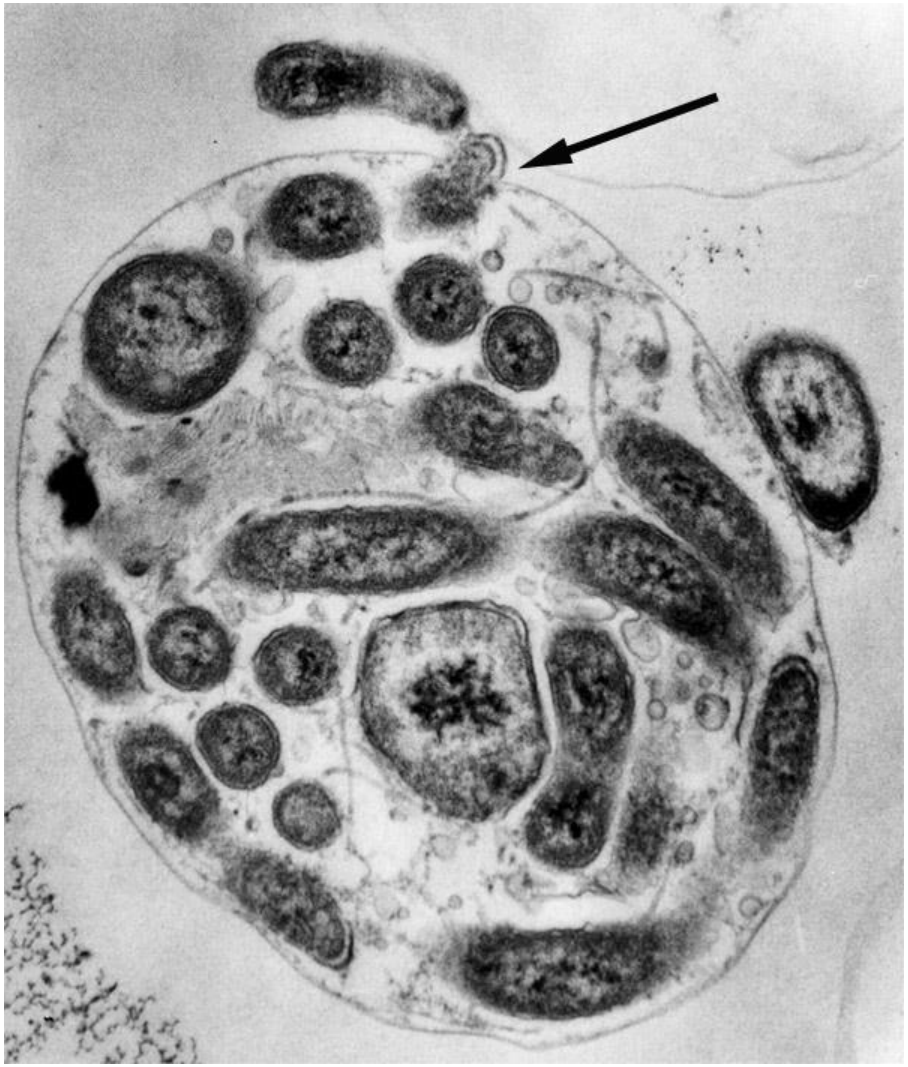
Inside to outside: these are serial sections, and thus NOT circumstantial: 6-10 (arrows) show bacterium crossing from inside to outside



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; in many cases cell wall degraded; many free bacteria in medium.



D3, most recent fixation: host completely consumed: (R)many free bacteria outside host cells;)(L) bacteria may escape by breaking through host cell wall (arrow).

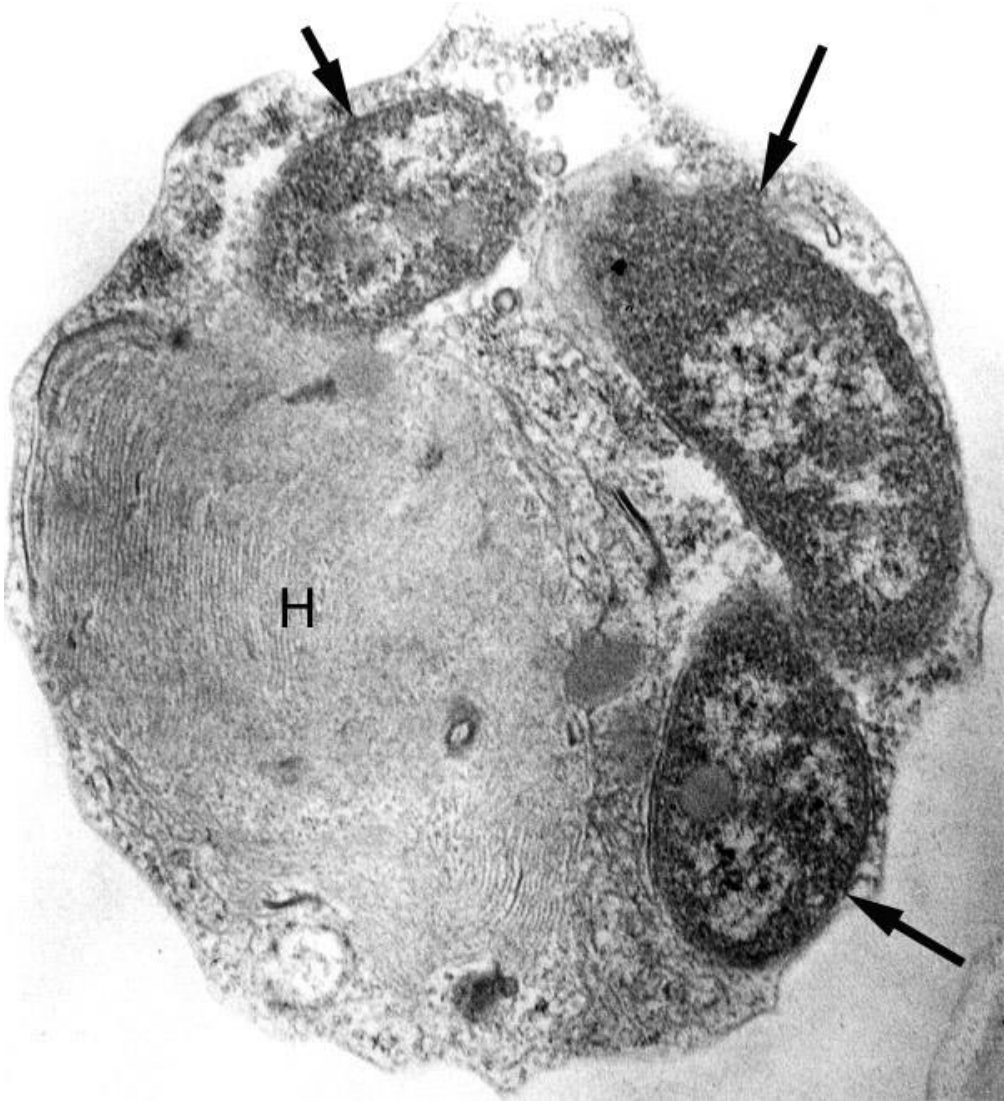


Now, let's go back to D2 AM and look closer at early infection, when the bacterium is inside, but some of the host still remains.

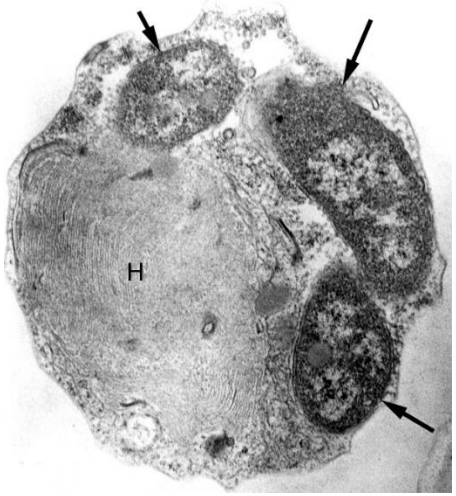
Is there any evidence of host plasma membrane, as in Bdellovibrio as illustrated in Guerrero et al. 1986, in which the bacterium is restricted to the "periplasmic space", as a periplasmic predator?

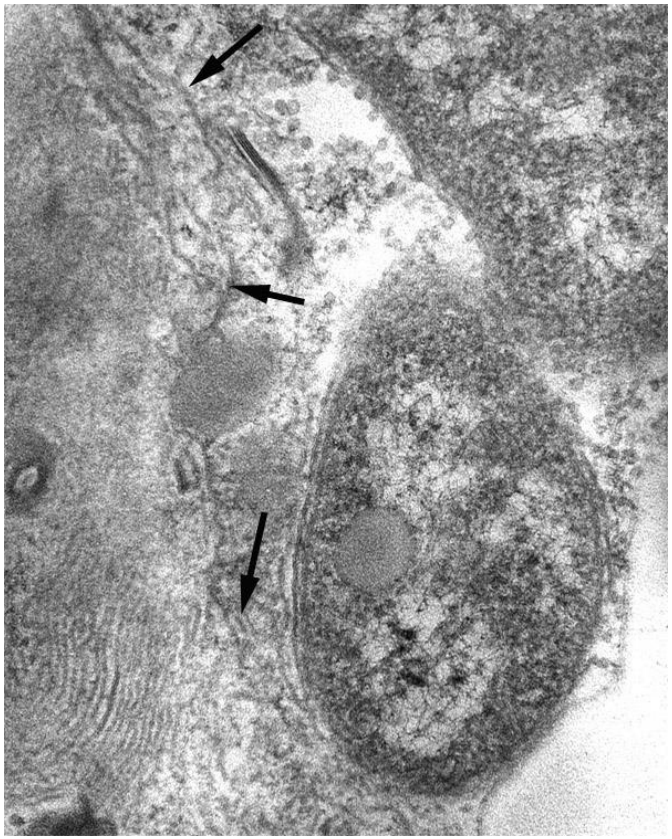
See slides 15/16, 17, 18, 19, 20.

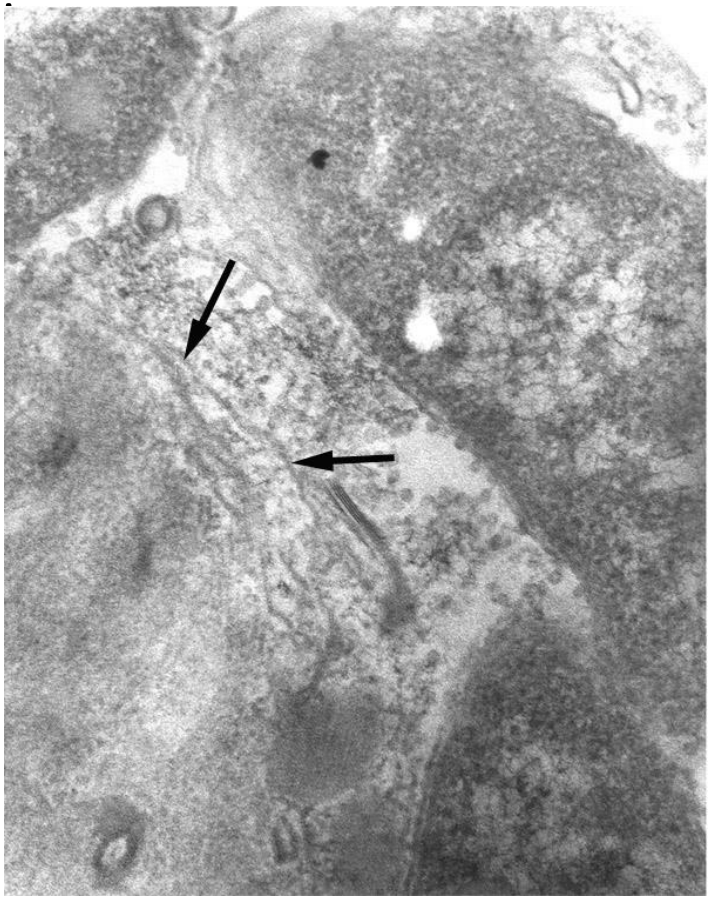
D2 AM: early infection, when part of the host (H) is still intact, but bacterium is inside (arrows)



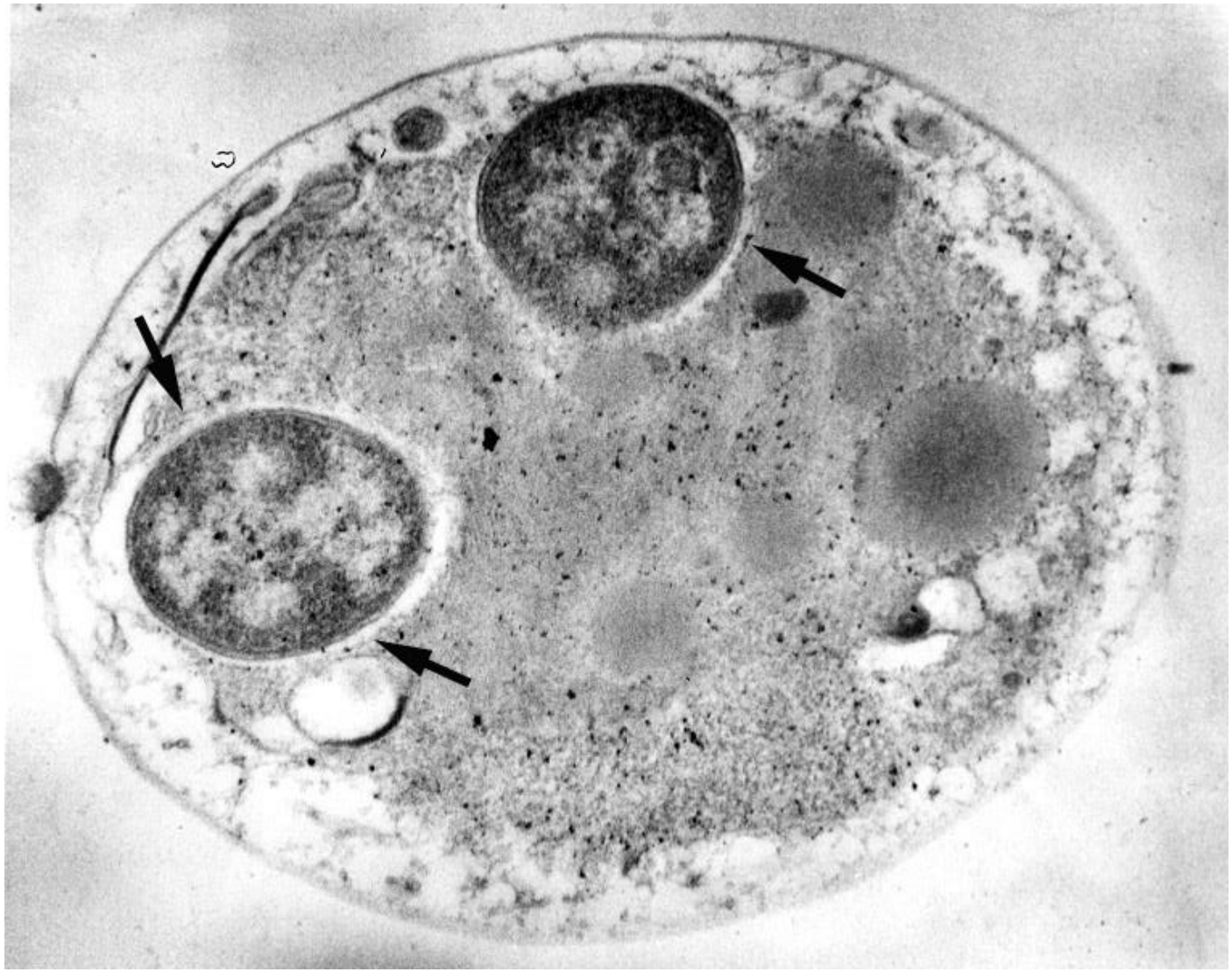
D2 AM: early infection, when part of the host (H) is still intact, but bacterium is inside (arrows). In enlargements, arrows indicate possible host plasma membrane (although it does not look very good).



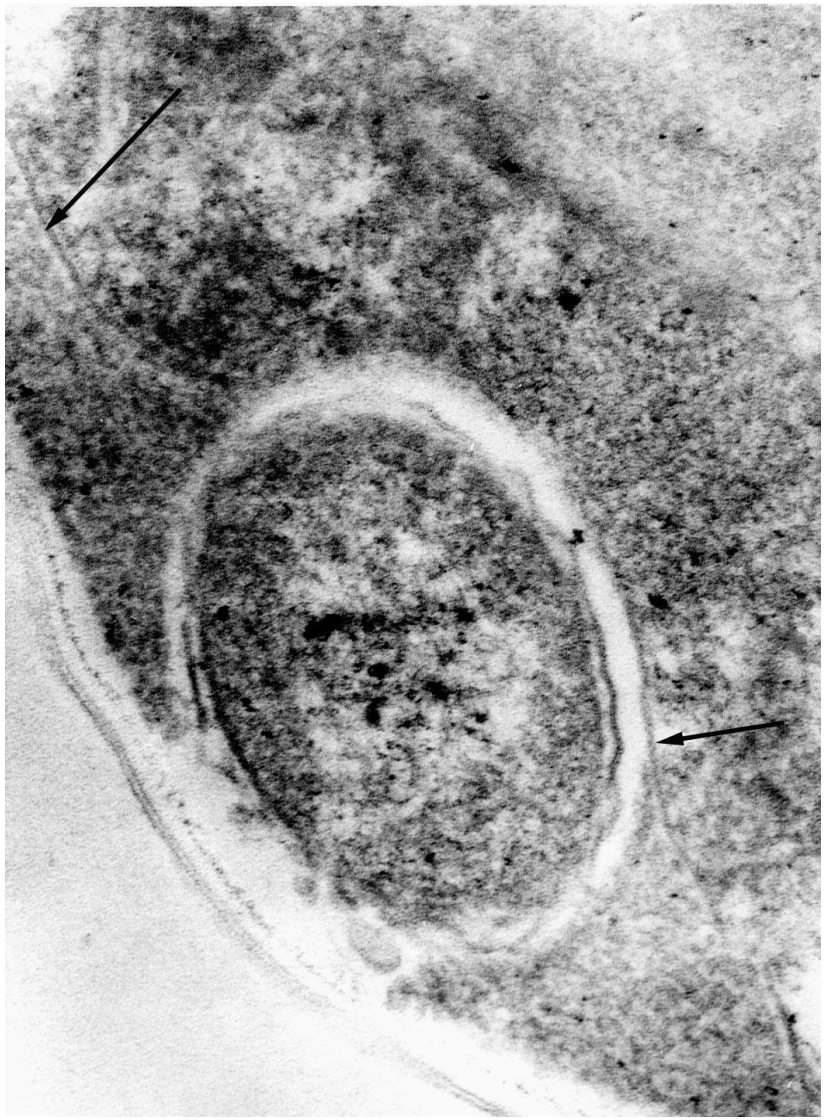




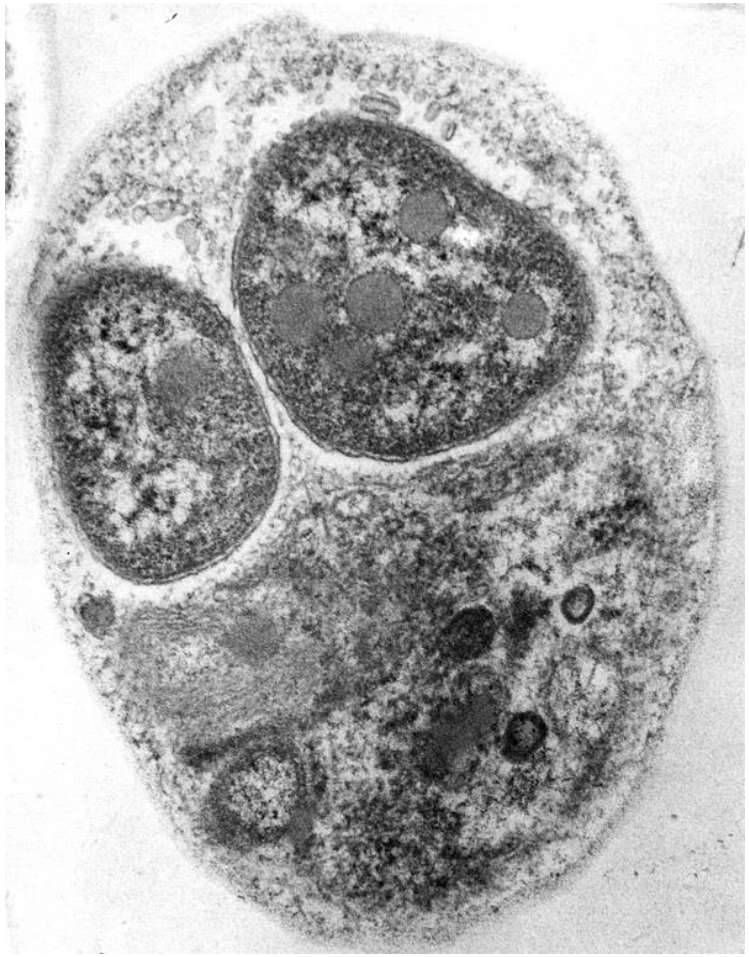
D2 AM: early infection, when part of the host (H) is still intact, but bacterium is inside (arrows). Arrows indicate possible host plasma membrane.



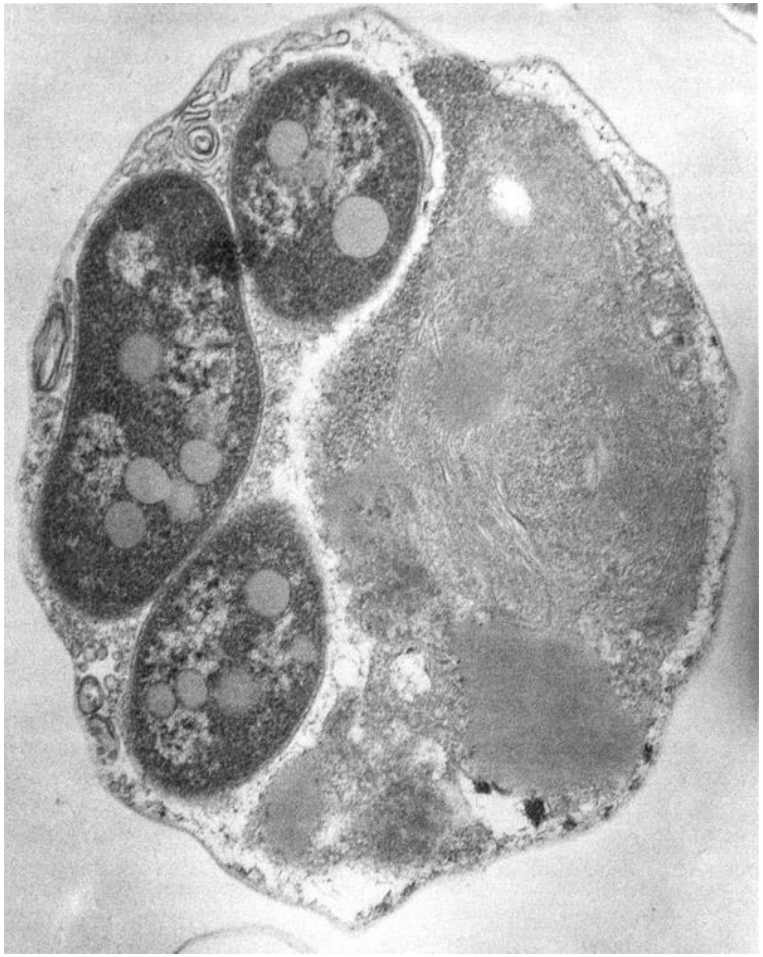
D2 AM: early infection, when part of the host is still intact, but bacterium is inside. In enlargement, arrows indicate possible host plasma membrane.

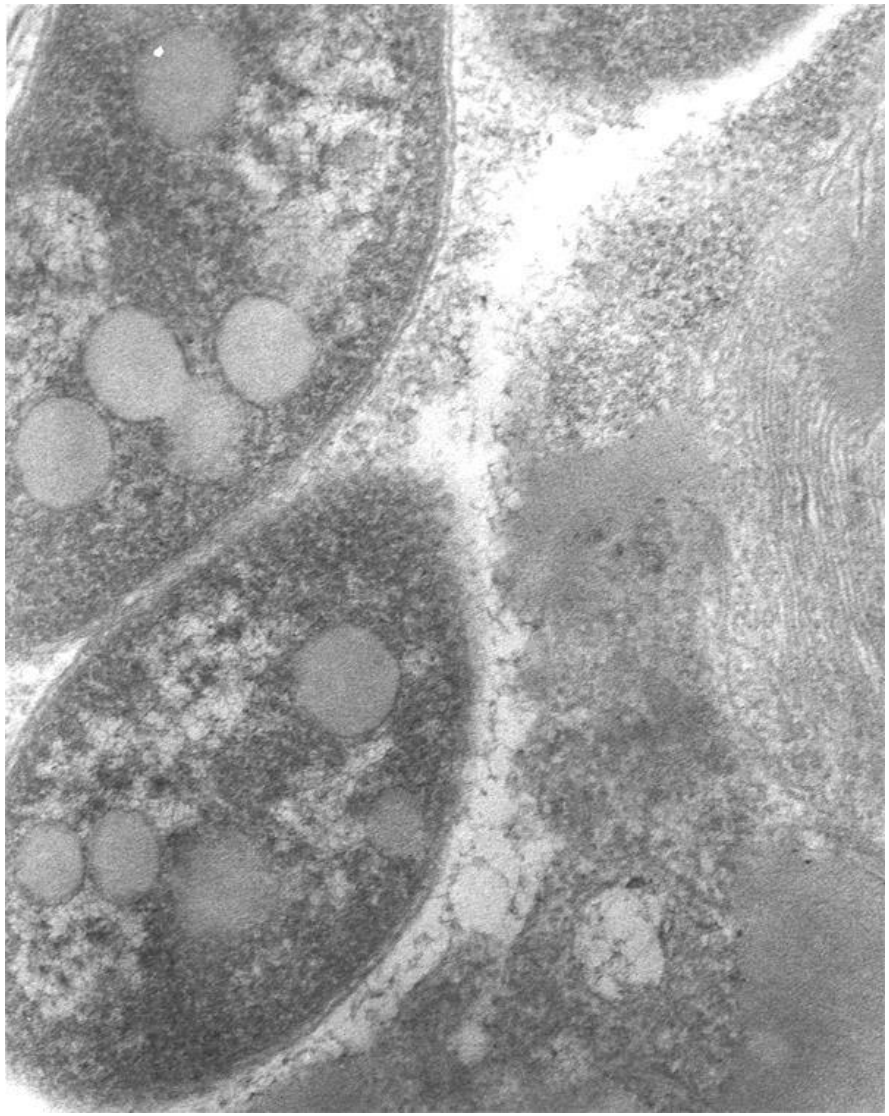


D2 AM: early infection, when part of the host is still intact, but bacterium is inside. In enlargement, no indication of remaining host plasma membrane.



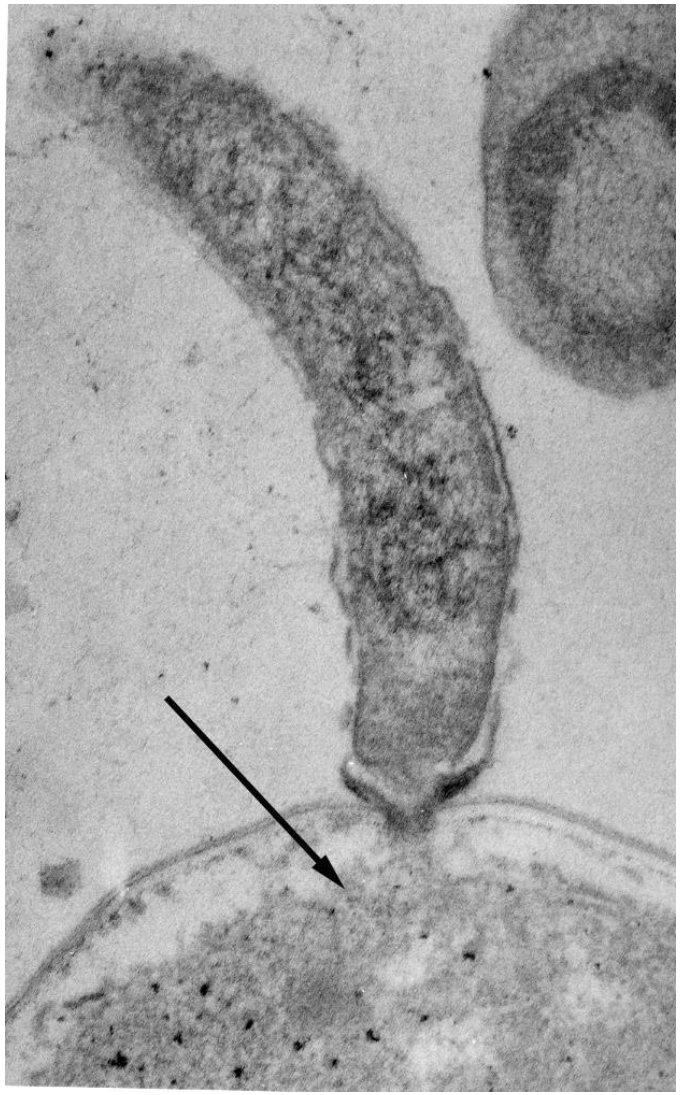
D2 AM: early infection, when part of the host is still intact, but bacterium is inside. In enlargement, no indication of remaining host plasma membrane.



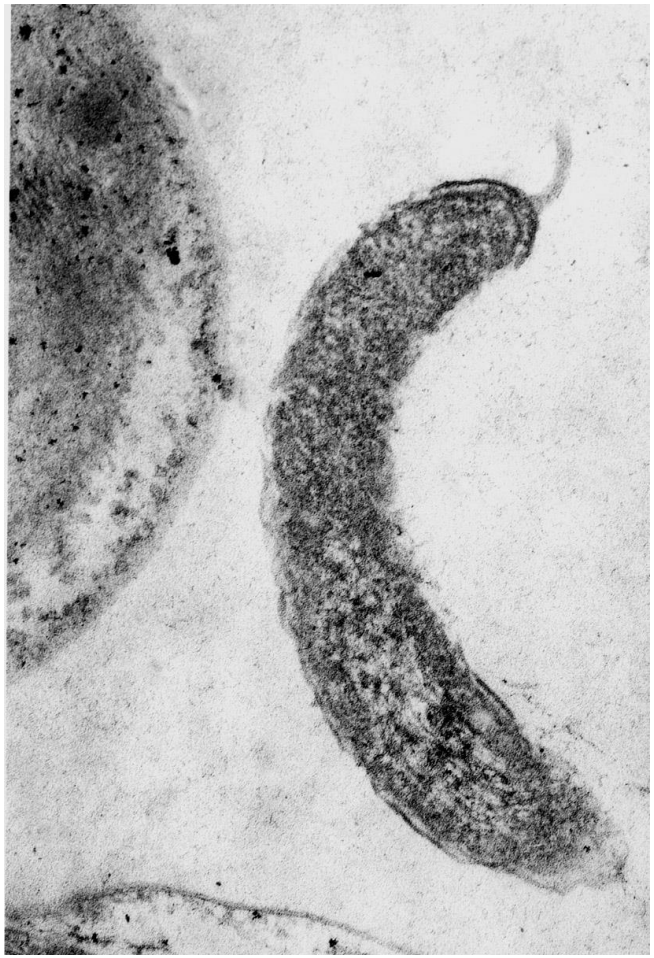


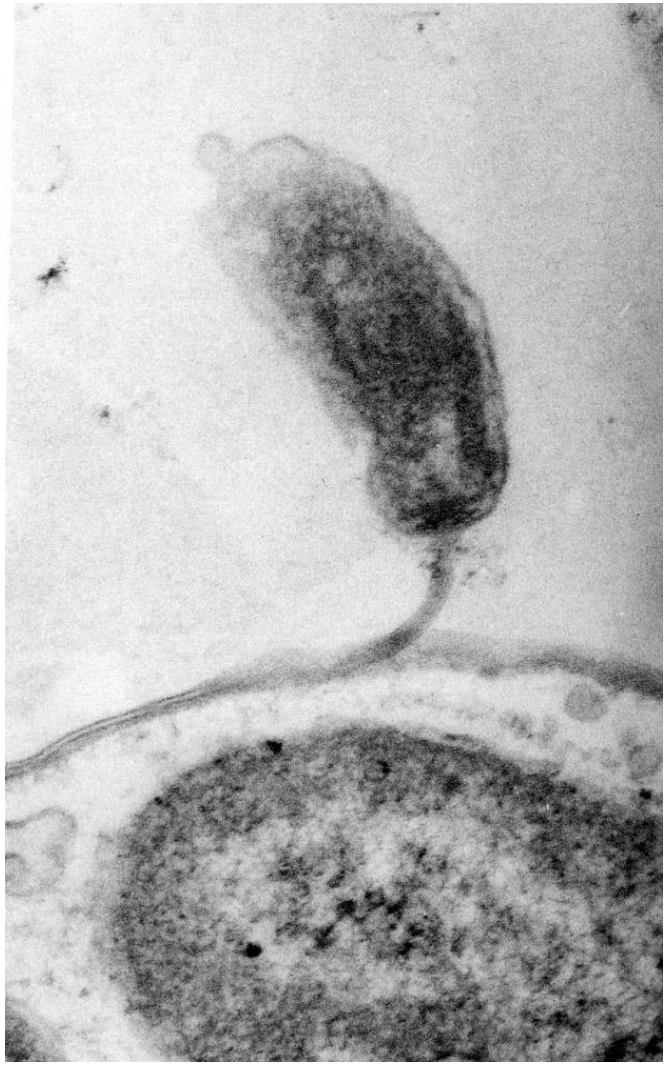
My gut feeling is that the host pm is there early on (slides 15-18), but the bacterium degrades it as it consumes the host (slides 19, 20). I think it just breaks everything down and eats it as it grows and consumes the host, and thus is not restricted to the periplasmic space. Rather, it is a “cytoplasmatic” predator (see attached pdf, “The quest for Daptobacter”). The “clear” area around the bacterium (sort of an interface between the bacterium and host) may be a “lytic zone” (page 3 in the pdf) as degradation proceeds.

Additional stuff: it looks like very early in infection, a bulbous area (arrows) appears adjacent to the holdfast, which may be from lytic enzymes released by the bacterium following penetration of the host cell wall.



Additional stuff: This bacterium seems to have an appendage associated with the holdfast, and the appendage is seen contacting the host cell wall. I had thought until I saw this that it was a different bacterium, but I'm not so sure of that now.





Other bacterium? Here, the appendage seems to be at the opposite end of the bacterium from the holdfast……

Double trouble: this bacterium looks to be attacking 2 cells simultaneously….or not; can’t determine the attack on the right from this section. For both us and the bacterium, “food for thought”.