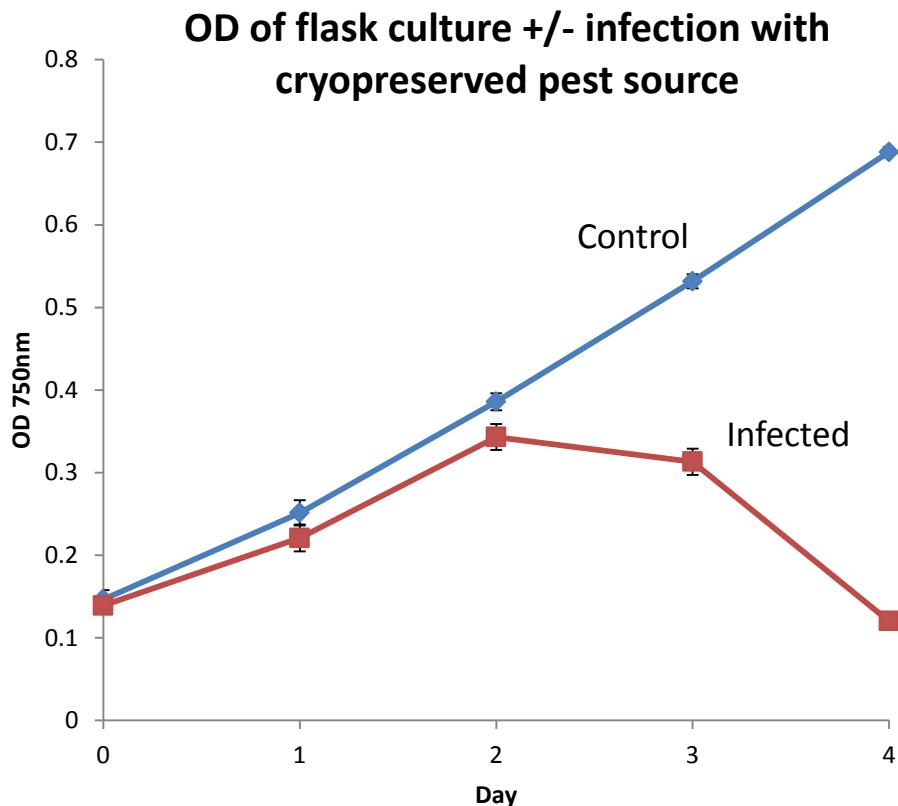


Pest Update

9/17/15

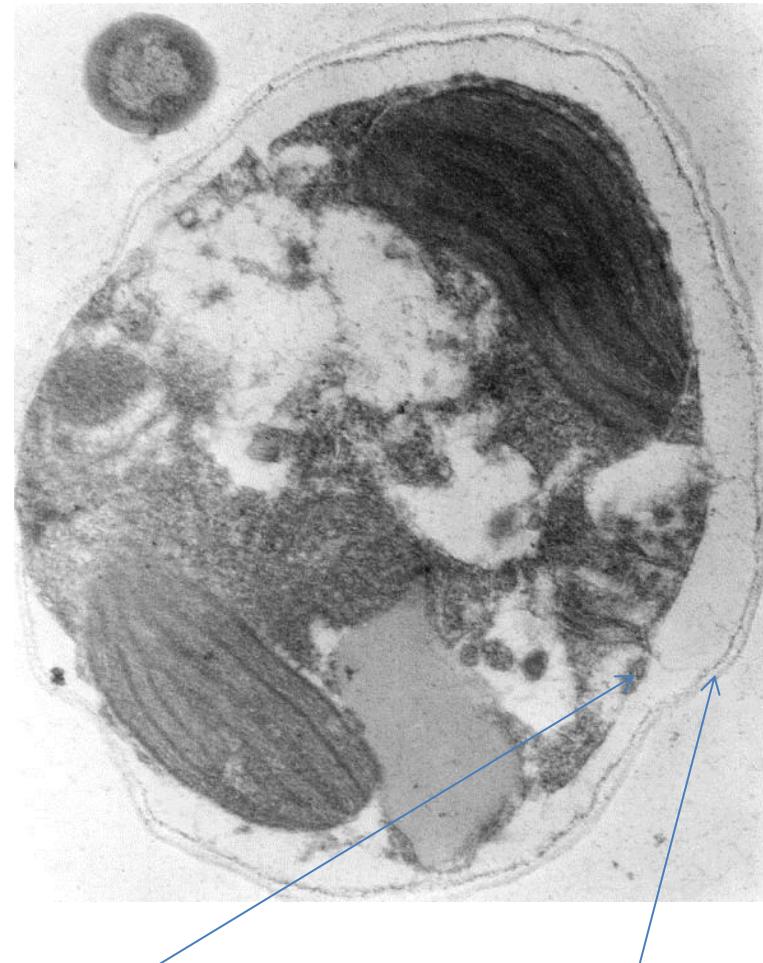
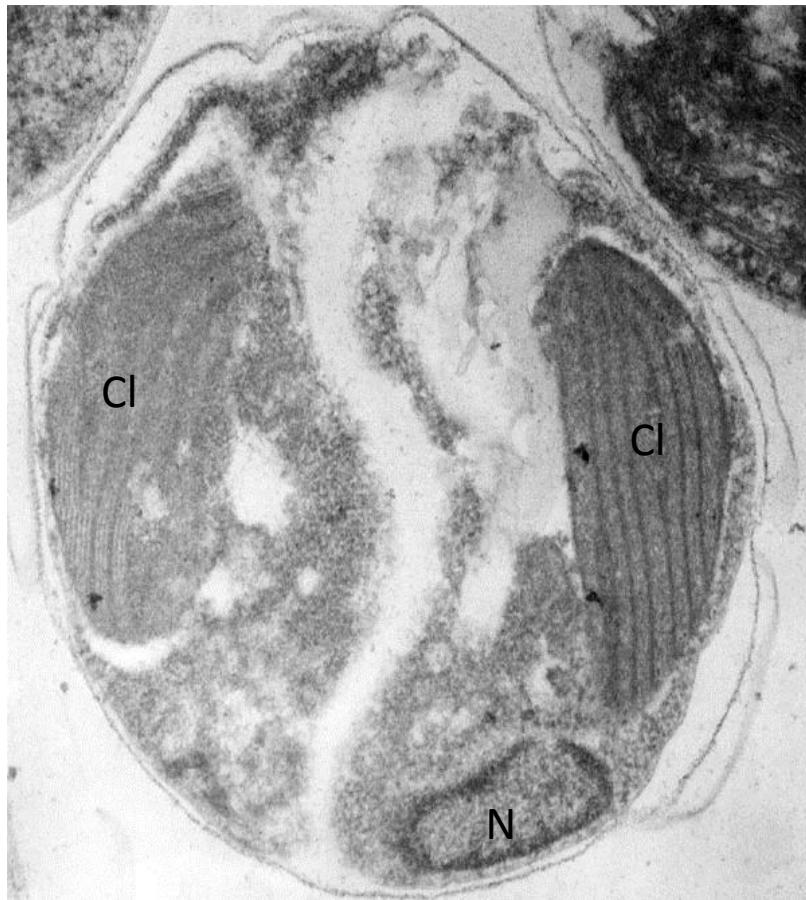
Infection in lab from cryopreserved stocks

- Lab culture was infected at 2% v/v with cryopreserved crash source
- Crash phenotype was replicated
- Samples fixed for EM day 1, 3,4 and sent to Pete Letcher



Error bars represent standard deviation of three analytical replicate samples from one flask

Day 1: healthy algal cells: notice chloroplasts and nucleus

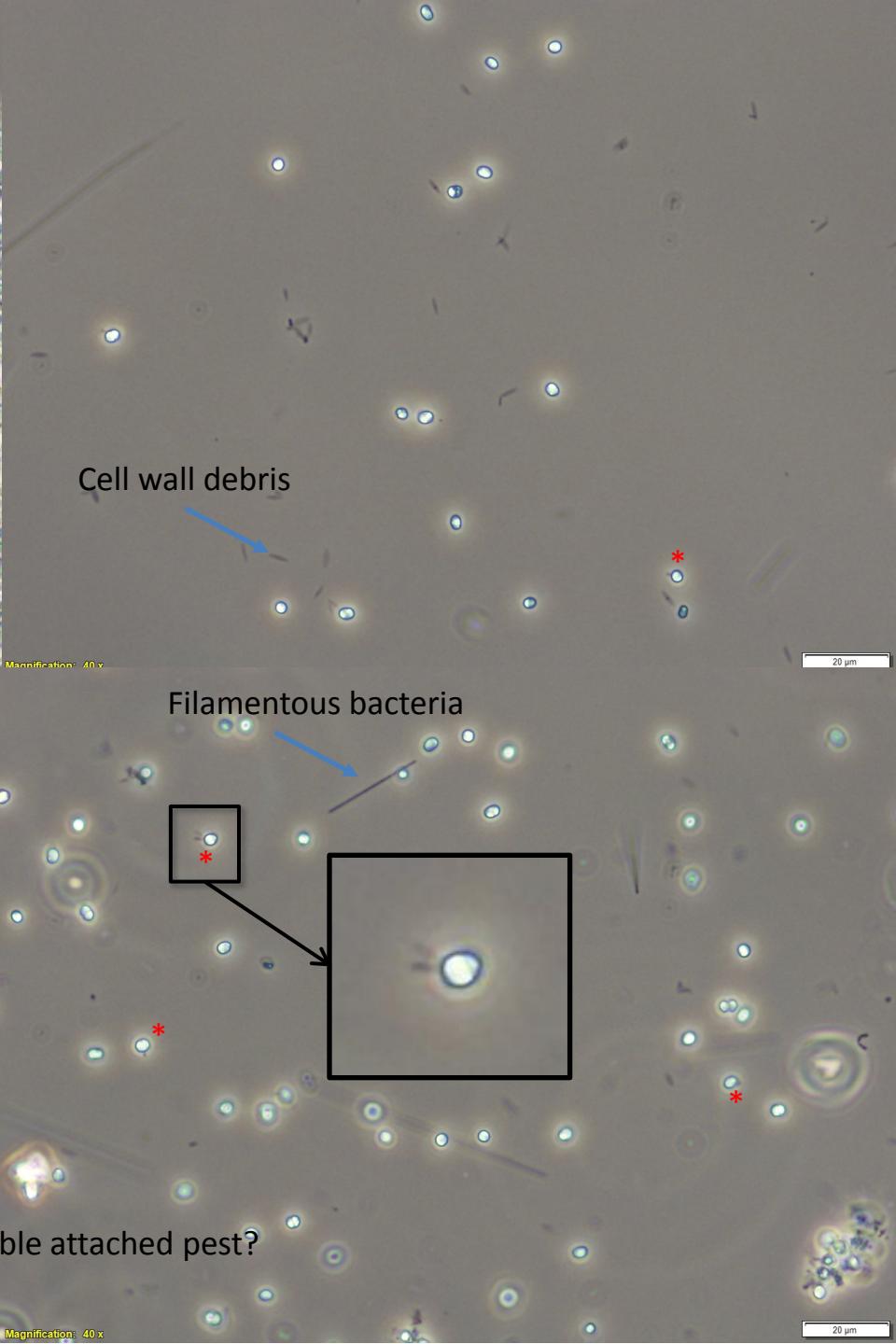
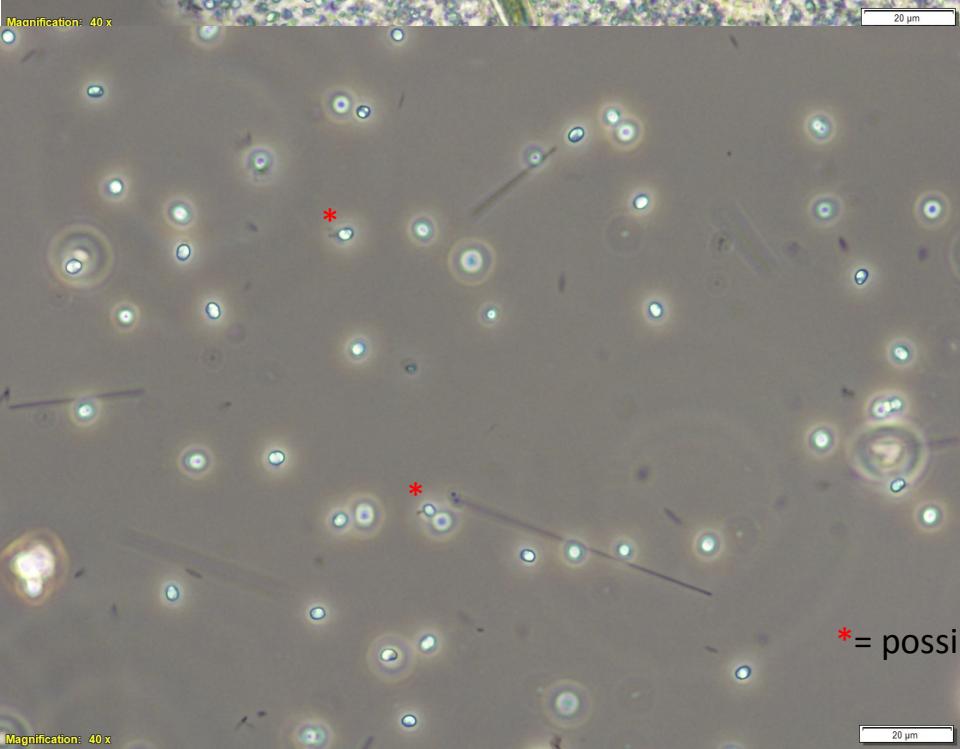
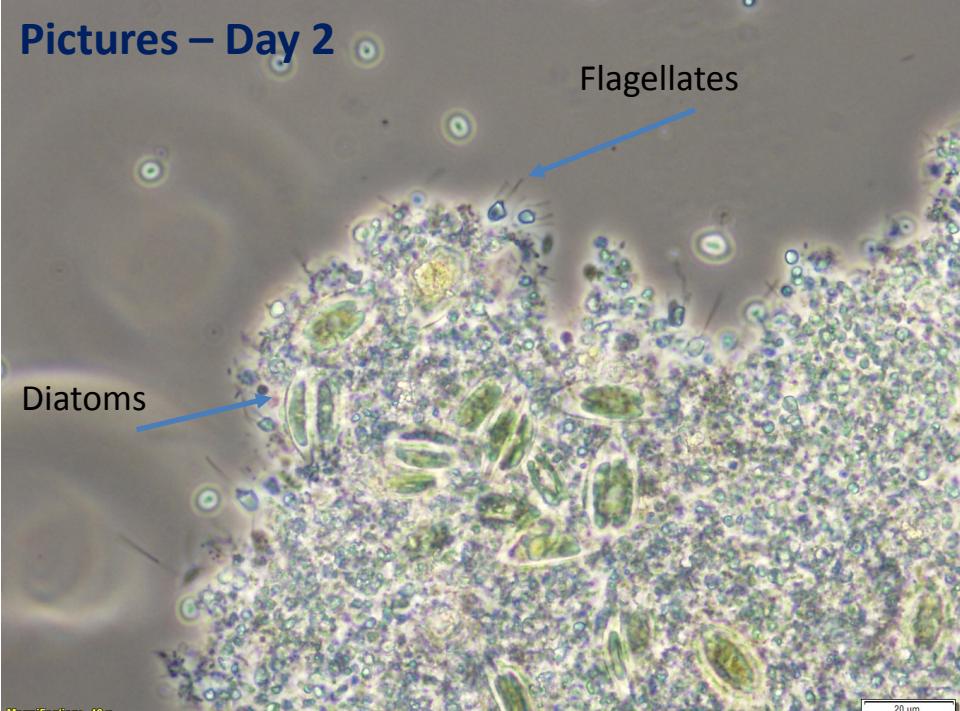


Cytoplasmic membrane

Cell wall

→ Pictures look similar to TEM pictures of *Nannochloropsis* from literature

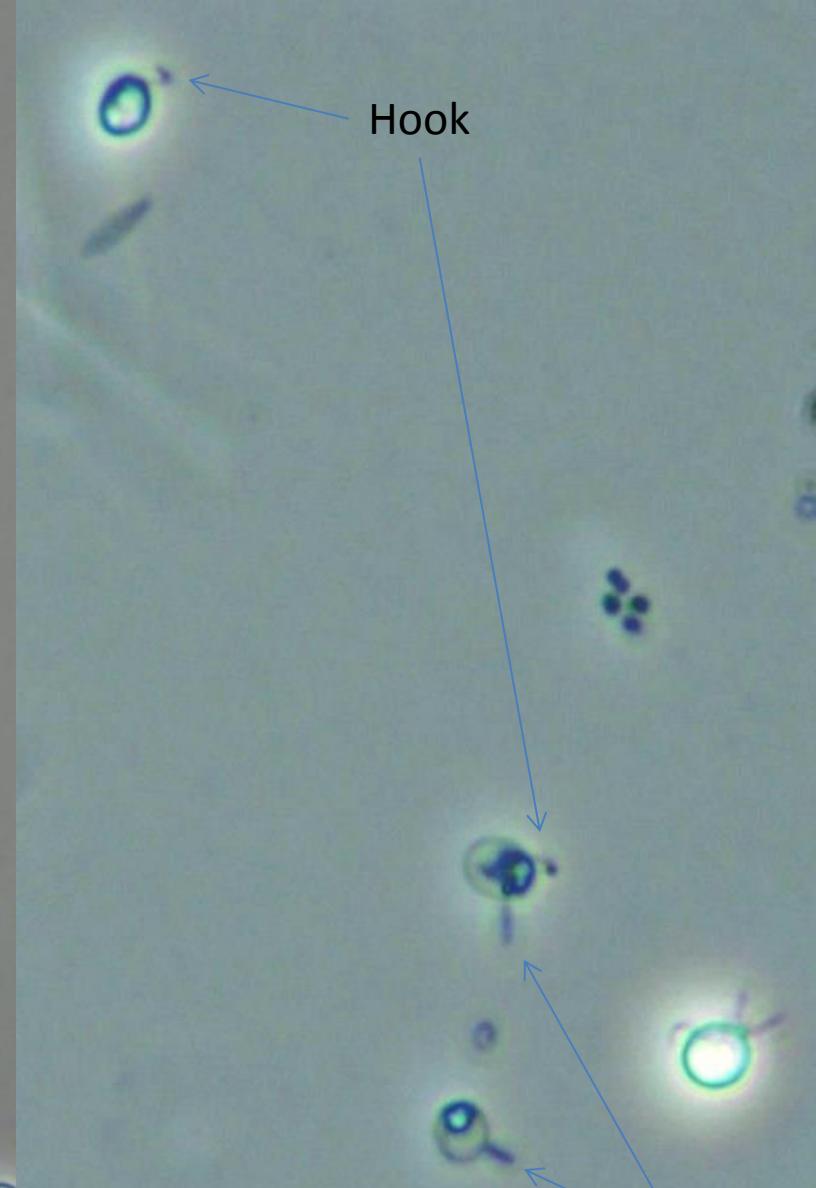
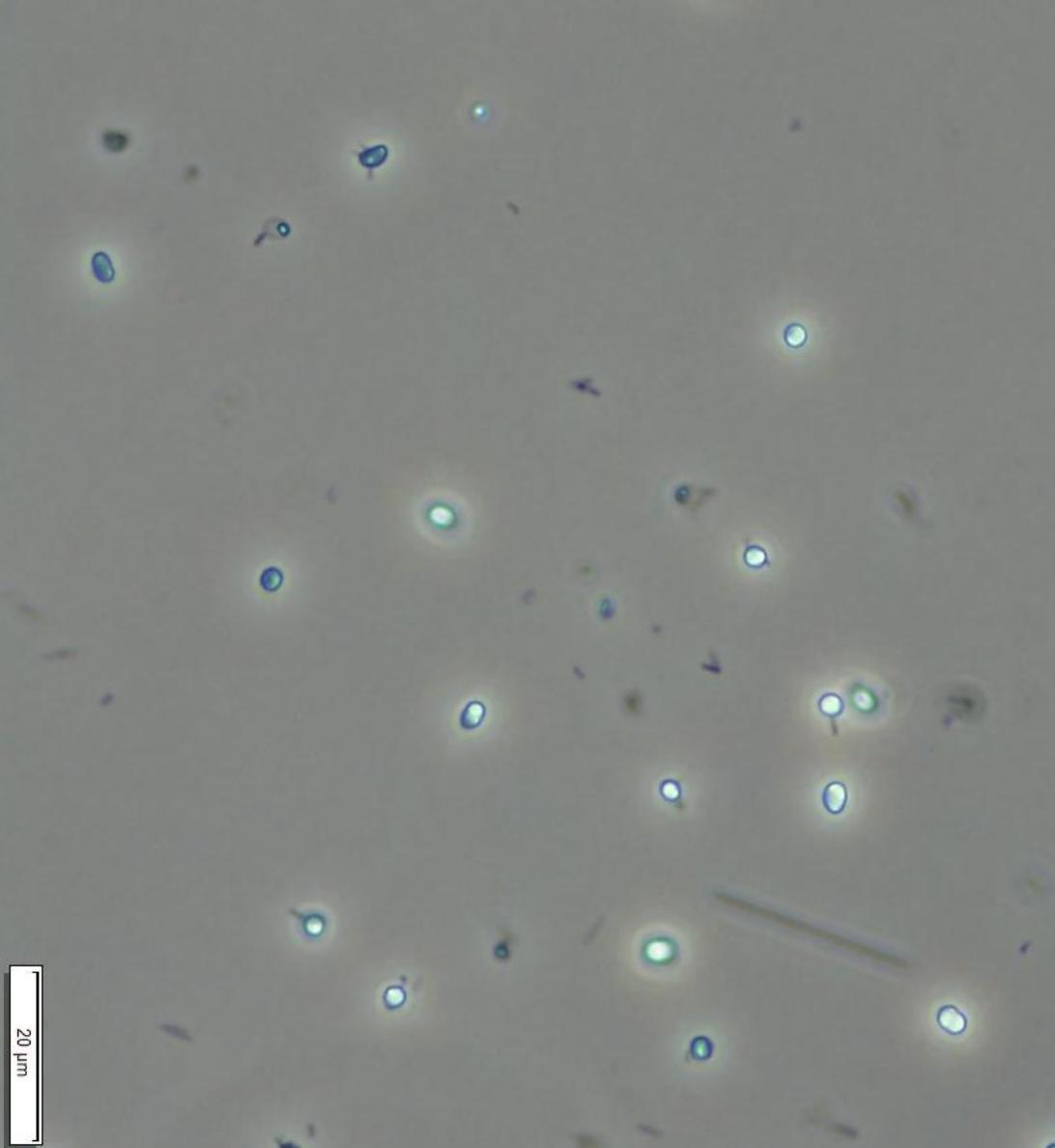
Pictures – Day 2



* = possible attached pest?

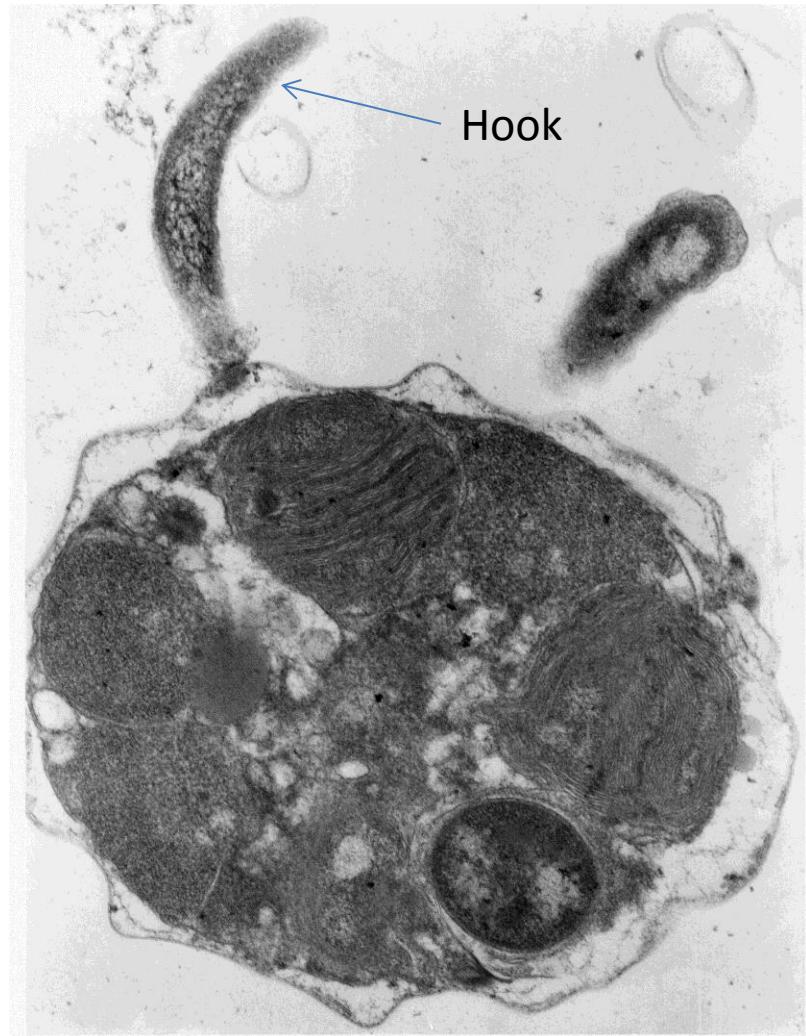
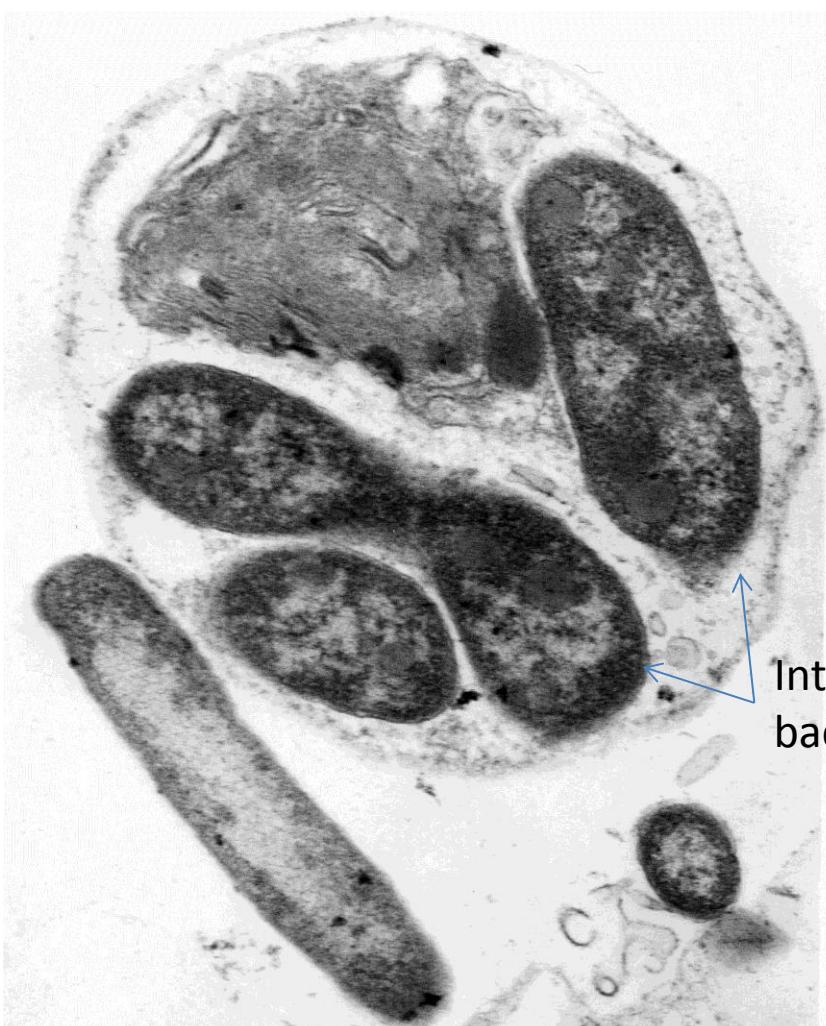
Pictures – Day 3 Infected flask

20 μ m



- Numerous cells with attachments, hooked and straight.
- Some cells displaying hollowed death phenotype

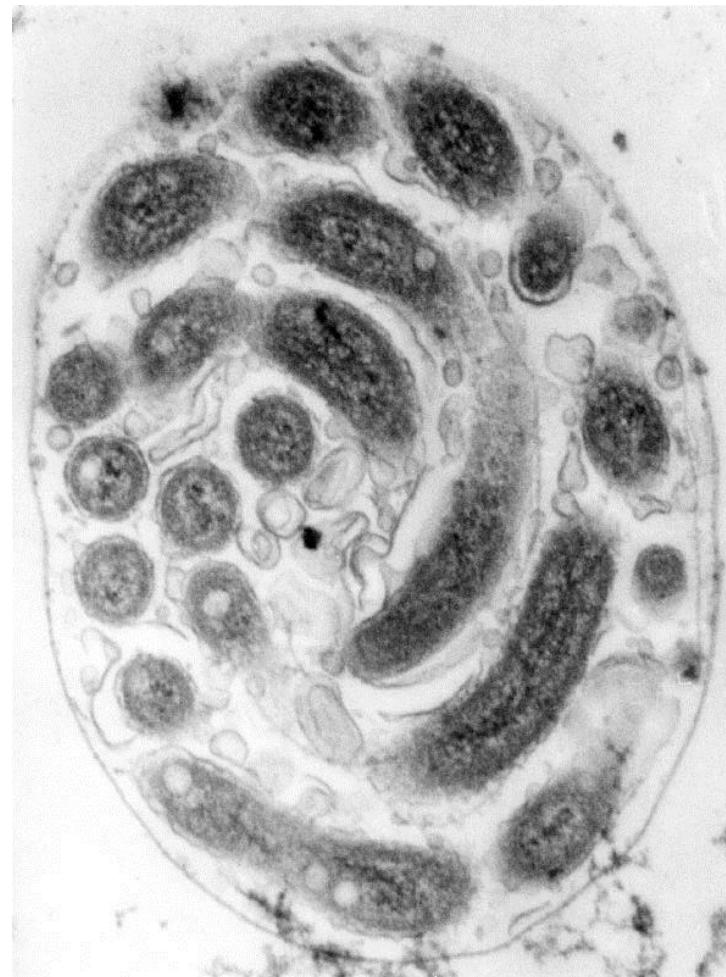
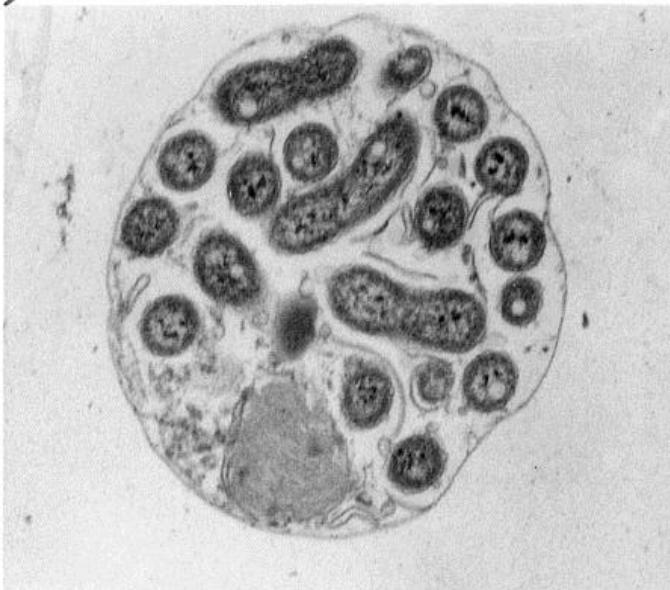
Day 3: infected algal cells: bacteria inside, and rod-shaped bacteria outside, also attached hooked bacteria



Rod

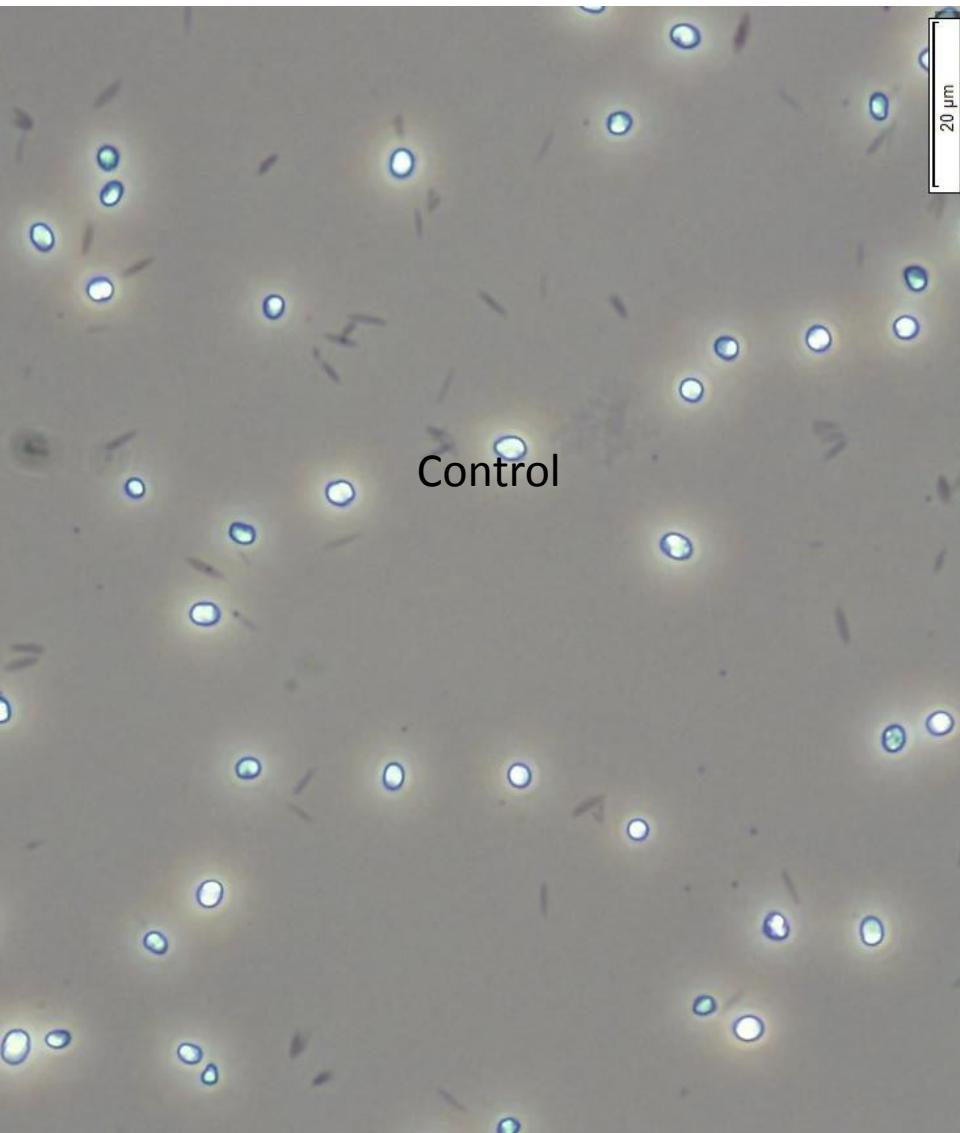
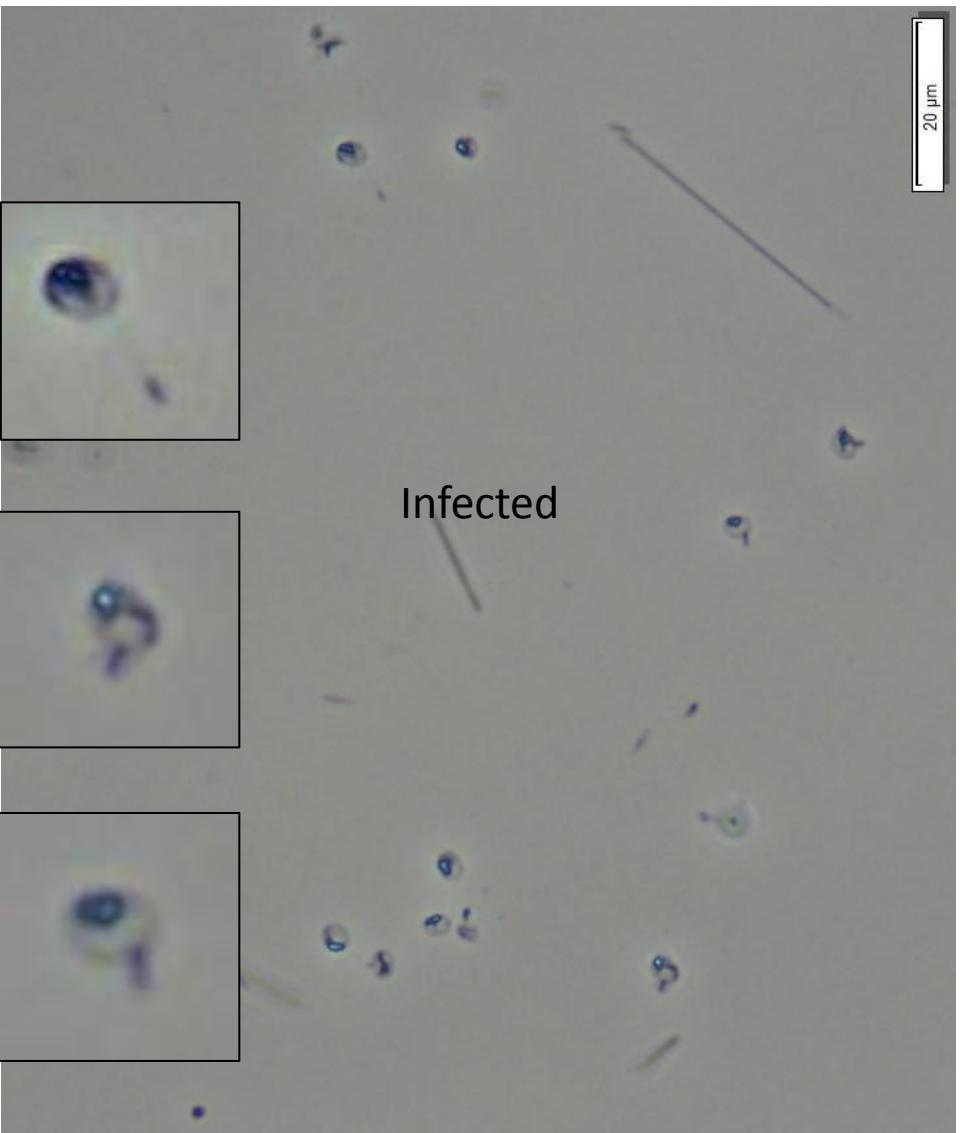
Hook

Day 3: infected algal cells: bacteria inside infected cells



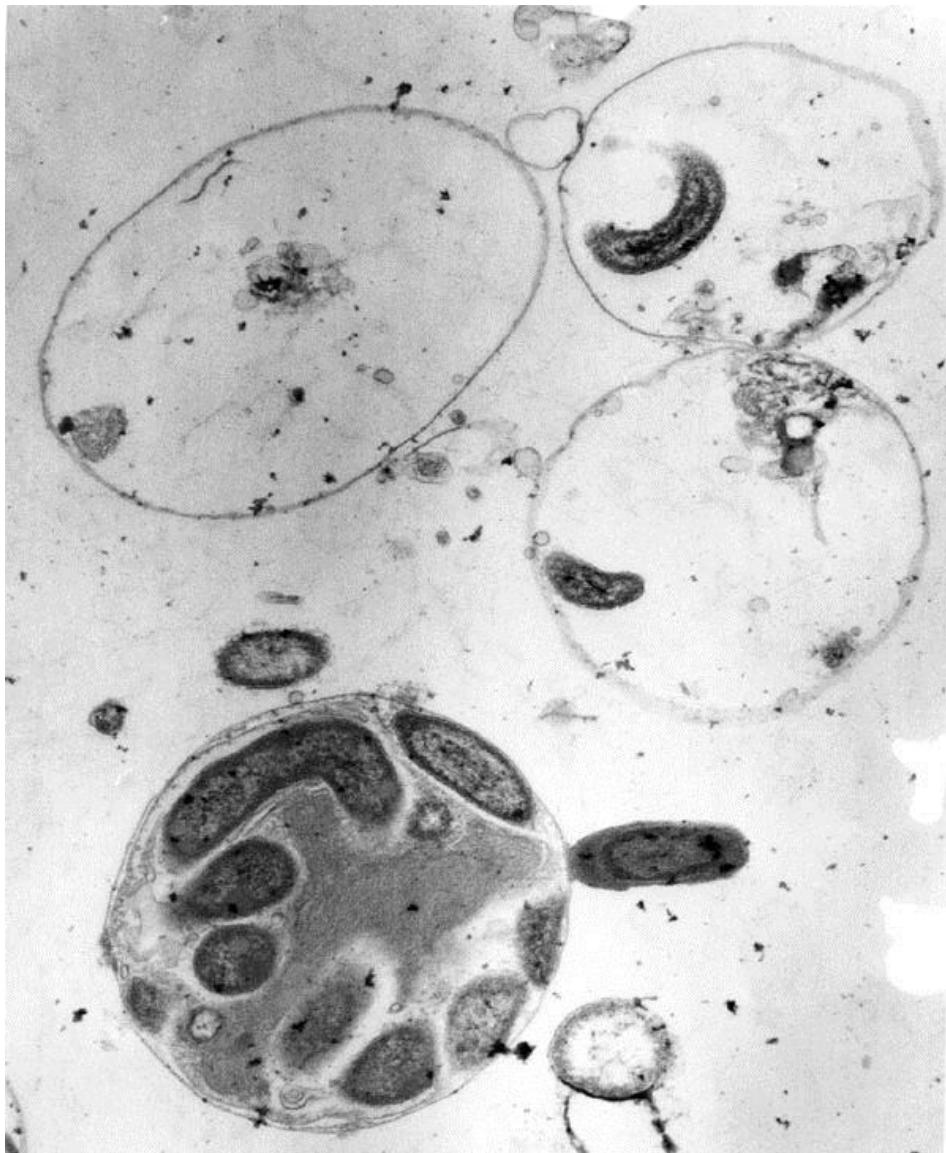
Division by fission, within host

Pictures – Day 4



- Most cells now hollowed shells with attachments
- In some cases organisms can be seen within dead cells

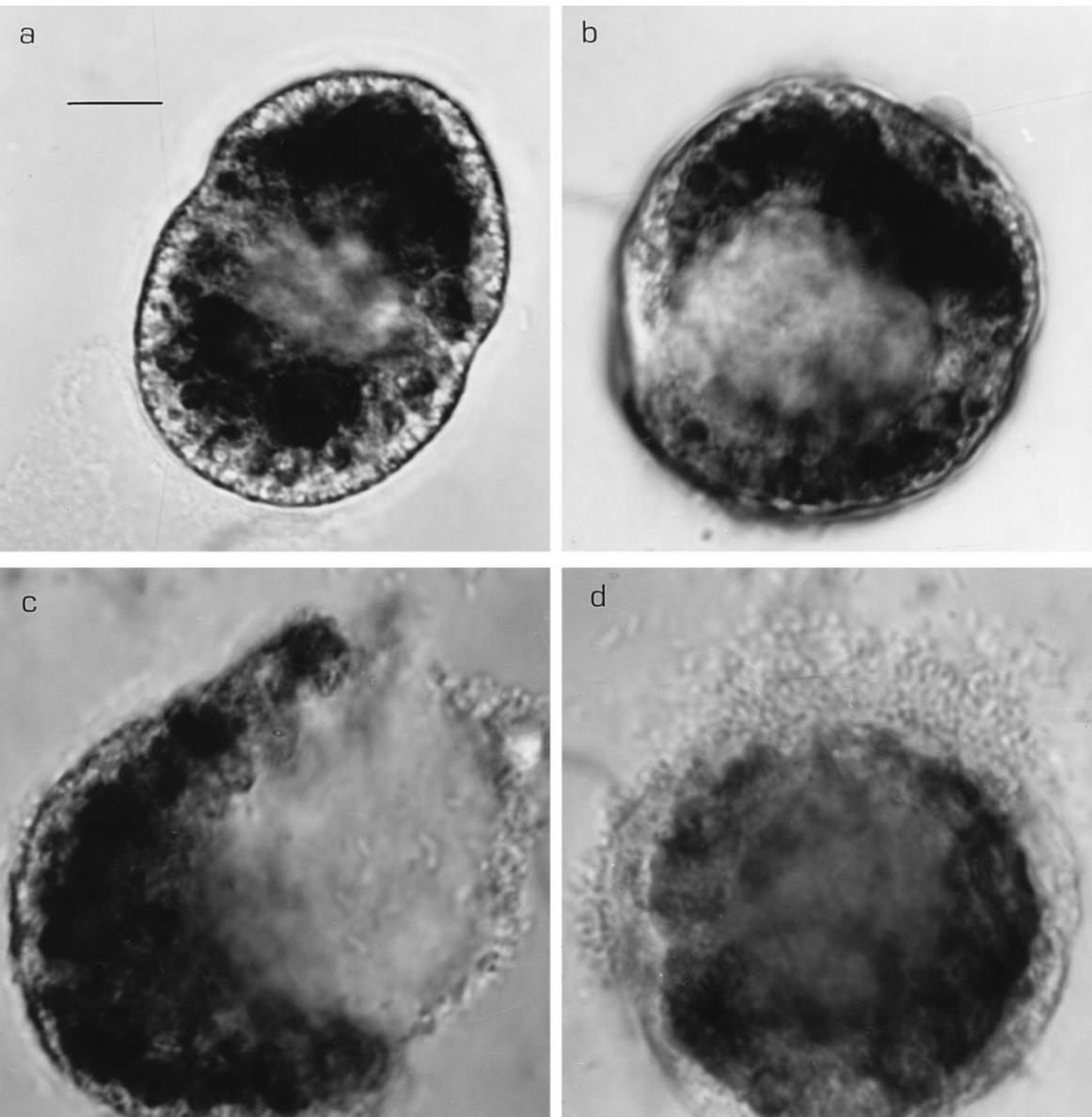
Day 4: algal remnants: many algal cells empty



Examples from literature of algicidal
bacteria

→ *Potential mechanisms of infection*

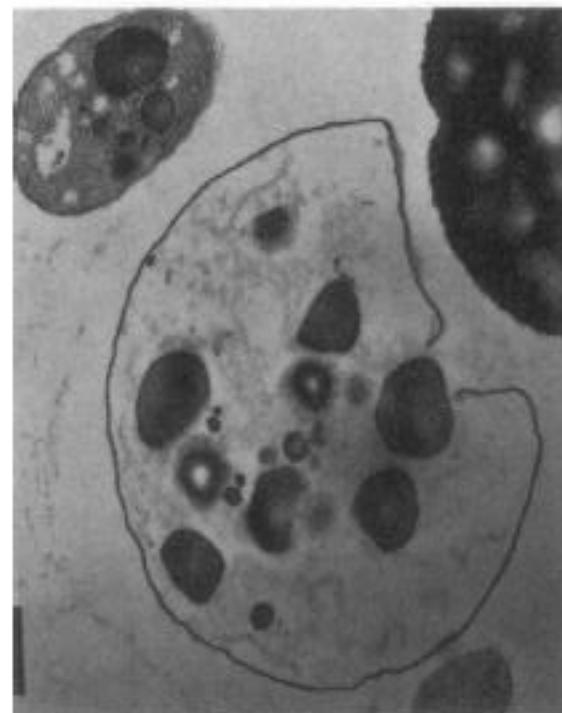
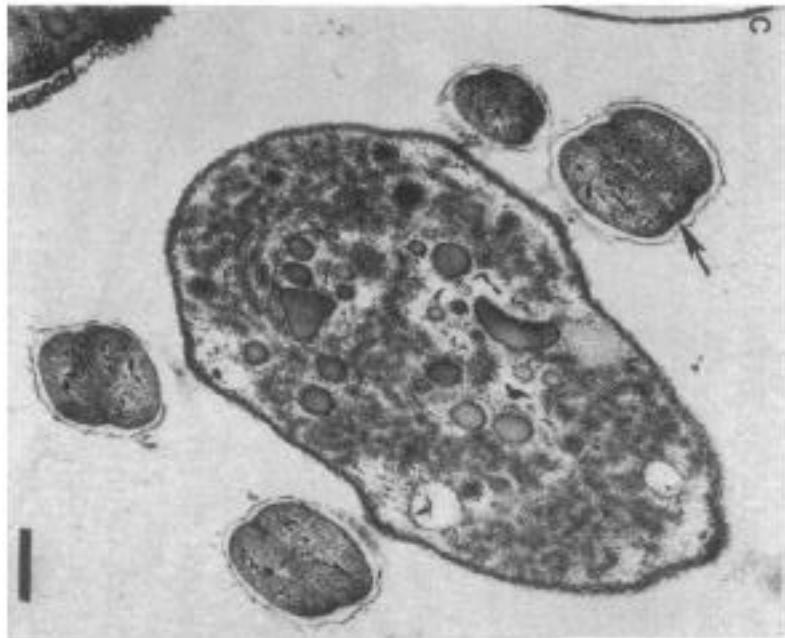
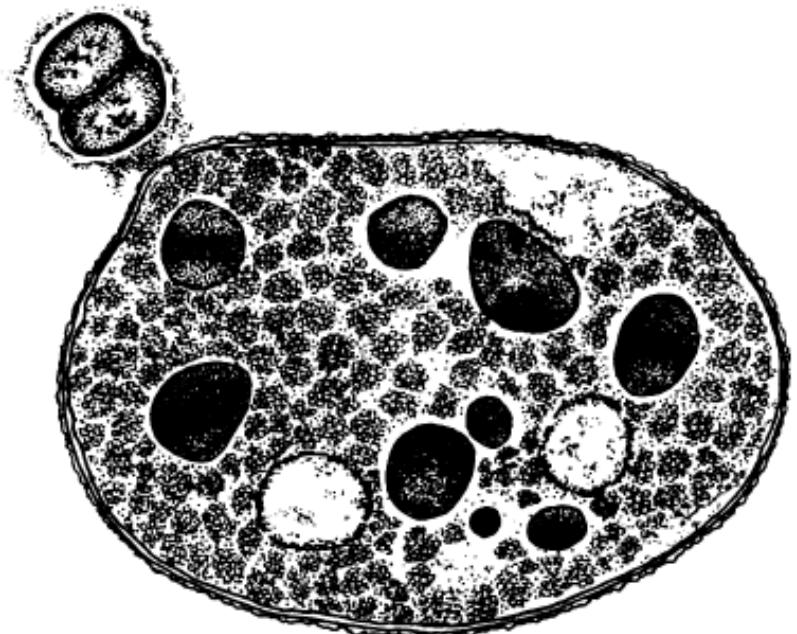
Indirect lysis of host by secretion of lysis inducing molecules



Example of a bacteria infecting algae and causing lysis. The most likely mechanism for this is secretion of something into the media by the bacteria.

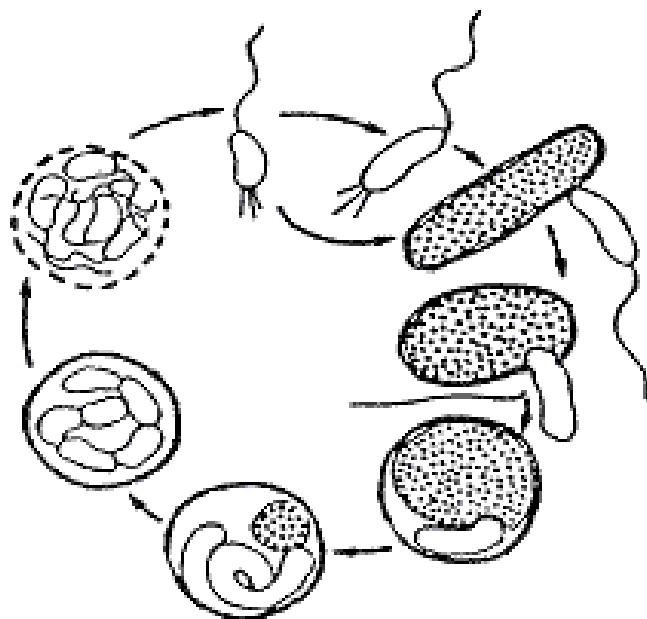
→ We don't seem to be seeing this mechanism

Epibiotic infection

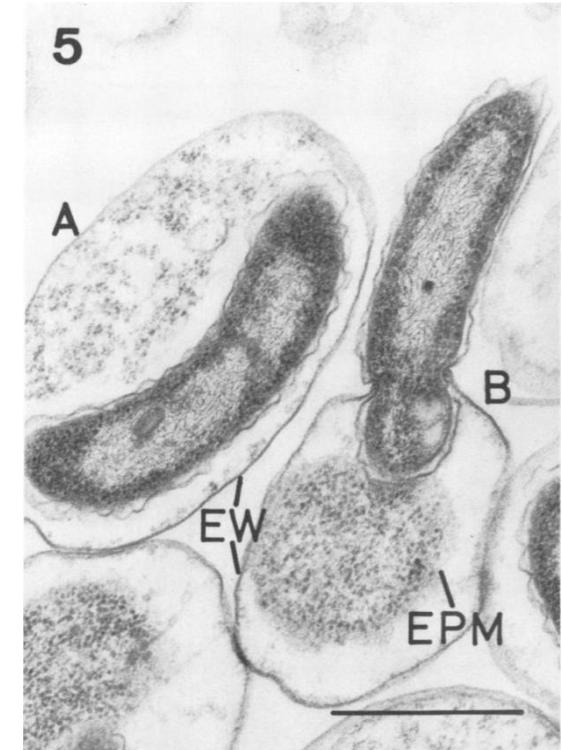
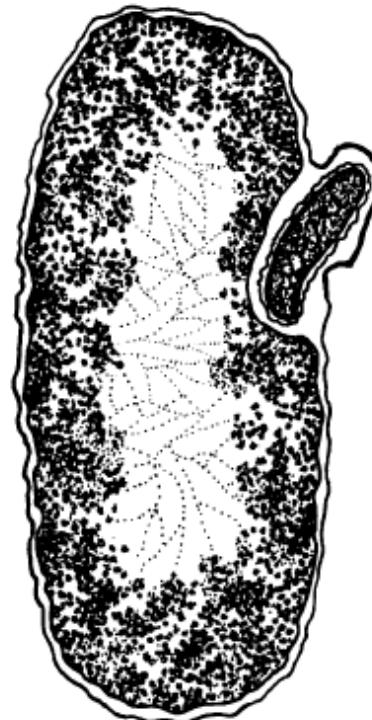


- Prey attaches to host and remains outside
- Injects enzymes etc to degrade the host
- Divide/bud from attachment point
- Leaves (mostly) empty cell behind

Periplasmic infection



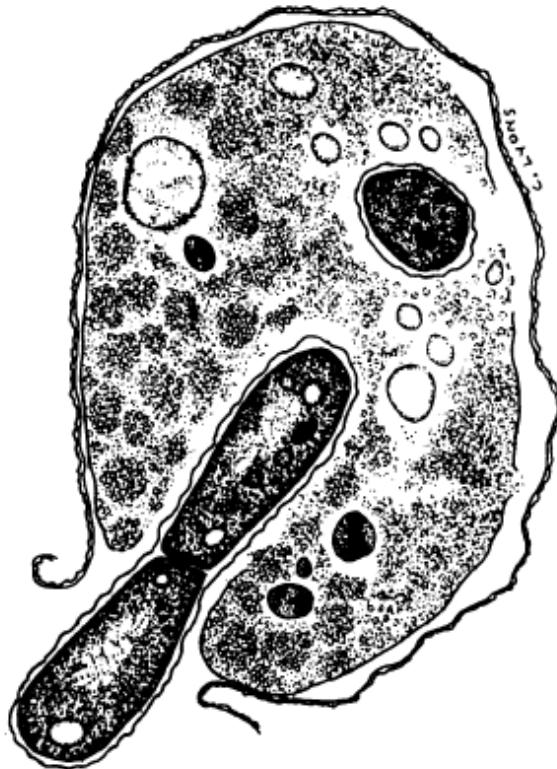
<https://es.wikipedia.org/wiki/Bdellovibrionaceae>



J. Virology, 1973 12(6) p1522-1533

- Pest enters through cell wall and resides in between the cytoplasmic membrane and cell wall
- In the case of *Bdellovibrio*, the bacteria elongates prior to dividing into daughter cells
- It is possible we could be seeing something like this – are all the bacteria in the infected cell pictures individuals or one long interconnected cell?

Endobiotic infection



<http://www.pnas.org/content/83/7/2138.full.pdf> Guerrero et al., 1986

- Pest enters through cell wall and cytoplasmic membrane
- Bacteria divide within the host while consuming cell contents
- We could be seeing this mechanism but so far it is unclear if the cytoplasmic membrane is still intact and an entry / egress point has not been observed

Conclusions/ Summary

1. The organisms attached and within the host are bacterial
2. There is no evidence of a eukaryotic organism present during infection
3. There is no evidence of viral infection
4. There may be at least two different bacteria associated with the host
(unclear if both, one, or neither are the pest but seems likely that one is)
5. We cannot see how the pest gets into or out of the cell and integrity of the cytoplasmic membrane during infection is unclear

Next steps

1. Continue isolation and sequencing efforts
2. Further sections on current samples for EM – looking for contact points, entry/egress mechanism, flagella presence on pest, are hooks/rods different organisms?, are internal bacteria individuals or one?, integrity of cytoplasmic membrane.
3. Generate more infection source and fix again – try to capture more samples between healthy and infected stages to look for initiation of infection (i.e. attachment and entry into the host)