FD101 Crash Validation

The initial slides in this presentation summarize an experiment that was done to try and determine if FD101 was a true pest of not. Early on, it was regularly tracked at Sapphire Energy in Desmodesmus ponds since it appeared to indicate when a crash was occurring – i.e., it proliferated during periods of poor culture health. As this presentation suggests, we later discovered that it was not a pest but likely a saprophyte proliferating on dead or dying algae. As we discovered the true causes of the algal death and developed tracking tools for those pests, we eventually stopped regularly tracking FD101 via qPCR but always investigated when we observed it.

Background

Experimental Design

Results

Discussion

FD101 was isolated from pond 16 and grown without a host on PmTG media

- A new chytrid phenotype (zoospores and sporangia) was observed in ponds of SE0107
  - Sequencing was done on pond samples and chytrid was designated FD101
- Isolation efforts were initiated to isolate FD101
  - Isolation was successful on PmTG media yielding FD101 colonies
- Isolated FD101 colonies were sequenced and confirmed identical to FD101 in outdoor cultures

ITS1&2 sequence generated to design qPCR primers

>FD101ITS1&2

ACAGcTATGACCATGATtATGCGAAGCTATTTAGGTGACACTATAGsATACTCAAGCTAT
GCATCAAGCTTGGTACCGAGCTBGGATCCACTMGTAACtGGCCGCCAGTGTGCYGG
AAcTTCGCCCTTTCCTCCGCTTATTGATATGCTTAAGTTCAGCGGGTAGTCCTACCTGA
TTTGAGACCAAGGTTCAAAGAGTTGTAAACAACCCTTTATAAGGTTATGAACTGCCTT
GAAAGATACCACTCCCCTATACAAATAACTTAATTAGTATCATAGAGAAACCAAGGTTT
CAGTCAAATACTTTTCTGTTATTATATAGTAAAGGATCAACATAGTACCCTATACAAAAC
AGTAATGAATTTCAAAGTACTCTGGGTAGAGACACTTCAAATCTTTACAGTGATACTA
GGTAAAACCACATATCAAGGTAAAAGAAATACATTAGATTCTCAAACAGGCATACTCT
AAAAGAGTGCAATGTGCGTTCAAAGATTTGATGATTCACGGAATTCTGCAATTCACAT
TACGTATCGCATTTCGCTGCGTTCTTCATCGTTGCGAGAGCCAAGAGATCCGTTGTCA
AAAGTTGTTTTTGTTTACTATATAAACAAGTCAGTCAATTTAAAACAGTGGTTTAATAT
AATGCTGGGTACACTCTATTACTAGAGCCTACCCAAAAGACATTGAATTGCACAAAGT
GTGAAAGAGTAGTACATTAGTGAGCTCAACTAGGAGCATCAACCGCAGTAAAACTCA
TAAATCAGTAATGATCCAACCGCAGGTTCACCTACGGAAAGGGCGAATTCCAGCACA
CTGGCGGCCGTTACTAGTGGATCCGAGCTCGGTACCAAGCTTGATGCATAGCTTGAG
TATTCTATAGTGTCACCTAAATAGCTTGCGTAATCATGgTCATAGcTGTTTTCYKGTGAD
KYCCTG

FD101 was isolated from pond 16 and grown without a host on PmTG media

- FD101 colonies were validated for pathogenicity
  - FD101 colonies did not elicit a crash in cultures of SE0107
  - Numerous zoospores were observed qualitatively up to 72 hours after infection, floc phenotype, no sporangia
- To date, an SE0107 crash from FD101 colonies has not been successful
  - Also, proliferation of FD101 in axenic cultures of SE0107 has not been successful

To date, an SE0107 crash from FD101 colonies has not been successful

- Why?
  - Saprophytic Pest?
  - Opportunistic Pest?
  - Mutualism?
  - Chemistry of ponds?
  - Biology of ponds?
  - Not a pest?
- A quick flask experiment demonstrated “new pest (FD107)” plaques + FD101 colonies yielded proliferation of FD101 in SE0107 cultures
  - A more elaborate/controlled experiment was designed

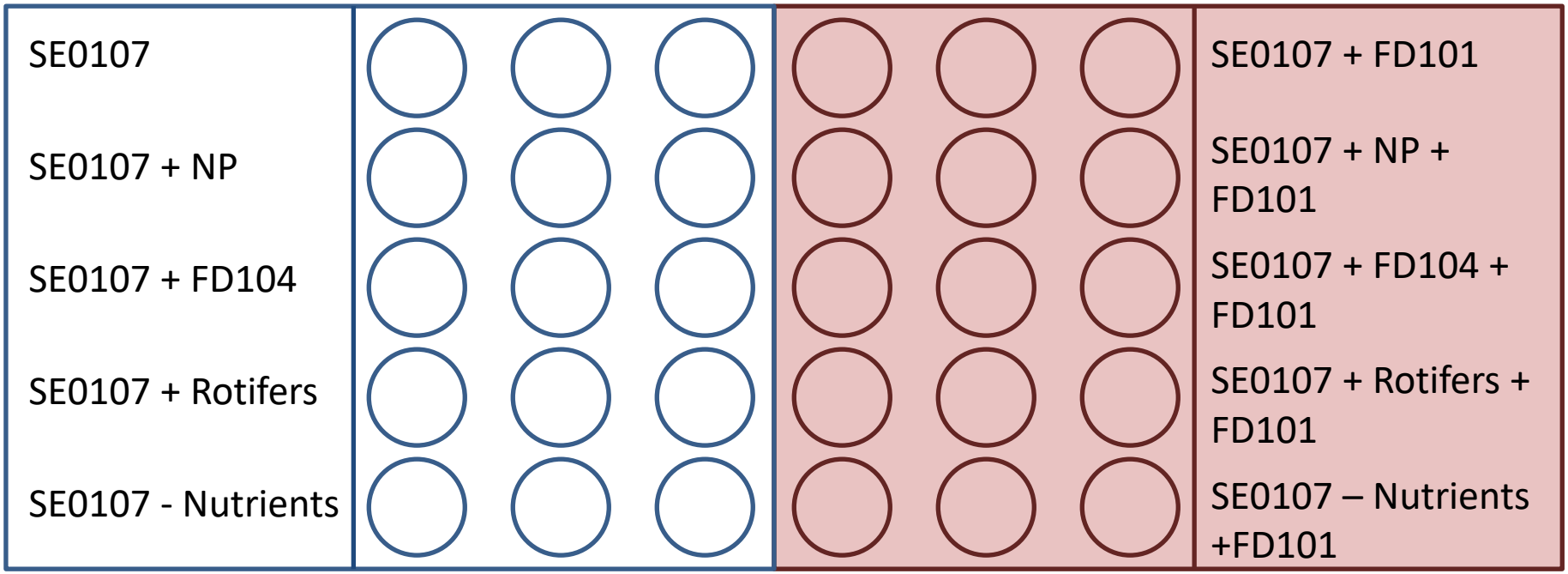
Capture and Isolate

Experimental Design

Results

Discussion

An experiment was designed to determine how to elicit proliferation of colony-derived FD101 in cultures of SE0107



Cultures started at OD 0.2 OD
50 ml culture in 125 mL flasks Fluorescence
FD104 normalized to CT 35 qPCR
FD104 normalized to CT 35 Week 1
Harvests

Note NP = New Pest aka FD107

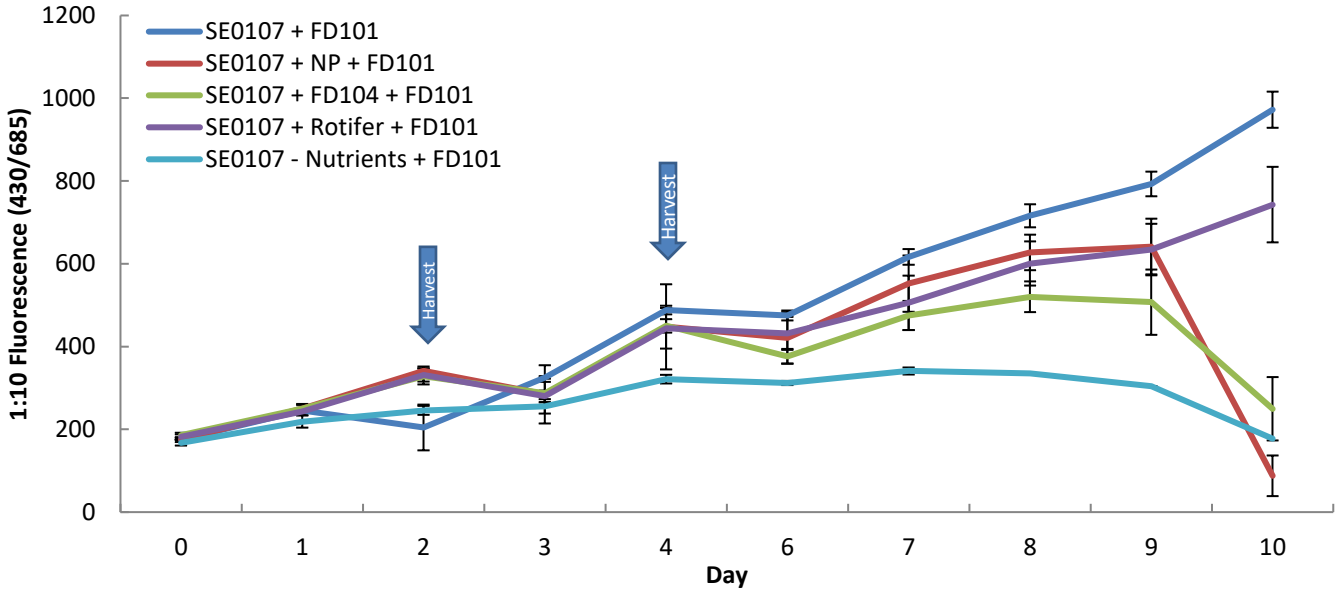
Background

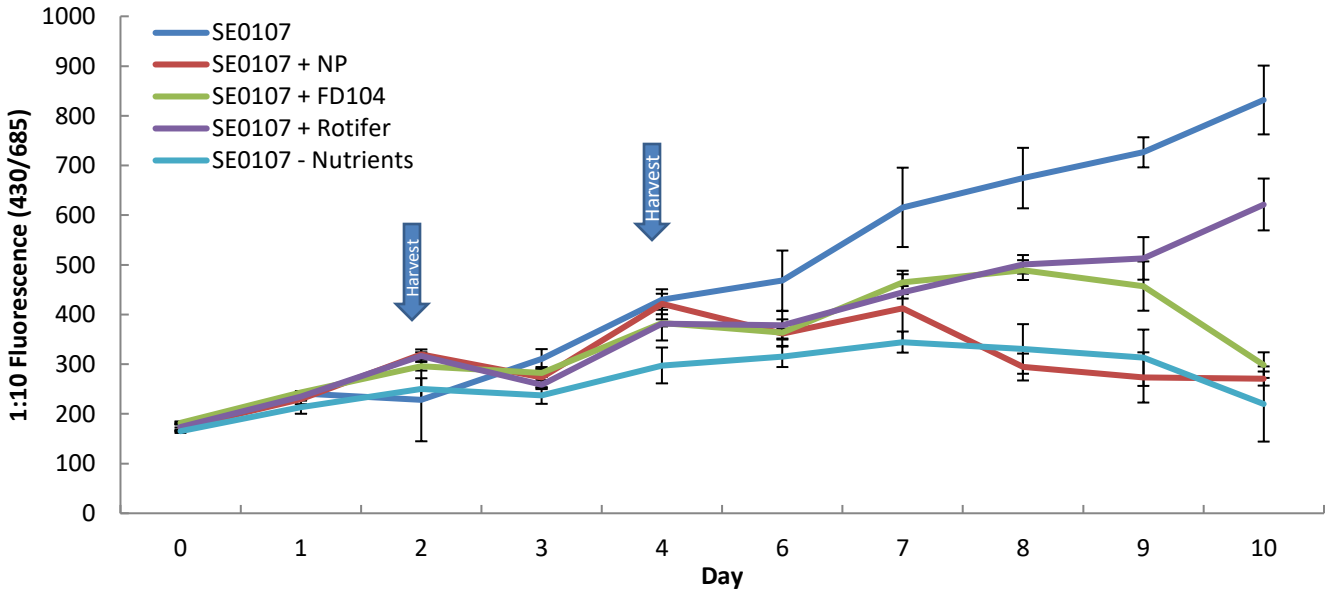
Experimental Design

Results

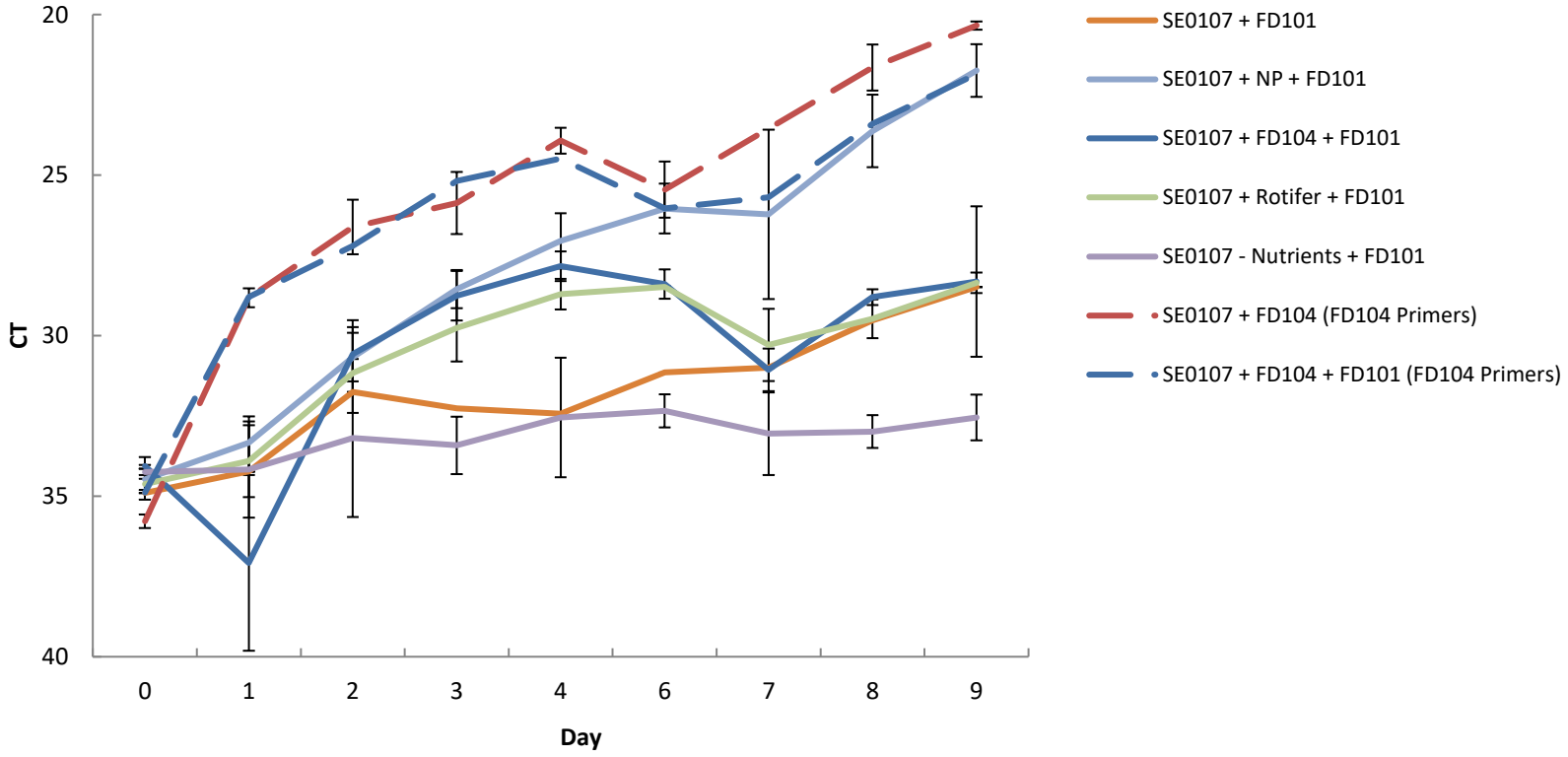
Discusssion

Fluorescence data indicates FD101 does not elicit a crash phenotype





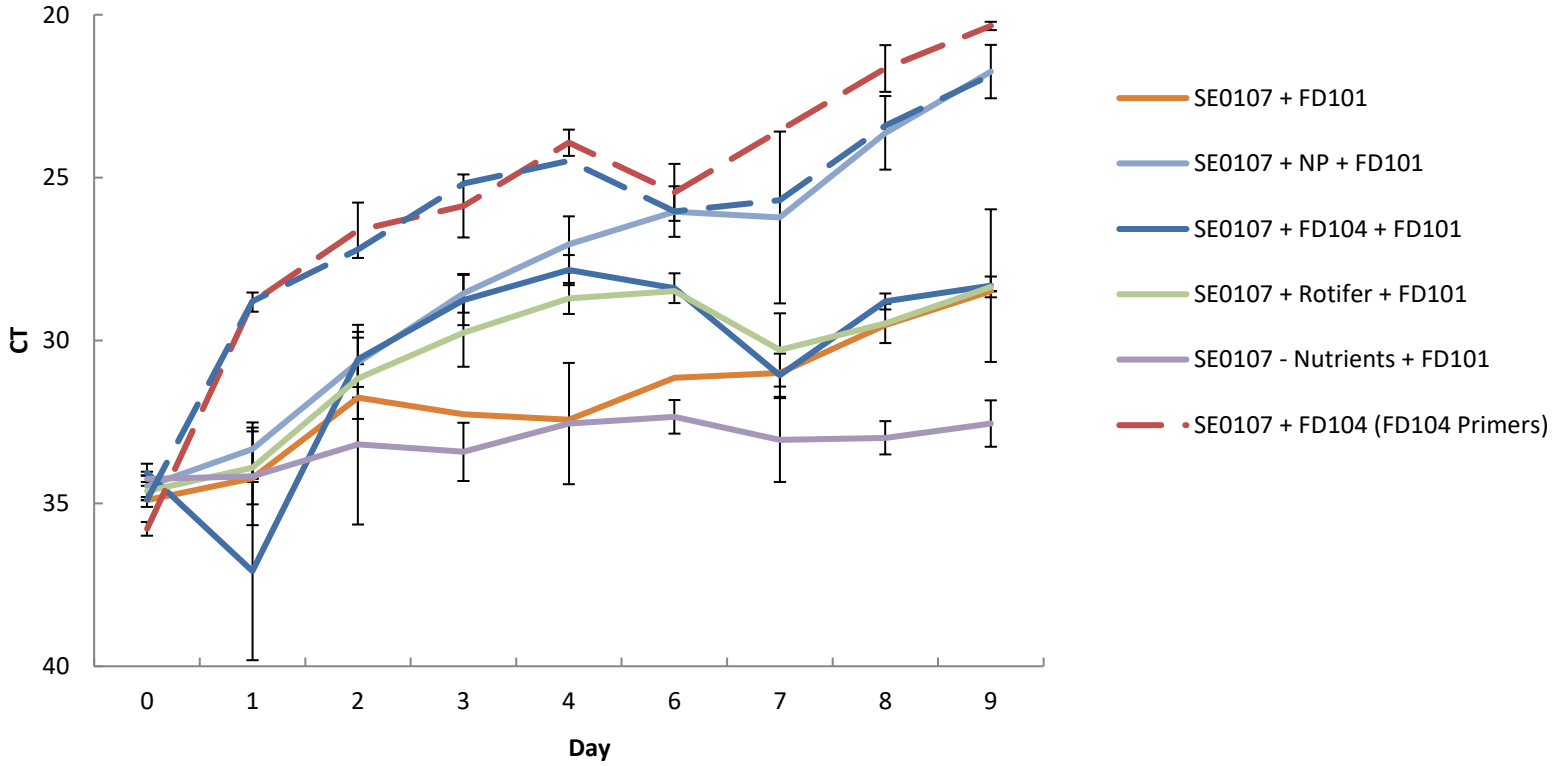
CT values indicate genomic DNA of FD101 increases drastically in cultures infected with new pest

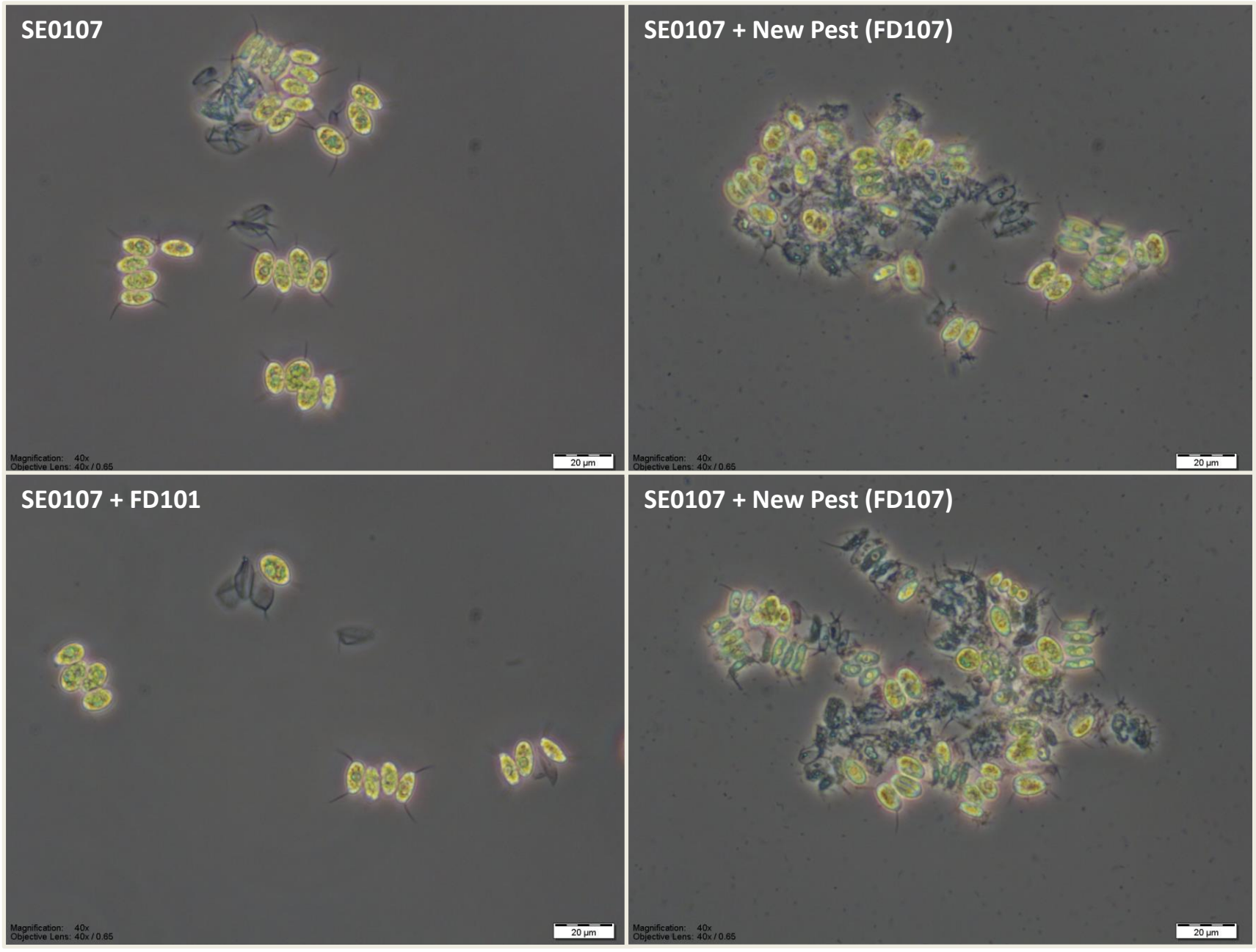


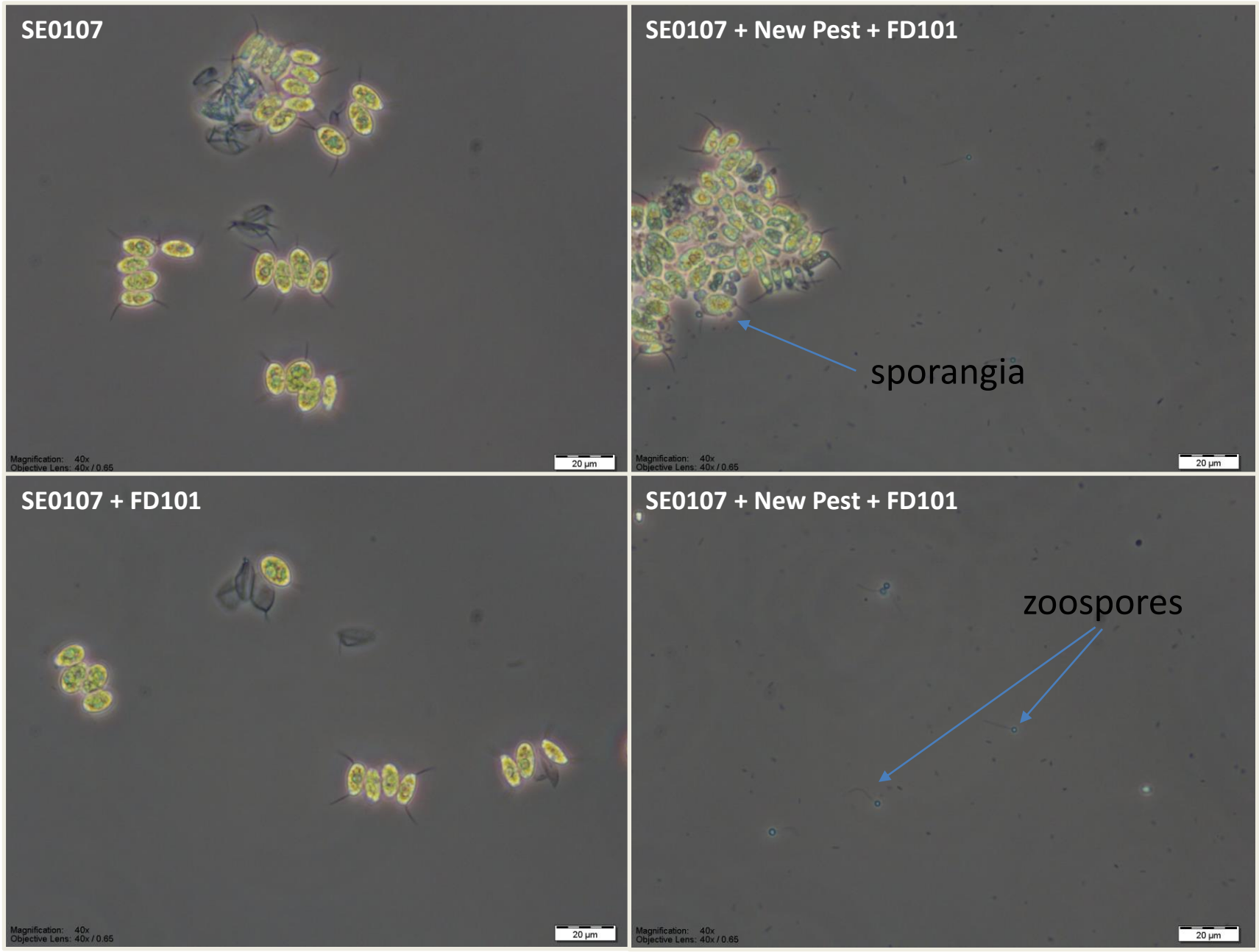
Solid Lines = FD101 Primers

Dashed Lines = FD104 Primers

Fluorescence data indicates FD101 does not elicit a crash phenotype




SE0107
Magnification: 40x
Objective Lens: 40x / 0.65
20 µm
SE0107 + New Pest (FD107)
Magnification: 40x
Objective Lens: 40x / 0.65
20 µm
SE0107 + FD101
Magnification: 40x
Objective Lens: 40x / 0.65
20 µm
SE0107 + New Pest (FD107)
Magnification: 40x
Objective Lens: 40x / 0.65
20 µm


SE0107
Magnification: 40x
Objective Lens: 40x / 0.65
20 µm
SE0107 + New Pest + FD101
sporangia
Magnification: 40x
Objective Lens: 40x / 0.65
20 µm
SE0107 + FD101
Magnification: 40x
Objective Lens: 40x / 0.65
20 µm
SE0107 + New Pest + FD101
zoospores
Magnification: 40x
Objective Lens: 40x / 0.65
20 µm

Background

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Discussion

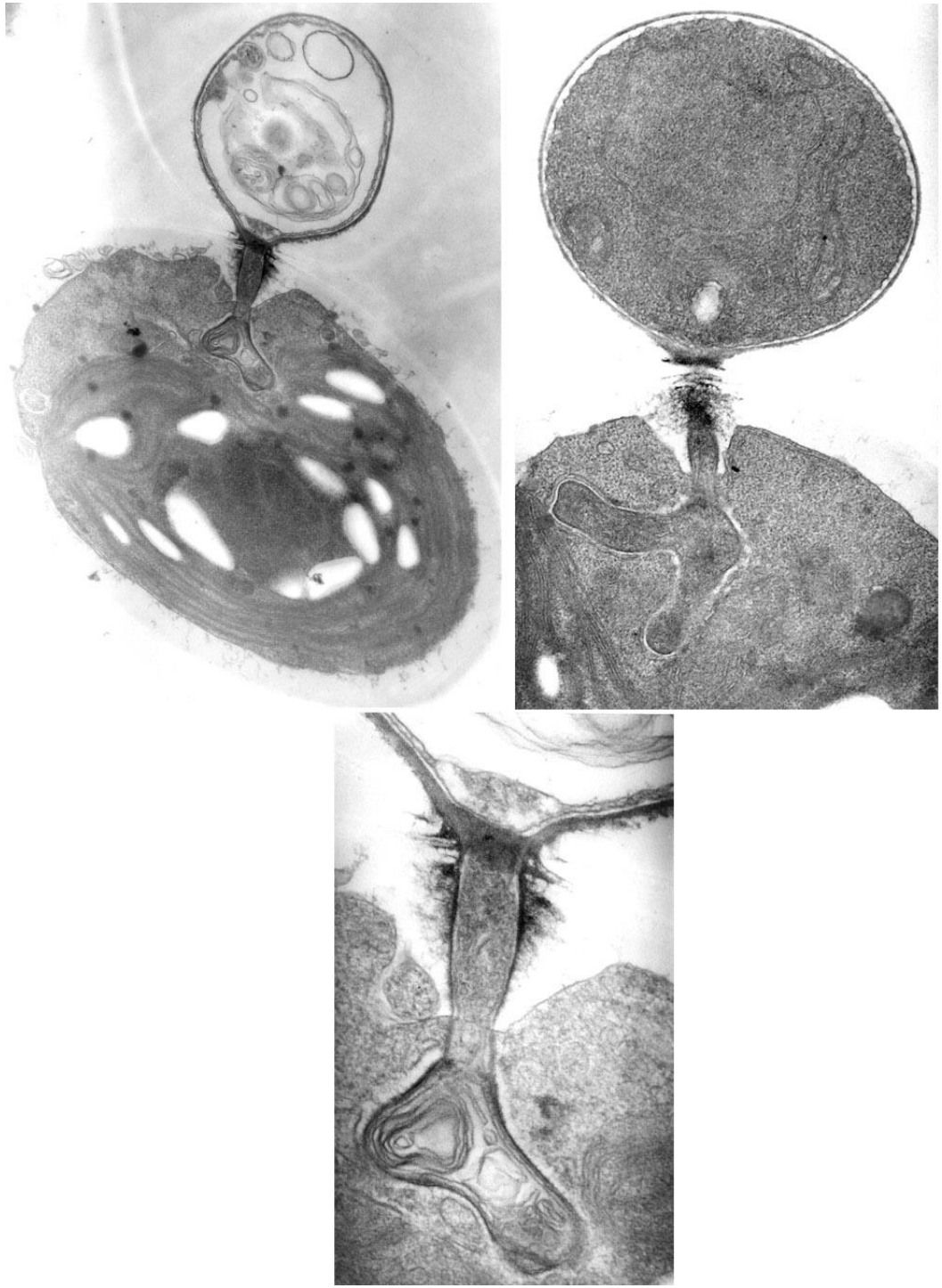
FD101 cannot crash axenic cultures of SE0107

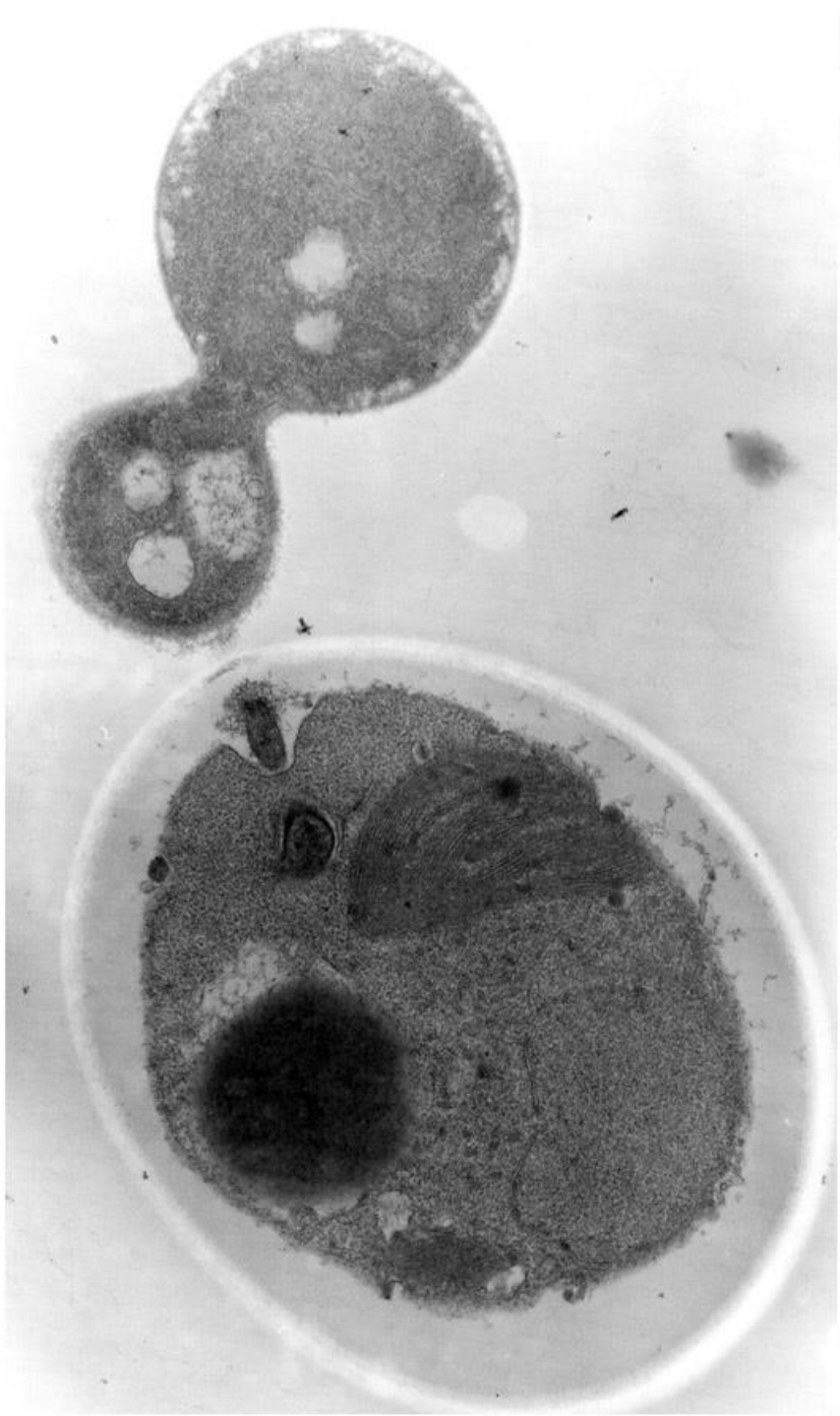
- FD101 derived from colonies did not crash cultures of SE0107
- FD101 proliferates when “new pest (FD107)” is present in culture *(note that other pests did not cause a crash in the same timeframe and Ct’s were dropping so FD101 may have propagated in the days following the end of the experiment, so this observation may not be specific to FD107)*
- Other biotic stresses did not elicit proliferation of FD101
  - Specifically, rotifers and FD104 (but see note above)
- Abiotic stress (nutrient deprivation) did not elicit proliferation of FD101
- This is the first successful propagation of FD101 from colony isolates

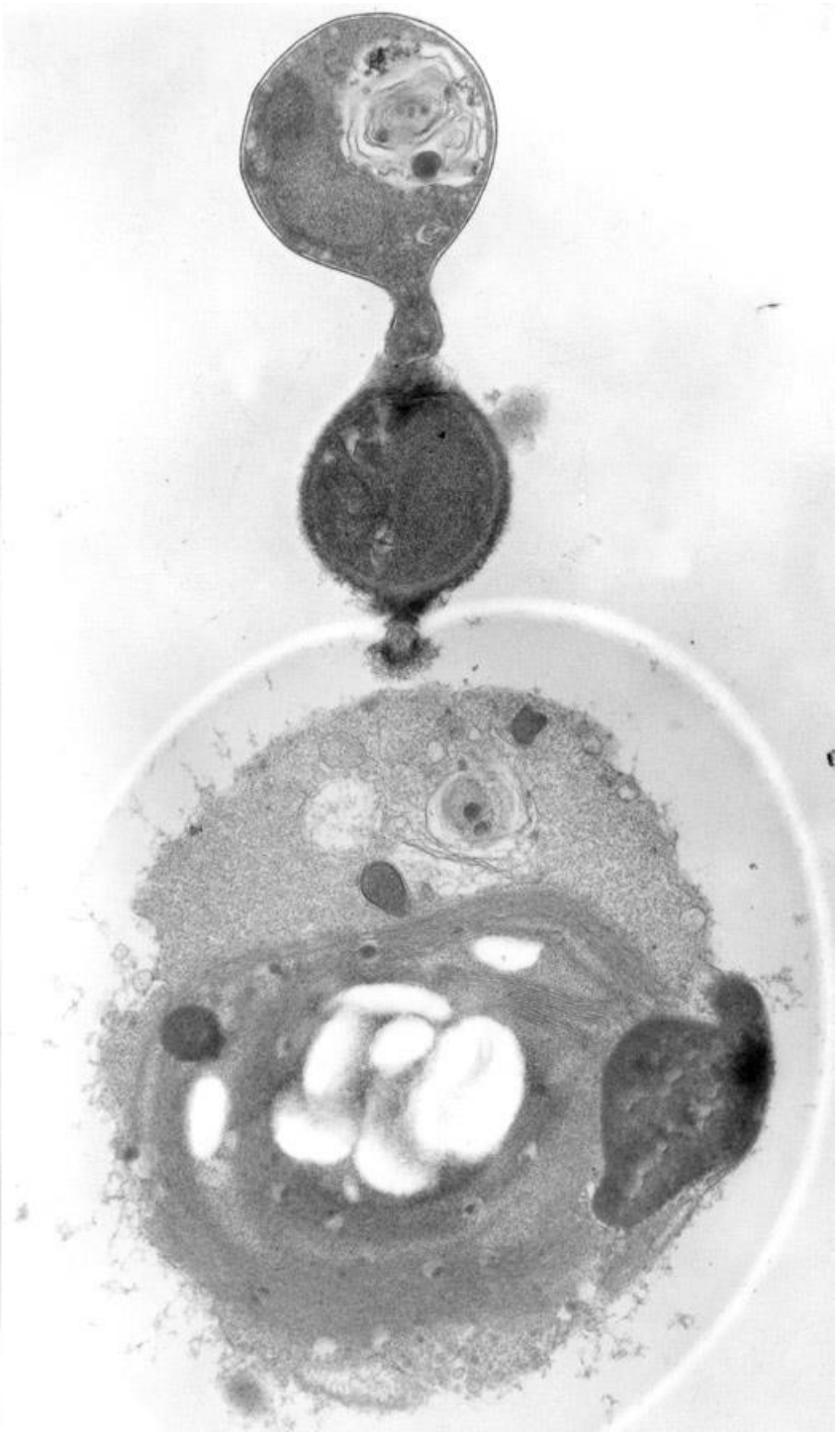
Microscopy Examples for FD101

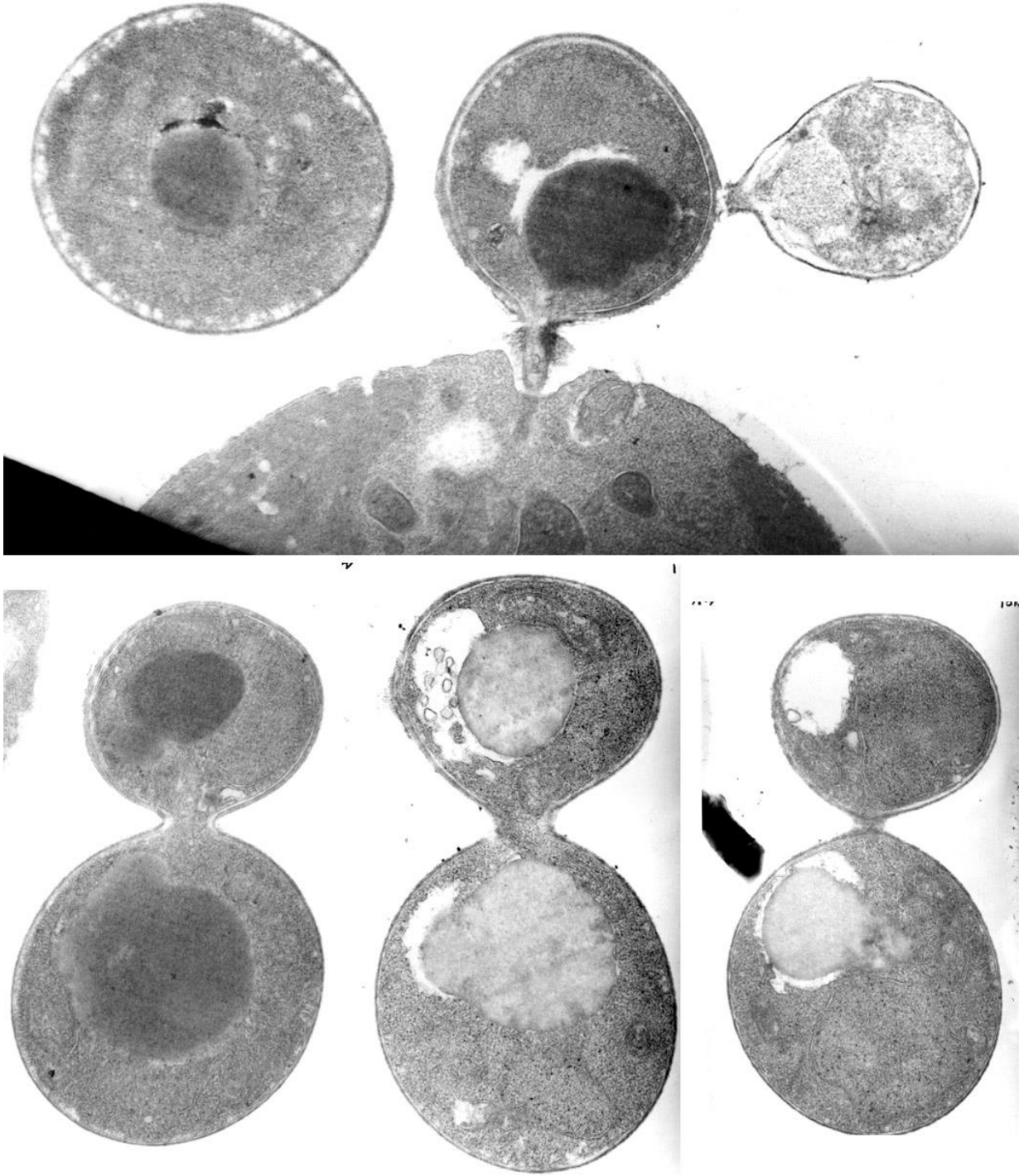
Light microscopy from Sapphire Energy

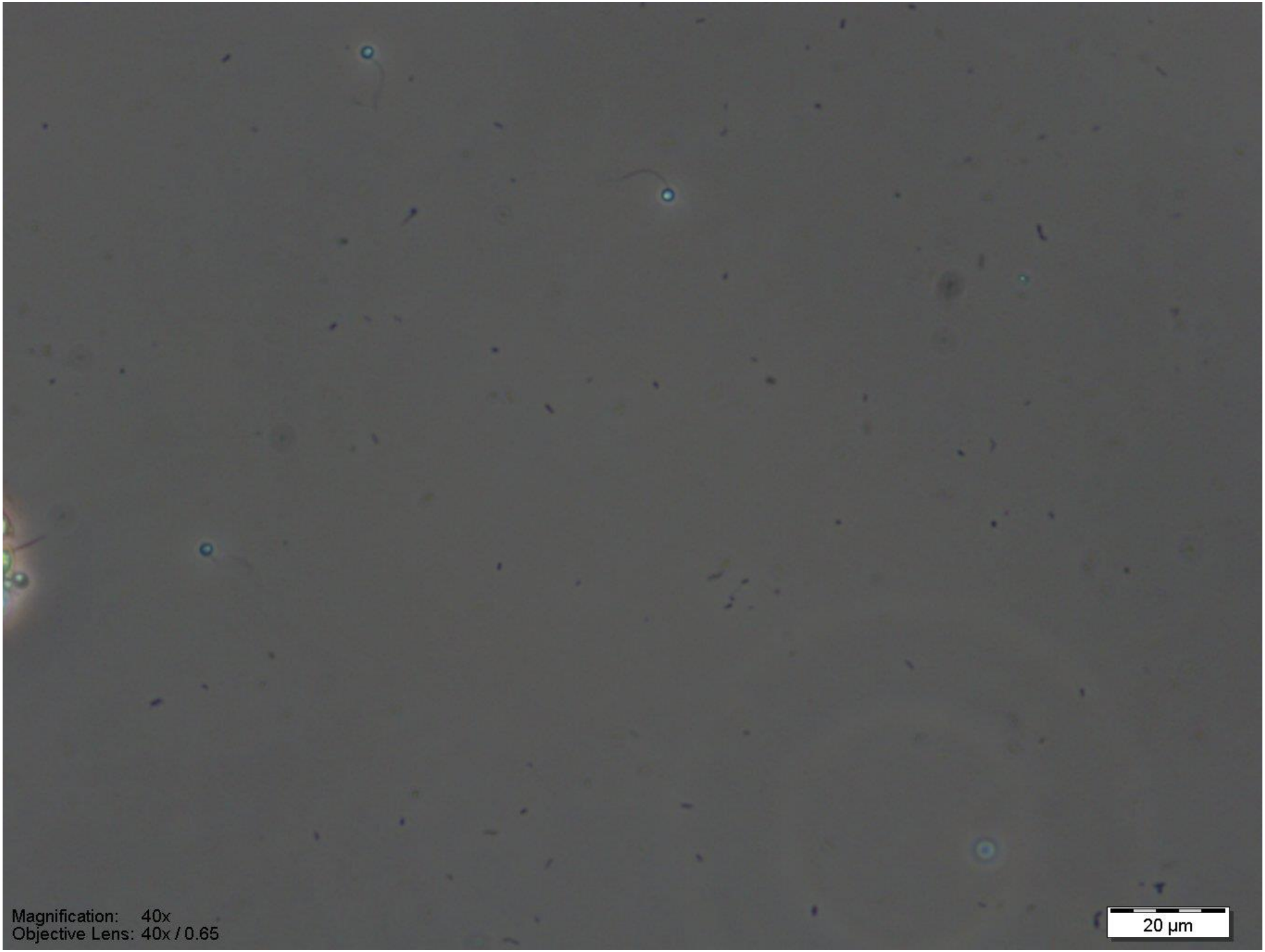
Pete Letcher, University of Alabama

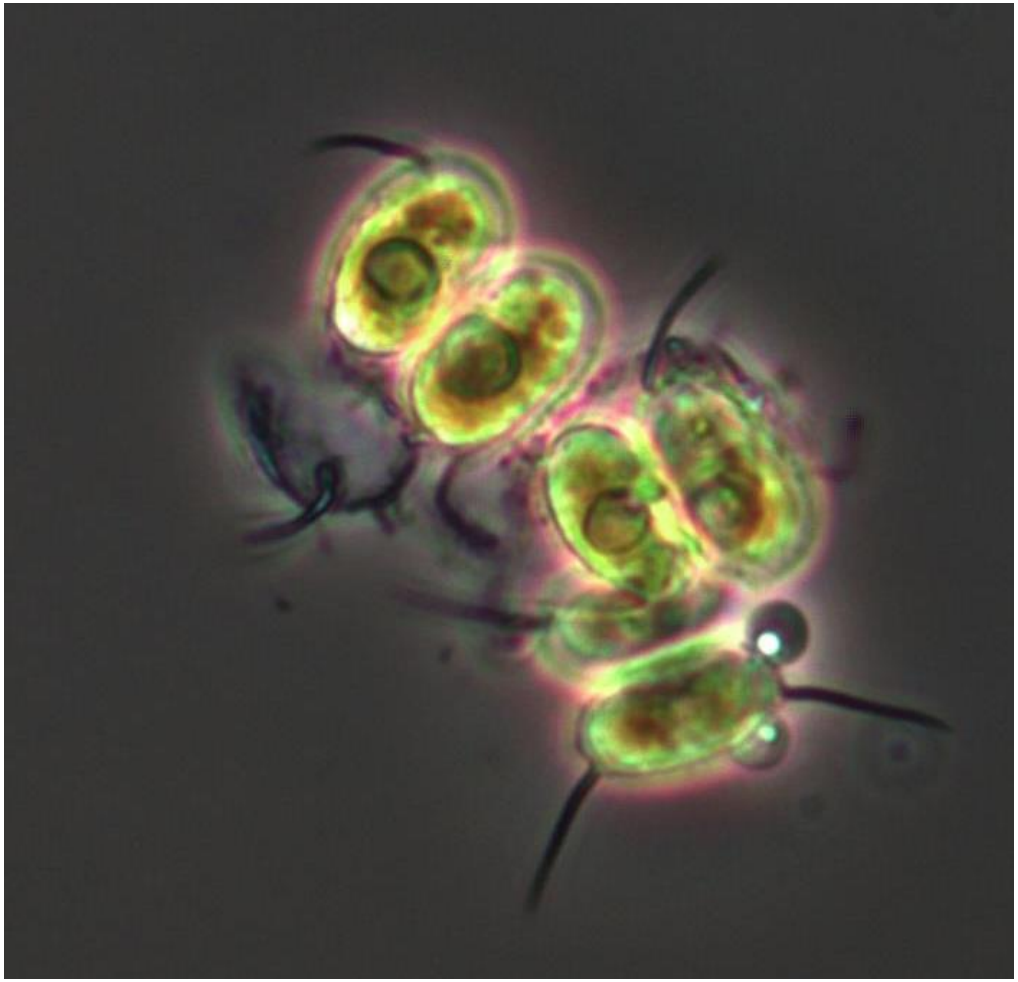


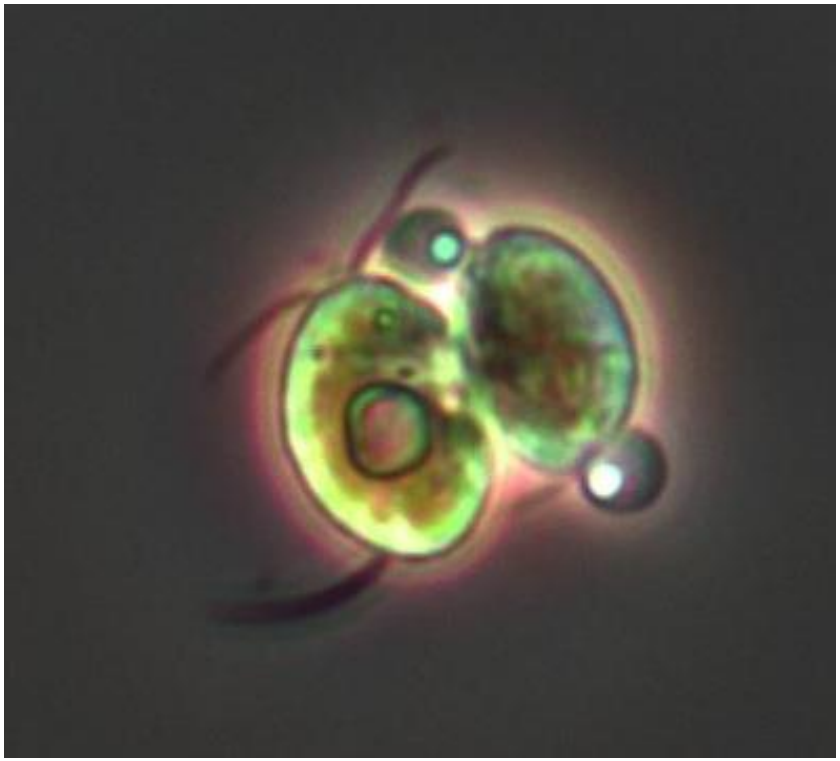


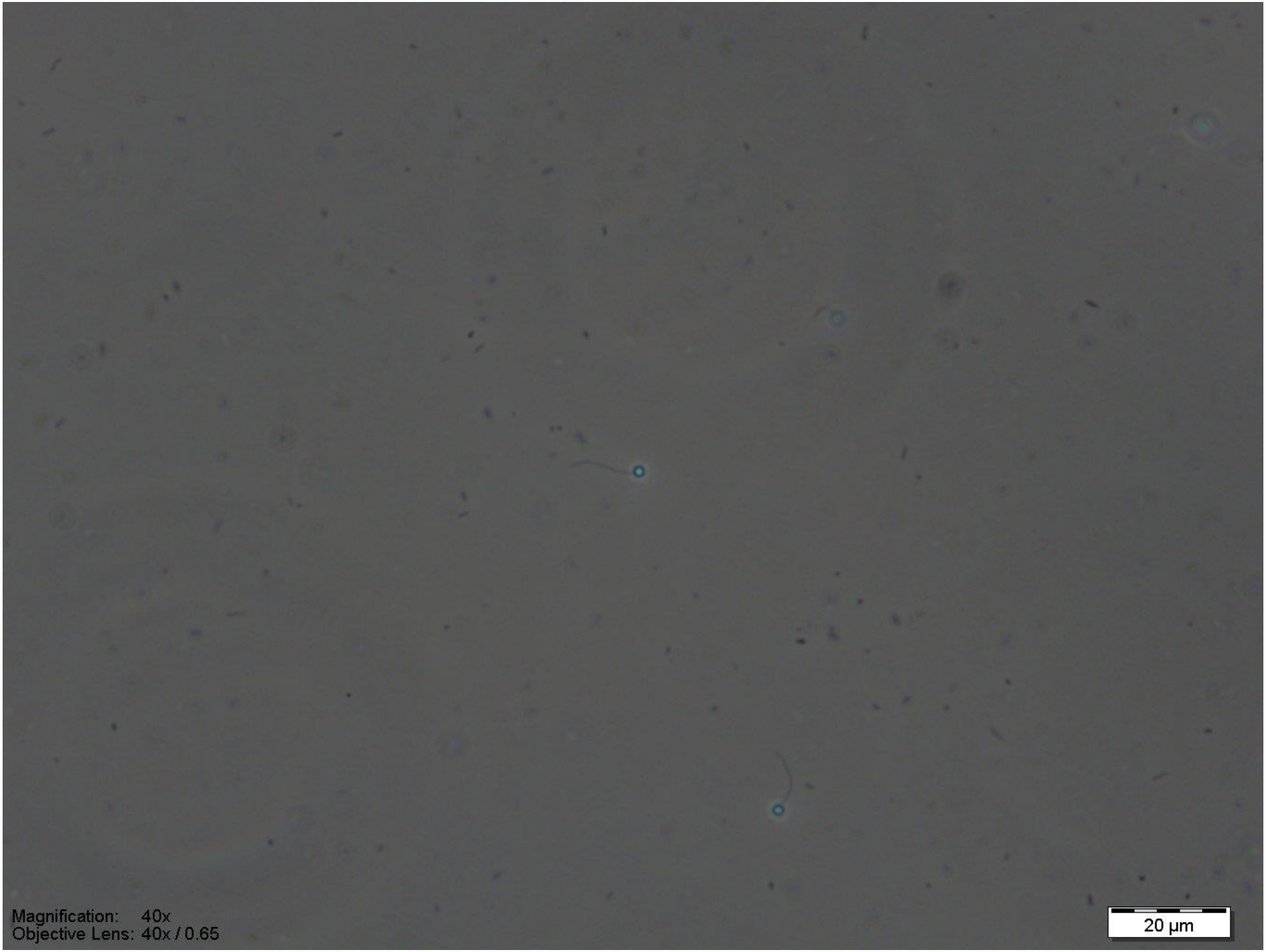





Magnification: 40x
Objective Lens: 40x / 0.65
20 µm






Magnification: 40x
Objective Lens: 40x / 0.65
20 µm


Magnification: 40x
Objective Lens: 40x / 0.65
20 µm

The following are snippets from email conversations regarding the isolation and culture of FD101:

FD101 did not elicit a crash in SE0107, and that it grows on PmTG. I'll bet that its a facultative parasite, able to infect and reproduce on "living" hosts, but also able to exploit senescent hosts and other dead organic matter, and thus grow quite well on PmTG. If you can find it, let's see if it revives. If so, I expect it will complete its life cycle on synthetic media (PmTG) just as well as on an alga. So, maybe the easiest route is to put it on PmTG first, then go to an algal host (SE0107) and see if additional stages of the life cycle can be documented.

xx

FD101 was a bit sensitive to antibiotics, if I remember correctly. I primarily used a combination of penicillin and ampicillin:

1x Penicillin: 500 mg/L

1x Amp: 100 mg/L

If you have the time/resources, doing a plate at 1x and 0.5x Penicillin/Amp might be worthwhile. Pete may also have recommendations regarding ideal concentrations, and I would likely take his recommendations over mine.

I do remember once I had isolated colonies, that when I streaked colonies onto plates with and without antibiotics, I had roughly 40-50% less colonies on the plates with antibiotics. They also grew slower.

Also, pour your plates thick. It took about 14 days when I first isolated, for very VERY small colonies to pop up. When you get your first colony, they grow much faster when transfered to fresh media, ideally without antibiotics to improve growth speed.

Once I dealt with the bacteria, I also had problems with mold and/or other types of fungus taking over the plate before chytrids colonies would form. Try to limit how often you open the plate to minimize this and observe the plate closed under the microscope when possible.

The FD101 colonies are a "bright" white, and tend to have a textured surface as they mature. At first, they will not reach a very large size--maybe the size of a period. Especially, if there is other bacteria and/or fungus on the plate. Once you transfer, colonies can get to the size of a small pea.

I know bright white is vague, but I remember them standing out amongst other white bacterial colonies. I would visualize all these brighter colonies under the microscope. It was easy to identify the chytrids colonies, as there was a clear halo around the colonies where you could observe the zoospores swimming around; satisfied, content, and at one with themselves. I remember envying them at this stage.

xx

Some chytrids seem adversely affected by antibiotics; maybe (just as in us) the antibiotics kill beneficial as well as harmful bacteria when applied. We use .5 g penicillin and .5 g streptomycin per liter, but I doubt if this makes much difference. If you can find a chytrid colony on a "contaminated" plate within the first few days, while the bacteria are initially being held at bay, then pick off that colony and put it on another antibiotic plate (as you may inadvertently take a stray bacterium with the colony). Keep on transferring every 4-5 days to get a chytrid colony clean of bacteria, then maintain/feed the chytrid on a non-antibiotic plate ("chicken soup"), once it looks to be free of infection.