

# Laboratory Tests Using EnSoil Algae product in Creeping bluegrass (*Poa reptans*), Clover (*Trifolium repens*) and Tomato (*Solanum lycopersicum*)

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# Methodology

## Treatments

- Control, EnSoil Algae product, Ensoil Algae only Boiled for 1 minute to kill algae, Ensoil Algae only autoclaved for 20 minutes to kill both algae and bacteria, Ensoil Broth only, Chlorella Vulgaris product, and Commercial Broth

## Plant growth

- Seeds of Poa reptans grass were subjected to surface disinfection by shaking in a 3% sodium hypochlorite solution for 1 hr. 10 Seeds were subsequently placed onto agarose culture medium and then inoculated with 5% algae production suspension. 3 replicates per treatment.
- Seeds of tomato (*Solanum lycopersicum*) were surface disinfected by sequentially treating with 70% ethanol for 1 minutes, followed by a 7.5% sodium hypochlorite solution shaking for 1 hr. 8 Seeds were placed onto agarose culture medium and then inoculated with 100% algae production suspension. 3 replicates per treatment.
- Seeds of Clover (*Trifolium repens*) were surface disinfected for 1 hr by shaking in 4% sodium hypochlorite. 10 Seeds were placed onto agarose culture medium and then inoculated with 100% algae production suspension, or placed into algae production suspension directly. 3 or 4 replicates per treatment.
- Seeds on agarose were germinated and grown in lab ambient conditions for 7-13 days depending on the plant being tested. Plant growth promotional characters were assessed, including – germination, lateral root number, root length, root hair growth and Gravitropic Response.

## Microscopy

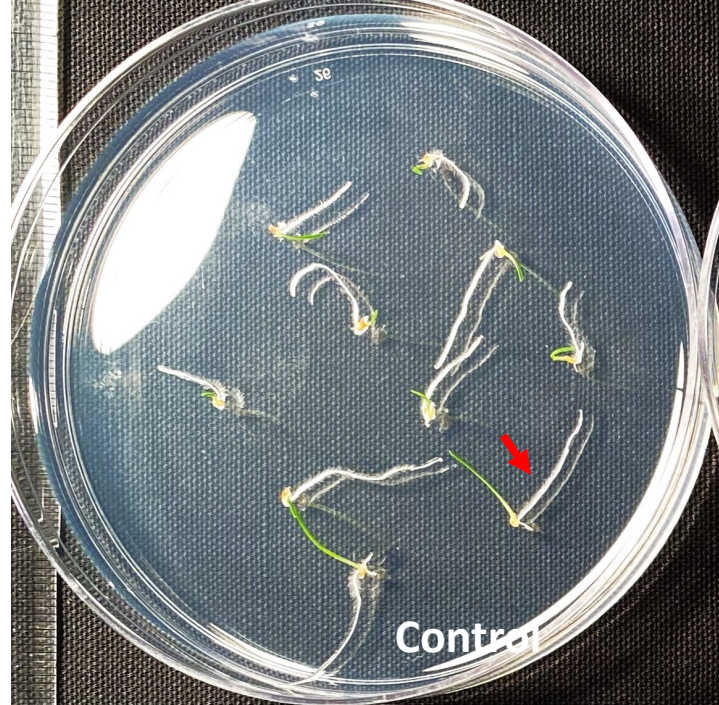
- Diphenylamine sulfate stain was used to visualize endophytic bacteria and nitrate (purple).

**Table 1. Stimulation of Root and Root Hair Growth in *Poa reptans* After Inoculation of Algae Product in agarose-based 7-day seedling assays (November)**

	Germination Rate	Gravitropic Response	Ave. Lateral Root per plant	Root Length	Root hairs
Control	93.3%	0	0.82 ± 0.55	1.78 ± 0.51	+
EnSoil Product	100%	0	1.10 ± 0.71	1.89 ± 0.49	+++
Boiled Ensoil Algae	83.3%	0	1.00 ± 0.71	1.74 ± 0.49	+++
Ensoil Broth	76.7%	2	1.04 ± 0.77	1.77 ± 0.60	+
Chlorella Vulgaris	76.7%	0	1.00 ± 0.60	1.91 ± 0.42	++
Commercial Broth	86.7%	0	0.96 ± 0.92	1.85 ± 0.41	++

**Results:**

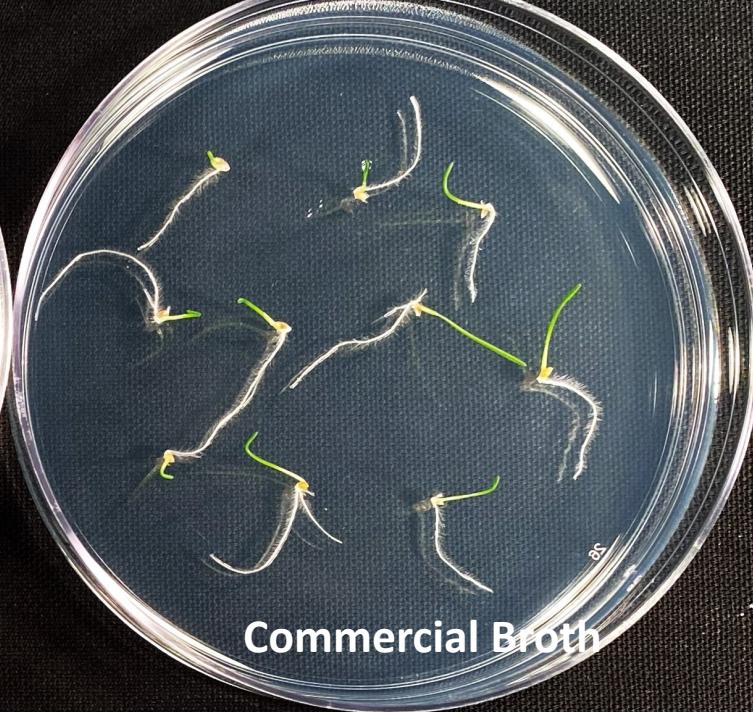
- After inoculation of Algae Product , *Poa* plants exhibited increased lateral root and root hair formation, longer root length, as well as a higher proportion of plants displaying gravitropic responses.
- Among these three treatment, the effect of plant growth promotional effect was: EnSoil Algae Product > Boiled Ensoil Algae > Chlorella Vulgaris> Commercial Broth and Ensoil Broth > control.



Control



Chlorella Vulgaris



Commercial Broth



EnSoil Algae product



Ensoil Broth



Boiled EnSoil Algae

**Table 2. Stimulation of Root and Root Hair Growth in Tomato (*Solanum lycopersicum*) After Inoculation of Algae Product in agarose-based 13-day seedling assays (Jan.)**

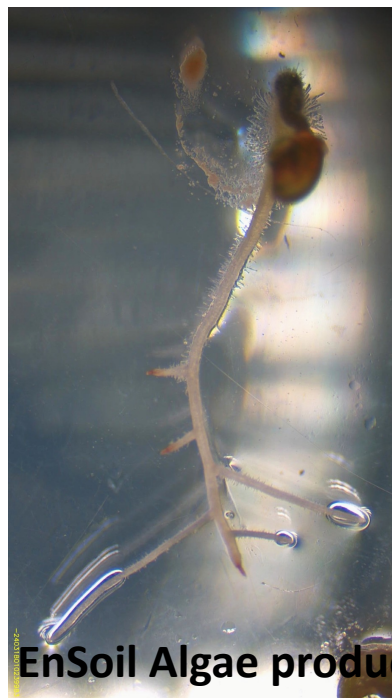
	Germination Rate	Ave. Lateral Root per plant	Root Length (cm)	Root Hairs	GR
Control	49%	0.50	0.94 ± 0.78	+	0
EnSoil Algae Product	50%	2.17	1.91 ± 0.41	++	2
Autoclaved Ensoil Algae	54%	2.54	1.78 ± 0.83	++	1
Boiled Ensoil Algae	42%	1.40	1.72 ± 1.03	++	1
Ensoil Broth	63%	1.41	1.96 ± 1.11	++	0
Chlorella Vulgaris	58%	1.47	1.65 ± 0.72	++	1
Commercial Broth	42%	2.43	1.52 ± 0.52	+	1

### Results:

- After inoculation of Algae Product , Poa plants exhibited increased lateral root and root hair formation, longer root length, as well as a higher proportion of plants displaying gravitropic responses.
- Among these three treatment, the effect of plant growth promotional effect: EnSoil Algae Product and Autoclaved Ensoil Algae > Boiled Ensoil Algae > Ensoil Broth > Chlorella Vulgaris> Commercial Broth > control.



**Control**



**EnSoil Algae product**



**Ensoil Broth**



**Autoclaved EnSoil Algae**



**Boiled EnSoil Algae**



**Chlorella Vulgaris**



**Commercial Broth**

**Table 3. Stimulation of Root and Root Hair Growth in Clover (*Trifolium repens*) After Inoculation of Algae Product in agarose-based 12-day seedling assays (March)**

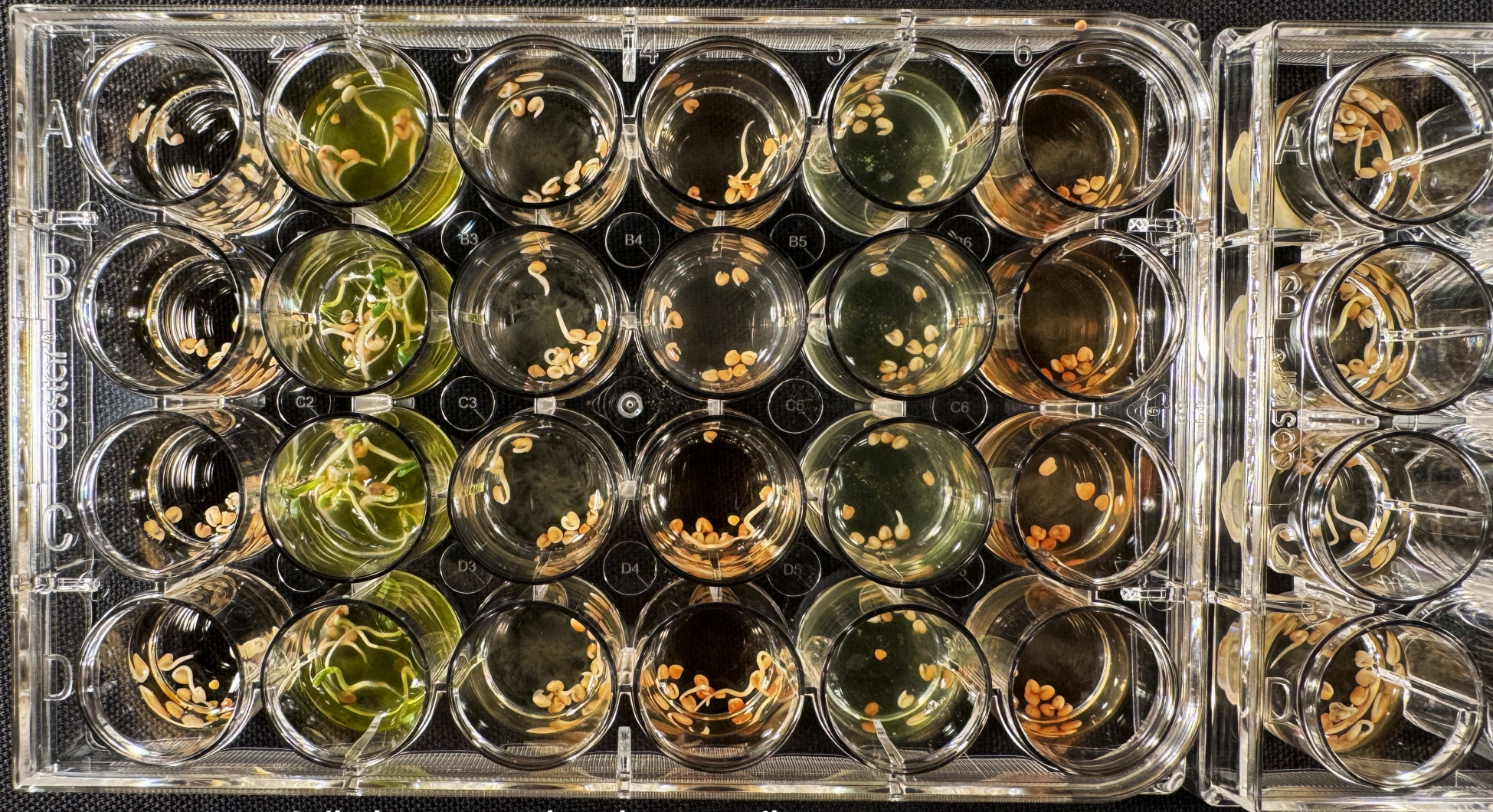
	Germination Rate	Ave. Lateral Root per plant	Root Hairs	Expanding Leaf
Control	85%	1.03	-	0
EnSoil Algae Product	93%	1.36	+	11
Autoclaved Ensoil Algae	77%	1.26	+	2
Boiled Ensoil Algae	93%	1.21	+	0
Ensoil Broth	87%	0.81	+	3
Chlorella Vulgaris	87%	1.25	-	2
Commercial Broth	87%	0.69	+	1

\* Due to fungi killed EnSoil Product treatment in agarose plate experiment, the data of EnSoil Product treatment came from production suspension experiment.

### **Results:**

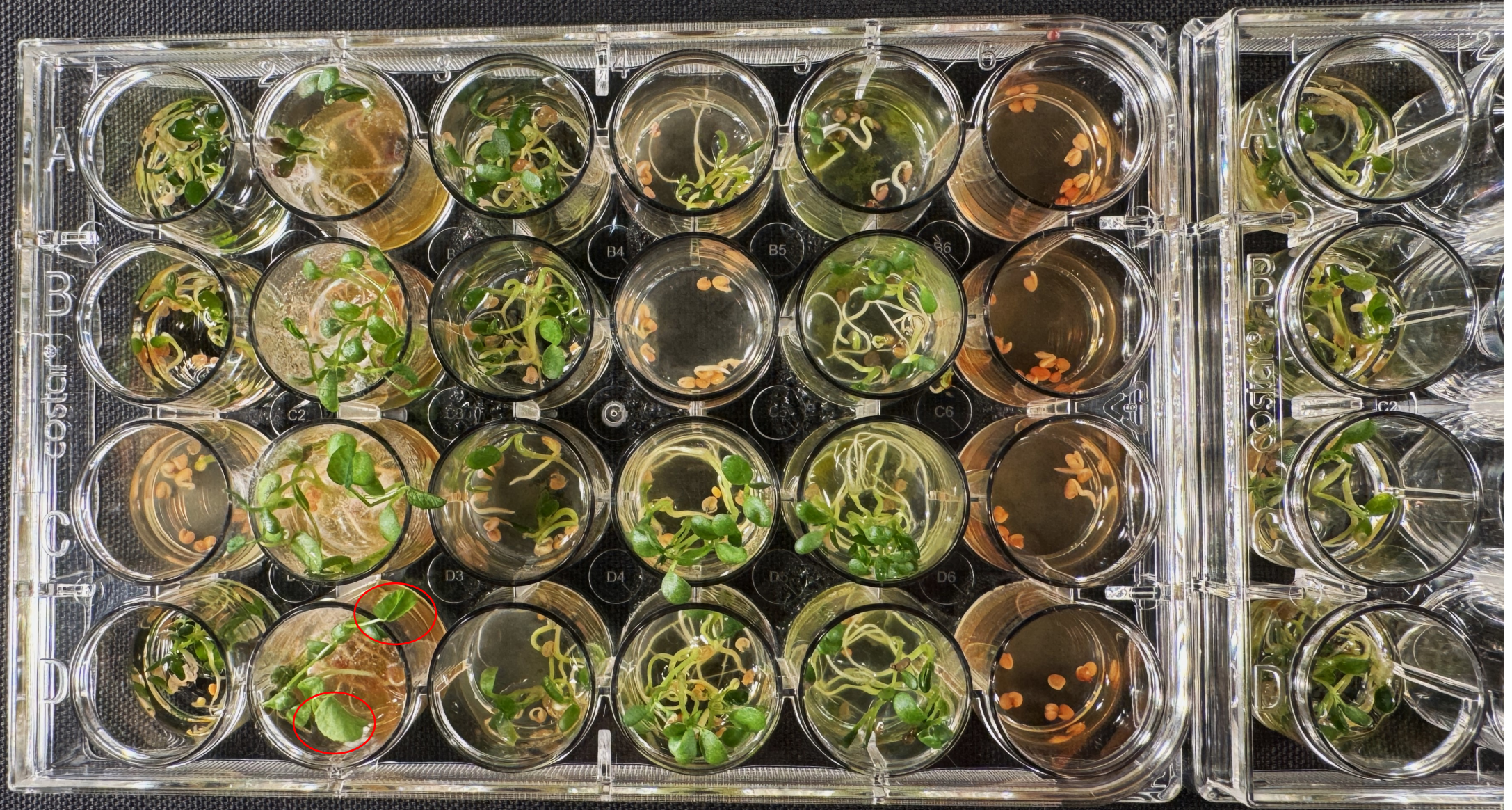
- Among these three treatment, the effect of plant growth promotional effect: EnSoil Algae Product > Autoclaved Ensoil Algae, Boiled Ensoil Algae, Chlorella Vulgaris, and Ensoil Broth > Commercial Broth and control.

# EnSoil Algae product stimulated clover germination and growth in Day 4



Control	EnSoil Algae product	Autoclaved EnSoil Algae	Ensoil Broth	Chlorella Vulgaris	Commercial Broth	Boiled EnSoil Algae

# EnSoil Algae product stimulated clover germination and growth in day 12



Control

EnSoil Algae  
product

Autoclaved  
EnSoil Algae

Ensoil  
Broth

Chlorella  
Vulgaris

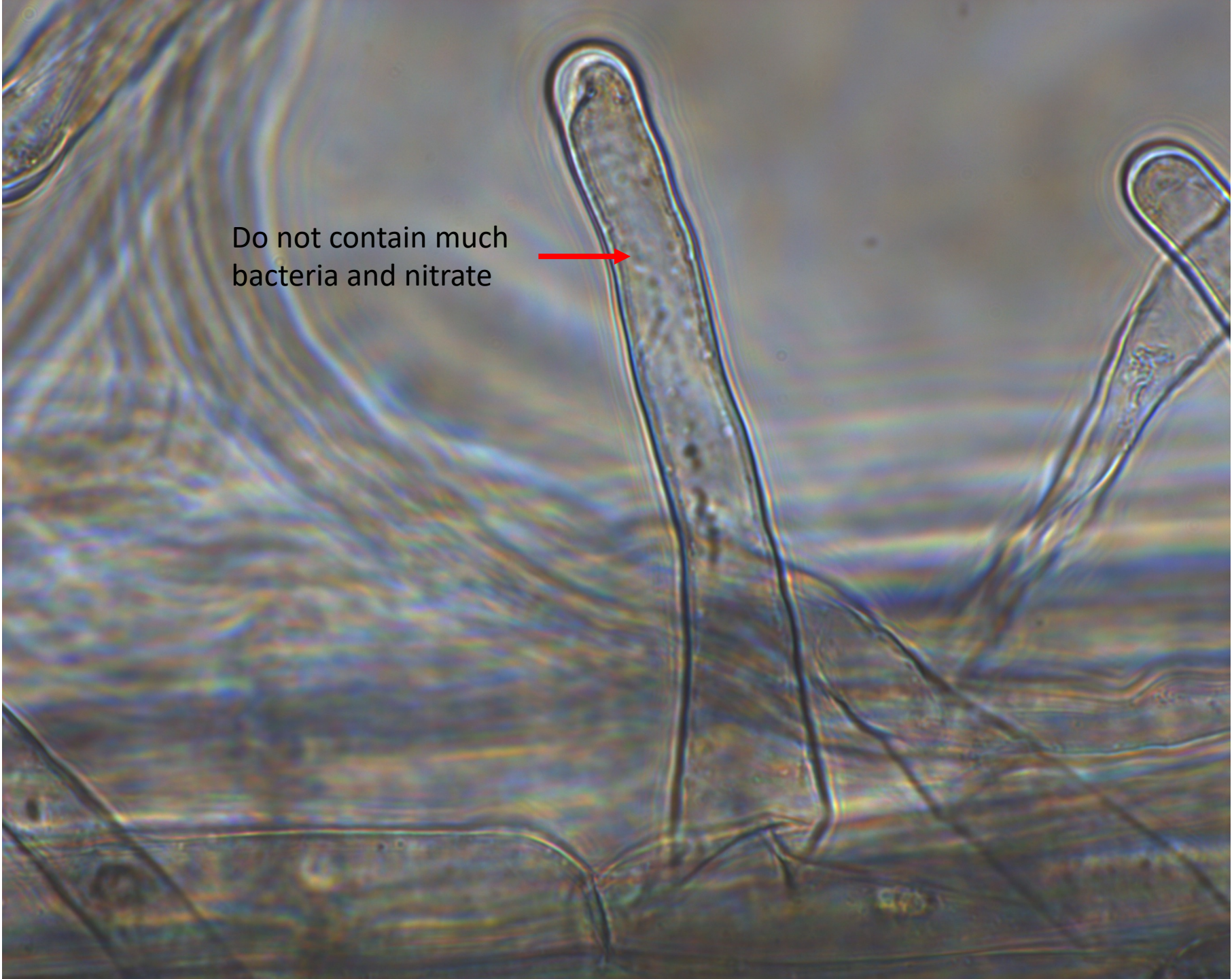
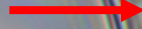
Commercial  
Broth

Boiled  
EnSoil Algae

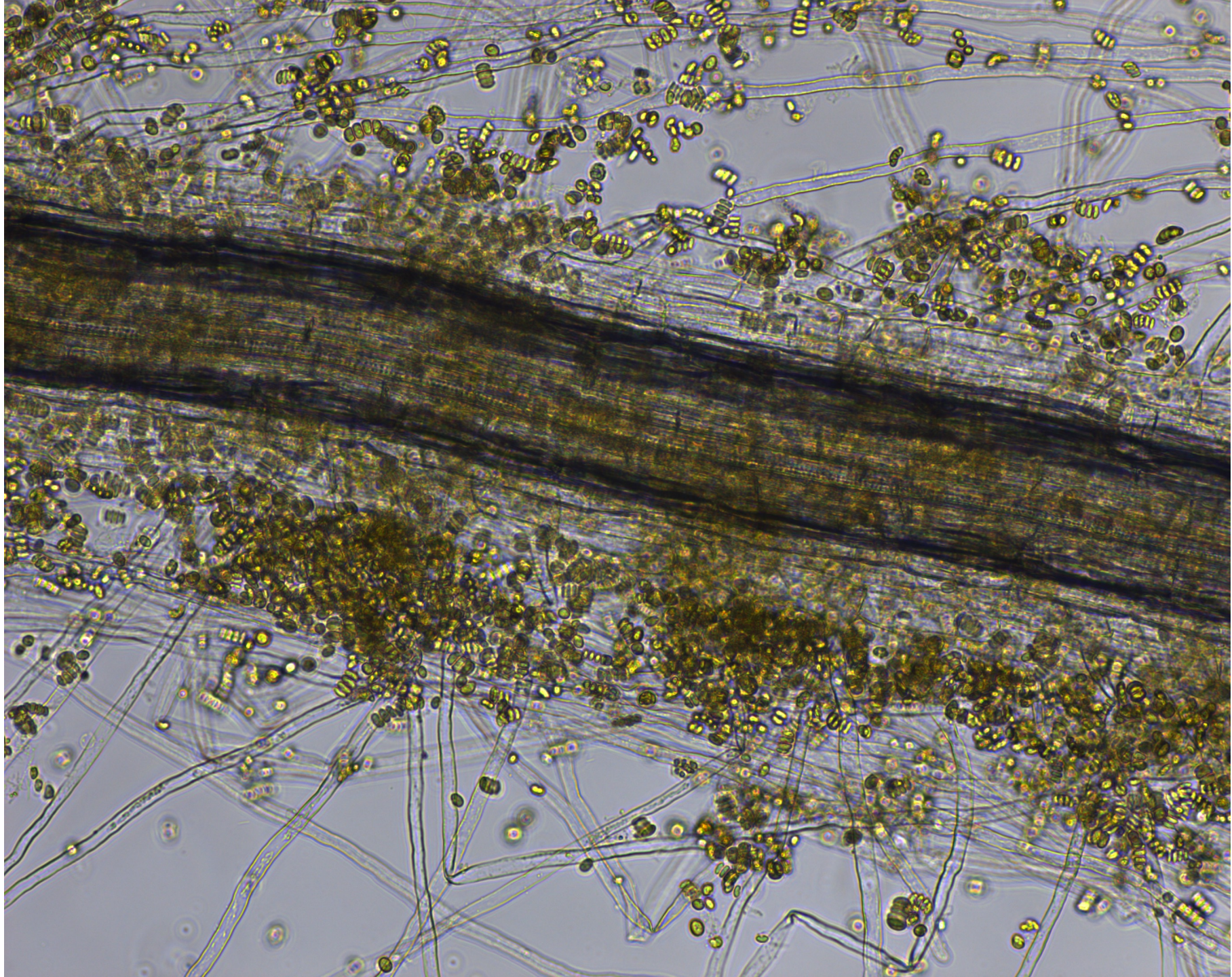
Control

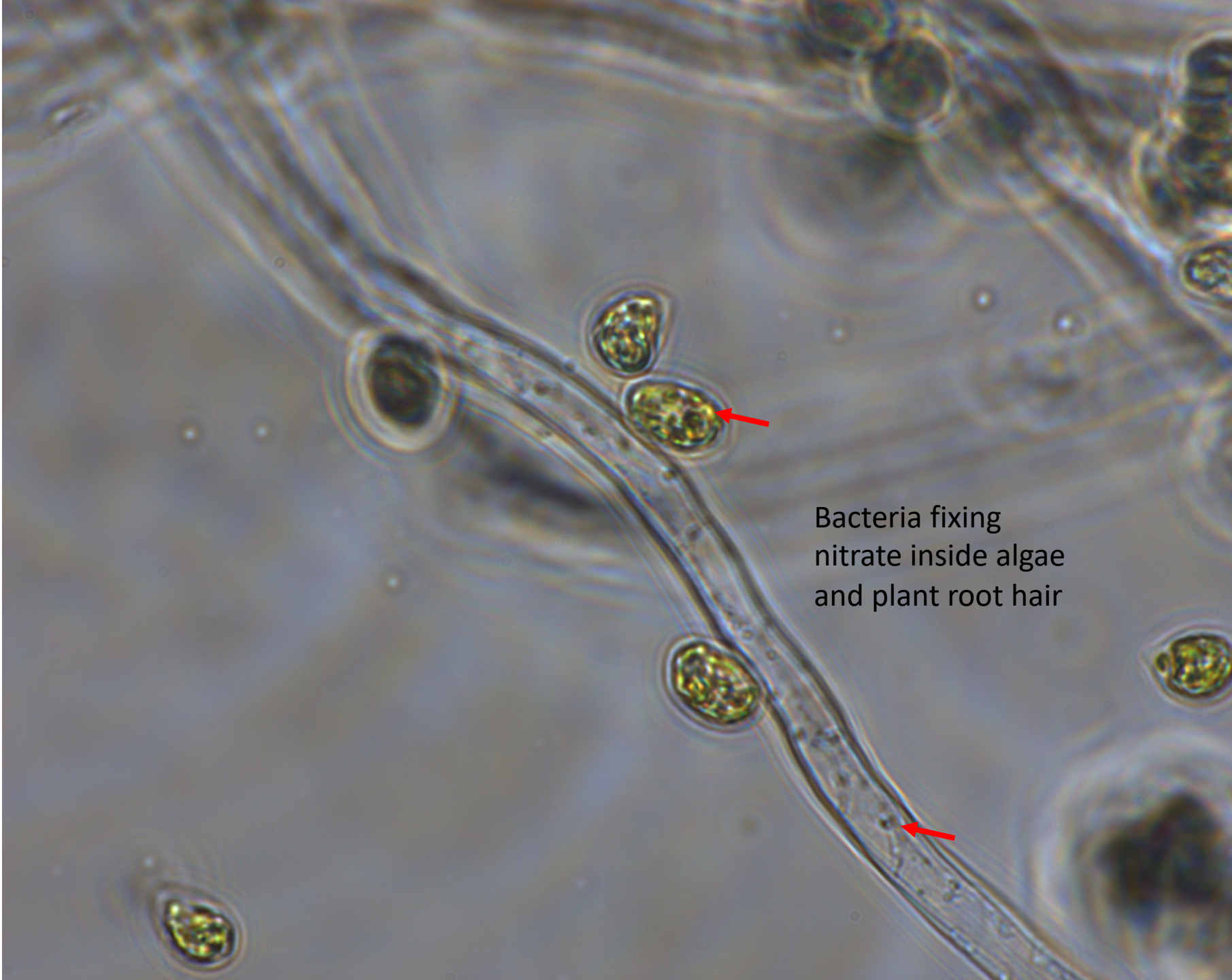


Do not contain much  
bacteria and nitrate

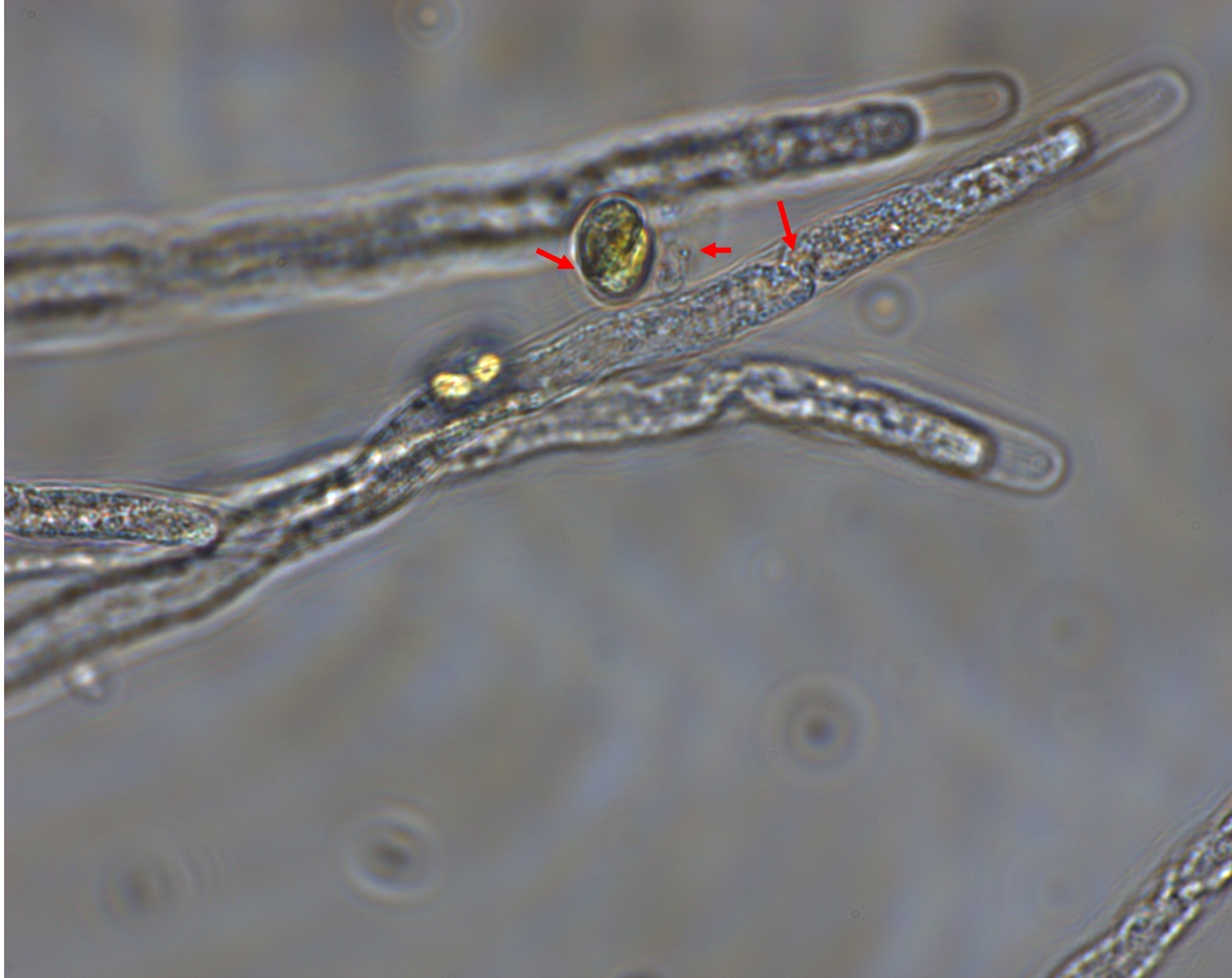


## EnSoil Algae





Bacteria fixing  
nitrate inside algae  
and plant root hair



**Boiled EnSoil Algae**





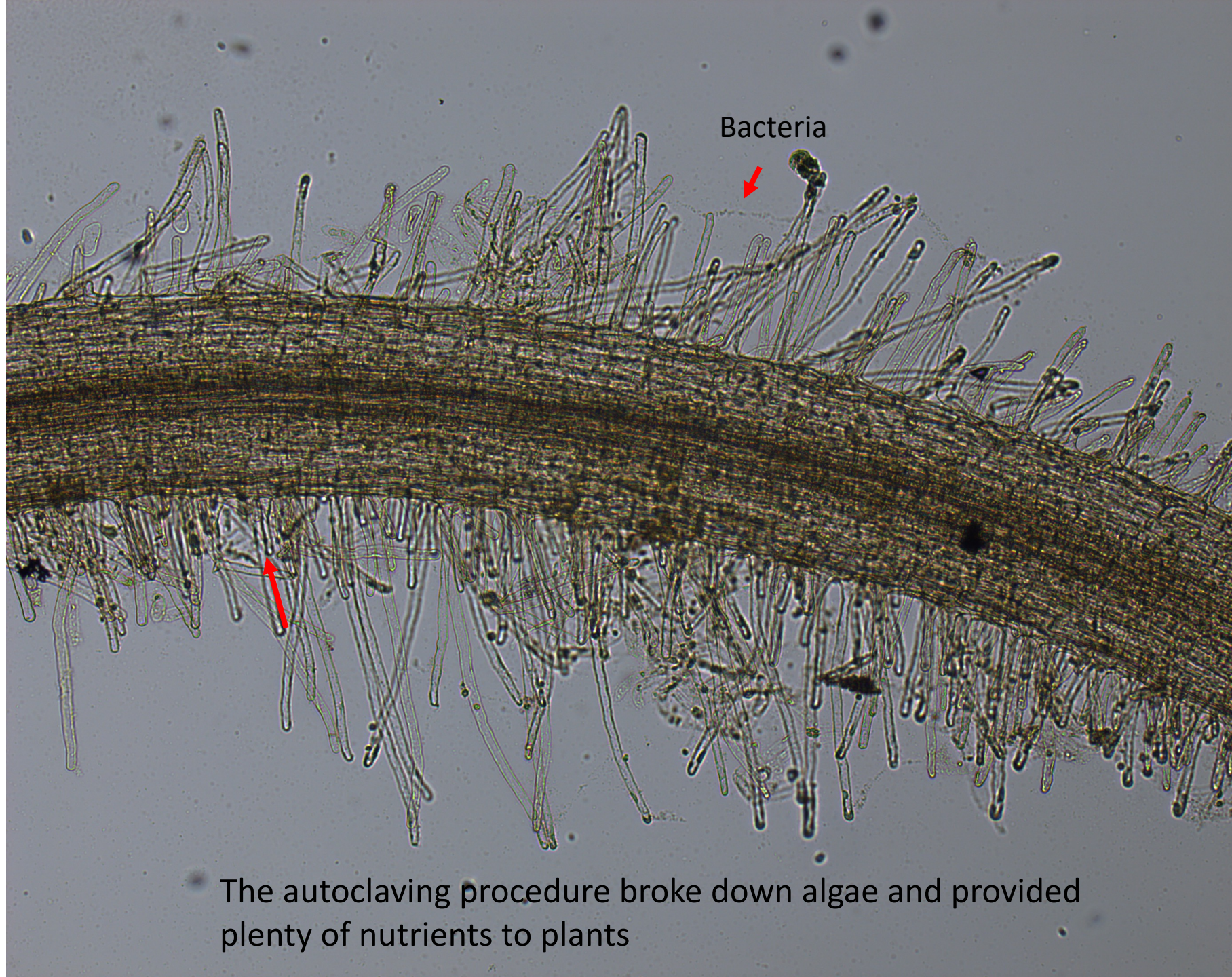
The Boiling  
procedure killed  
algae but not  
bacteria

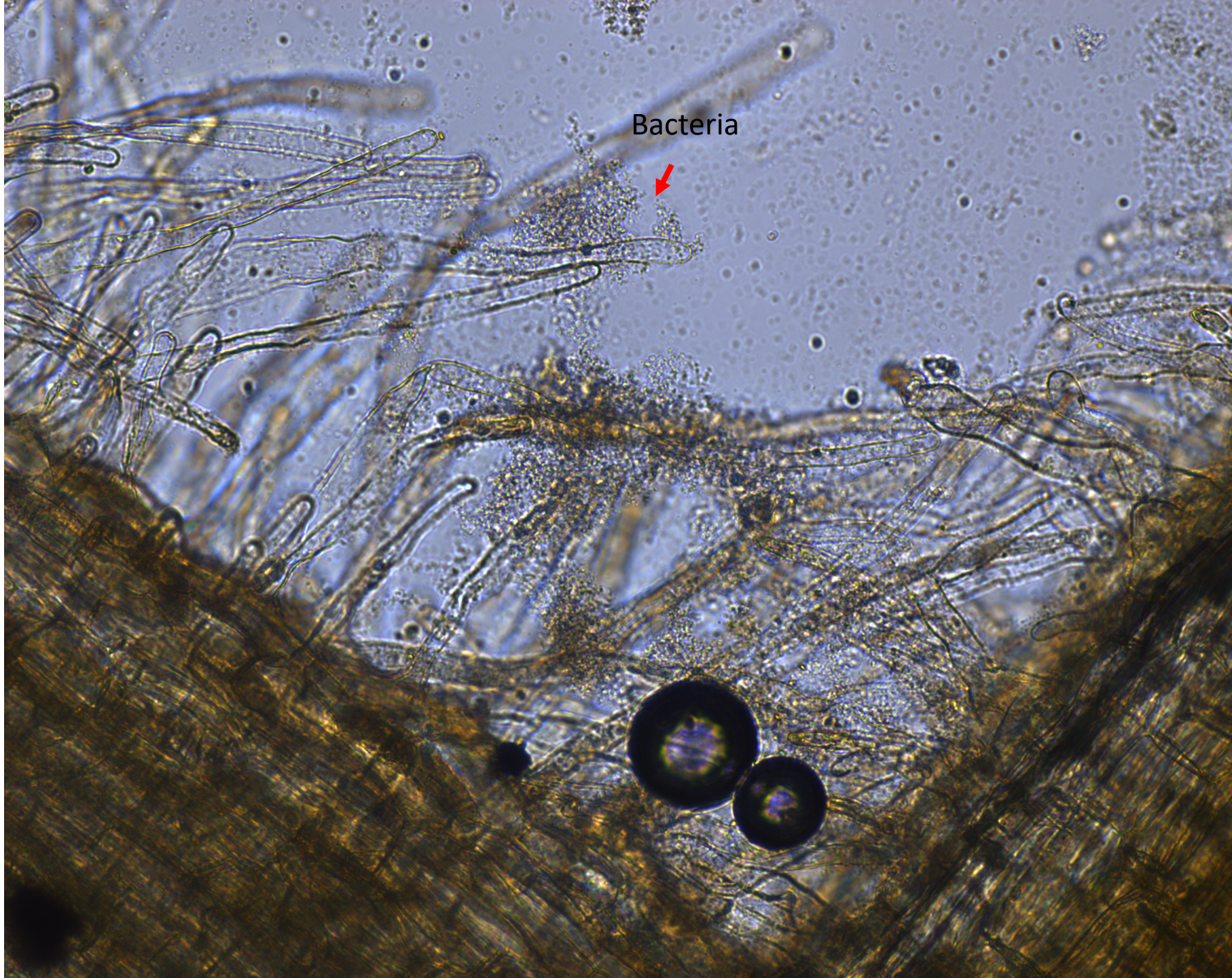
Algae inside  
plant root  
hair cell





## Autoclaved Ensoil Algae





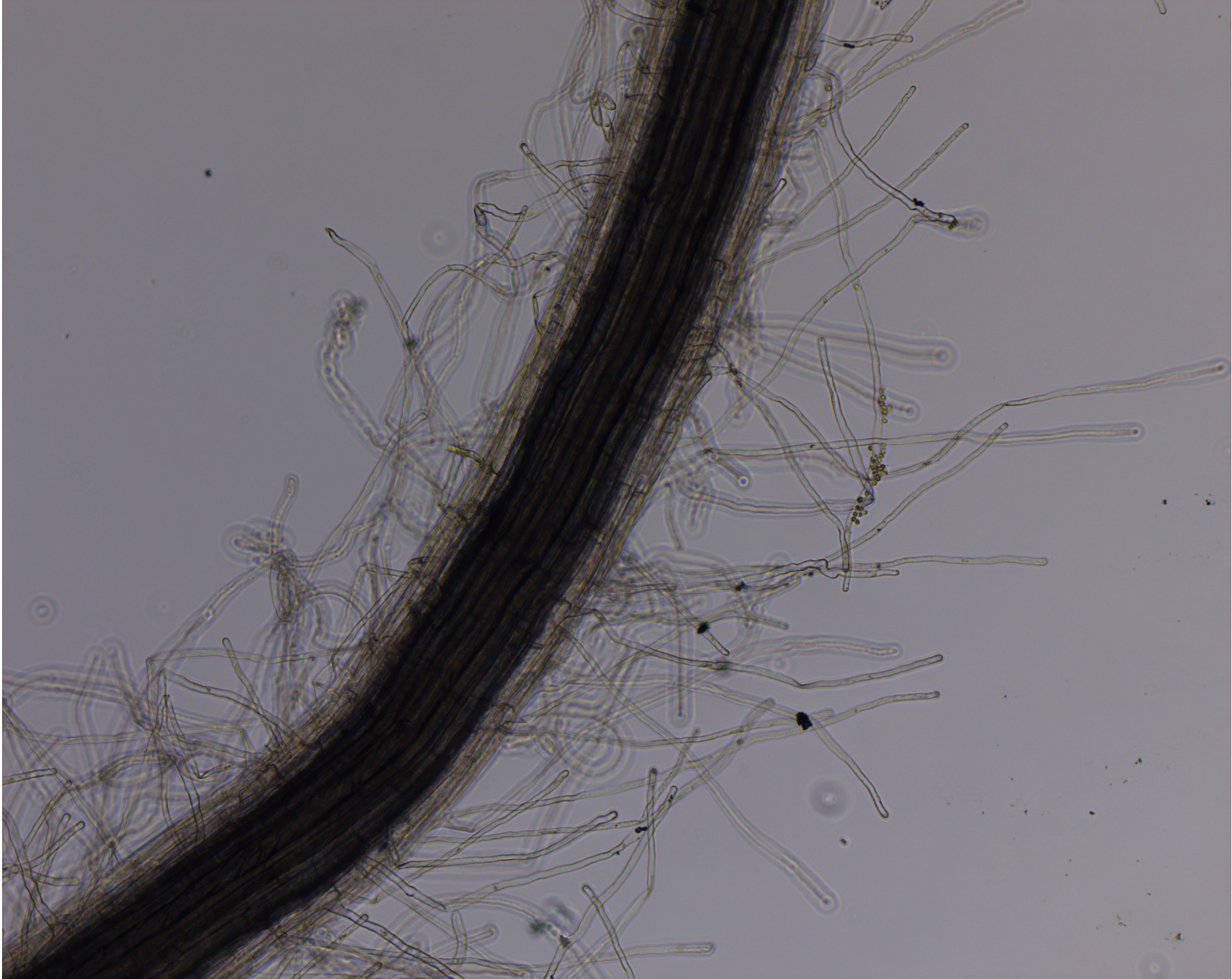
Bacteria



Bacteria



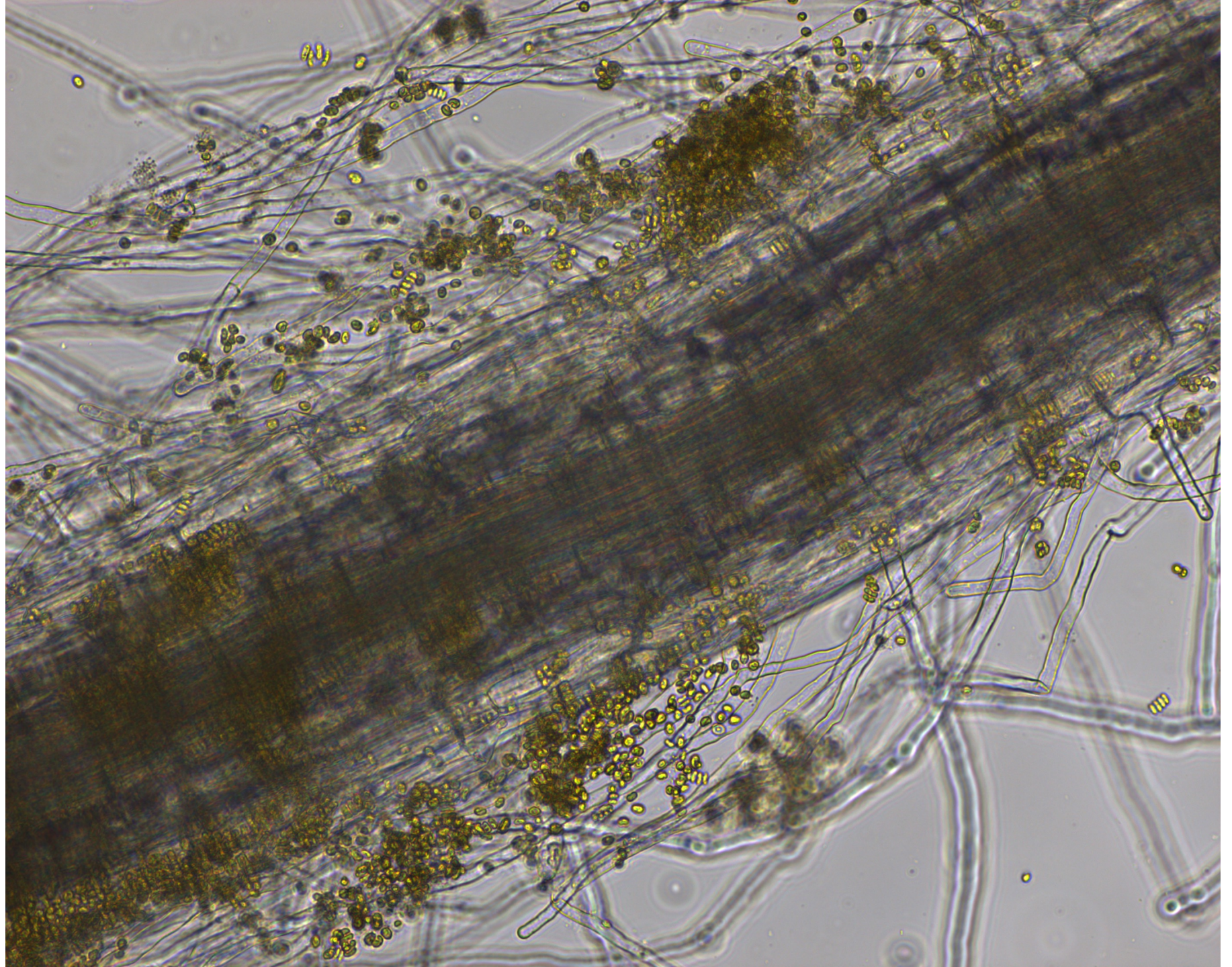
## EnSoil Broth

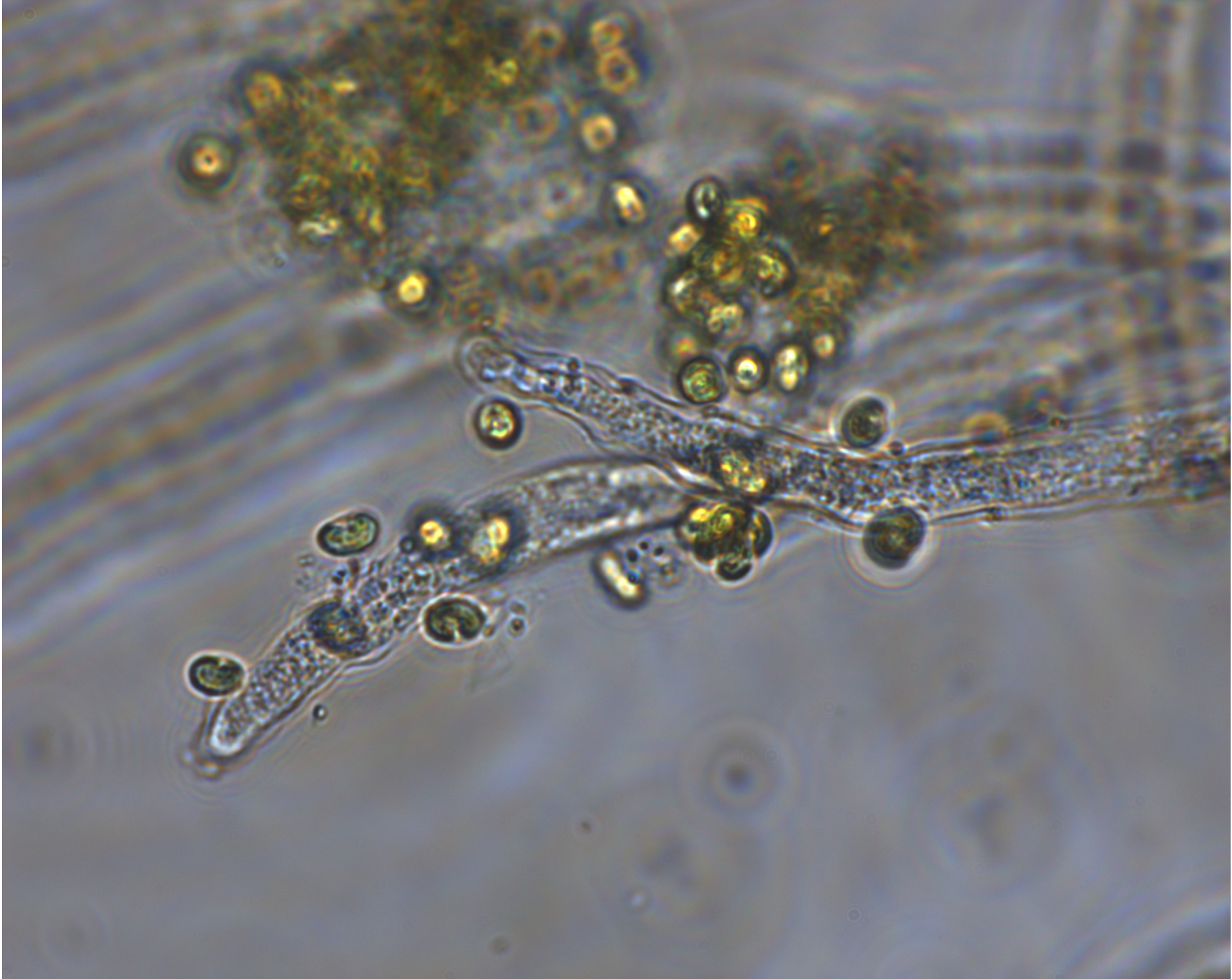




Plenty of nitrate

Chlorella Vulgaris







## Commercial Broth



# Conclusion

- For promoting growth in Creeping bluegrass (*Poa reptans*), Clover (*Trifolium repens*), and Tomato (*Solanum lycopersicum*), EnSoil Algae Product is better than Chlorella Vulgaris. Additionally, EnSoil broth is superior to commercial broth.
- EnSoil Algae Product demonstrated the best performance among the tested three plant species. Its growth-promoting effect stems from both algae and broth. Algae aid in plant branching and root hair growth, while broth supports primary root elongation.
- Dead algae provide nutrients to sustain plants and promote the growth of endophytic bacteria. It's possible that dead algae may be absorbed into plant cells entirely.
- Over time, the growth-promoting effect of EnSoil broth improved, possibly due to the secretion of secondary metabolites by algae.