Stains growth conditions

closely matches species	Category	Growth media	Approximatly growth time on plate or media
Sphingomonas melonis	HCom	King's B	48h
Methylobacterium goesingense	HCom	NBA including 1% Methanol	48h
Duganella zoogloeoides	DCom	NBA	48h
Pseudomonas viridiflava	DCom	NBA	48h
Cystofilobasidium macerans	HCom	PDA	48h
Plectosphaerella cucumerina	DCom	PDA	2-4 weeks ***
Rhogostoma epiphylla CCAP 1966/12	HCom	NCL:PJ	1-2 weeks *
Albugo laibachii Nc14	DCom	Obligate biotroph grow on the plant	**
Methylobacterium goesingense	SynCom	NBA including 1% Methanol	48h
Bacillus altitudinis	SynCom	NBA	48h
Rhizobium skierniewicense	SynCom	NBA	48h
Sphingomonas faeni	SynCom	NBA	48h
Pseudomonas koreensis	SynCom	NBA	48h
Flavobacterium pectinovorum	SynCom	NBA	48h
Microbacterium proteolyticum	SynCom	NBA	48h
Arthrobacter humicola	SynCom	NBA	48h
Frigoribacterium faeni	SynCom	NBA	48h
Aeromicrobium fastidiosum	SynCom	NBA	48h
Nocardioides cavernae	SynCom	NBA	48h
Paenibacillus amylolyticus	SynCom	NBA	48h
Dioszegia hungarica	SynCom	PDA	48h
Sporobolomyces roseus	SynCom	PDA	48h
Rhodotorulakratochvilovae	SynCom	PDA	48h

**Preperation of Rhogostoma epiphylla CCAP 1966/12*

Rhogostoma epiphylla can grow in New Cereal Leaf – Prescott Liquid (NCL:PJ) medium which is provided by Culture Collection of Algae and Protozoa (CCAP https://www.ccap.ac.uk).

Preparation of NCL:PJ Media

1. **Prepare Stock Solutions**
   Begin by preparing the stock solutions as detailed in the table below.

Composition of Prescott´s & James´s stock solutions (PJ).

Stock Solution No.	Ingredient	Amount per 100 ml
1	$CaCl_2.2H_2O$	0.43 g
	KCl	0.16 g
2	K_2HPO_4	0.51 g
3	$MgSO_4.7H_2O$	0.28 g

2. **Dilution of Stock Solutions**
   Take 1 ml of each PJ stock solution (1–3, as listed in the table) and dissolve it in 1 L of deionized water.
3. **Heating and Mixing**
   - Transfer 500 ml of the prepared solution into a microwave-safe container and heat until it begins to boil.
   - Add 1 g of wheatgrass powder to the boiling solution and boil again for approximately 5 minutes.
4. **Refilling Volume**
   - Allow the solution to cool.
   - Refill the missing volume (due to condensation during boiling) with deionized water to bring the total back to 500 ml.
5. **Filtering and Combining**
   - Filter the boiled solution through GF/C filter paper.
   - Combine the filtered solution with the remaining 500 ml of PJ solution.
6. **Final Mixing and Sterilization**
   - Mix the entire solution thoroughly.
   - Autoclave the mixture to sterilize it

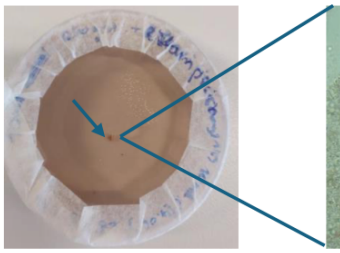
Subculturing of *Rhogostoma epiphylla*

1. **Maintenance of Cultures**
   Rhogostoma epiphylla was cultivated in NCL:PJ media. Initially, new subcultures were prepared by transferring 1 ml of an established *R. epiphylla* culture into a new Petri dish or a 50 ml culture flask filled to half the

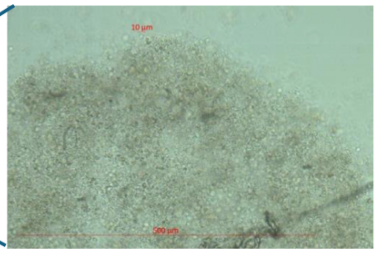
total volume with NCL:PJ media.
Stock plates were transferred to growth chambers set to 16°C with an 8-hour light/16-hour dark cycle for long-term storage, covered with a sheet of paper to protect them from direct light. Older cultures were maintained by replenishing the media every 3-6 months (dependes on the volume of media) weeks.

2. **Scaling Up for *In Planta* Experiments**
   To obtain sufficient cell concentrations for *in planta* experiments, the following strategy was employed:
   - Prepare 10 ml cultures by mixing 1 ml of *R. epiphylla* culture taken from stock plates with 9 ml of NCL:PJ media.
   - Apply one of three treatments:
     1. Add 10 µl of Rifampicin to the culture.
     2. Add diluted *E. coli* strain DH5αpFru46 (grown on NBA media, diluted in 1 ml of nuclease-free water, and 200–500 µl taken).
     3. Combine *E. coli* and Rifampicin.
3. **Incubation**
   The cultures were stored in a paper box at 22°C for 5–7 days until they reached the desired cell concentration. The cell density was checked under a light microscope.
4. **Determination of Cell Concentration**
   - Take 15 µl of the culture suspension and load it onto a Neubauer counting chamber.
   - Observe under a light microscope (epifluorescence Axiophot microscope) (10–20× magnification) to count cells.
   - Calculate the concentration of cells (cells/ml) and adjust to approximately 25 × 10^6 cells/ml in a total volume of 10 ml.
   - **Observation of Patches:**
     During growth, undisturbed plates sometimes formed patches on the liquid surface, visible to the naked eye. Microscopic examination of these patches revealed higher cell concentrations compared to liquid samples from the middle of the culture. These patches can be carefully harvested to obtain a concentrated suspension for experiments.

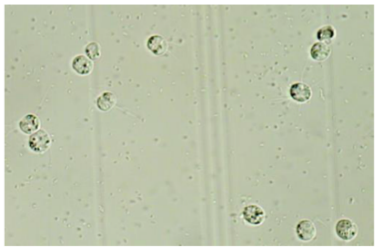
Below is an image illustrating a typical patch:



Patch pointed by arrow



Day 14 –patch of cells under microscope (10x objective)



Single cells (20x objective)

****Preparation of *Albugo laibachii* Nc14 Spores**

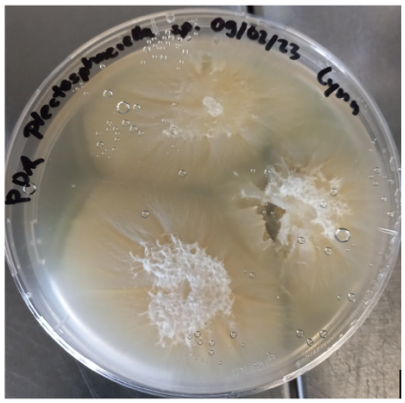
Albugo laibachii Nc14, which had been maintained on *Arabidopsis thaliana* Ws-0 for over a year, was used in this experiment. Infected leaves were collected and placed in a 50 ml Falcon tube, filling approximately one-third to half of the tube with plant material. The tube was then filled with cold tap water.

The tube was placed on a rotator for 30–60 minutes to facilitate spore separation from the leaves. Subsequently, it was stored on ice for 1 hour. After this, the contents were vortexed and filtered into a new Falcon tube to remove debris.

To determine the concentration of *A. laibachii*, 15 µl of the prepared spore suspension was loaded onto a Neubauer counting chamber. Using a light microscope ((epifluorescence Axiophot microscope), intact *A. laibachii* spores were counted, and the spore concentration (spores/ml) was calculated. The final concentration was adjusted to approximately 25 × 10^5 cell/ml in a total volume of 10 ml.

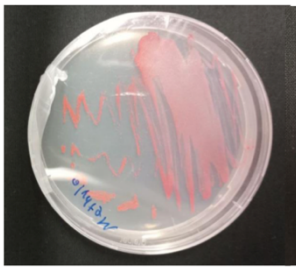
Preparation of *Plectosphaerella cucumerina* Spores

1. **Culturing on PDA Plates**
   Subculture *Plectosphaerella cucumerina* on Potato Dextrose Agar (PDA) plates. Incubate the plates at 22°C for approximately 3–4 weeks. During this period, the fungus will grow and produce hairy hyphae, indicating sufficient spore development. (Refer to the image below for reference.)
2. **Spore Collection**
   - Collect sufficient fungal material from the PDA plate and transfer it to a 50 ml Falcon tube.
   - Add 25 ml of sterile distilled water (NFW). Note: Adjust the volume as needed, but ensure a final volume of 10 ml of spore suspension is prepared.
   - Vortex the tube thoroughly to dislodge and separate the spores into the water.
3. **Spore Concentration Determination**
   - Load 15 µl of the spore suspension onto a Neubauer counting chamber.
   - Use a light microscope (e.g., an epifluorescence Axiophot microscope) to count the spores.
   - Calculate the spore concentration in spores/ml based on the counts.
4. **Final Adjustment**
   Adjust the final spore concentration to approximately 25 × 10^5 spores/ml in a total volume of 10 ml

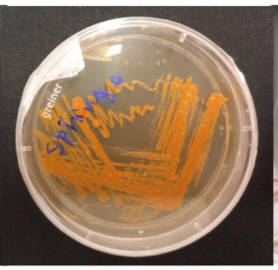


Plectosphaerella cucumerina

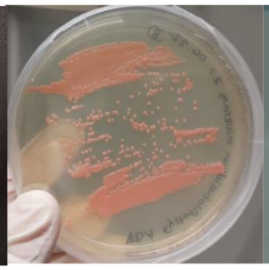
Image of the strains (Hcom and Dcom)



Methylobacterium goesingense



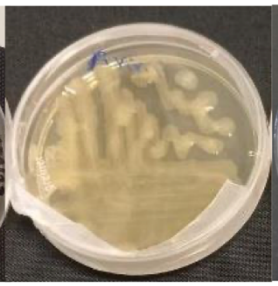
Sphingomonas melonis



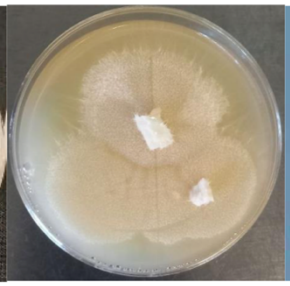
Cystofilobasidium macerans

Rhogostoma epiphylla

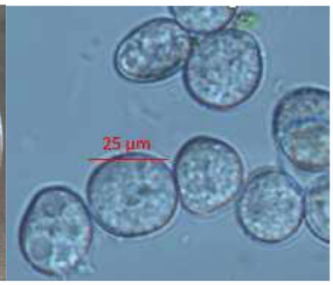
Duganella zoogloeoides



Pseudomonas viridiflava



Plectosphaerella cucumerina



Spors of Albugo laibachii Nc14

Image of SynCom strains