WP3.2 B. Subtilis CFU experiments

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Author: Daniel Steukers
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WEDNESDAY, 7/23/2025

Introduction

We performed a calibration experiment to establish a correlation between optical density (OD_{600}) measurements and the actual colony forming unit (CFU) counts of *B. subtilis*. Cultures were grown to defined OD_{600} values, serially diluted, and plated on agar. After incubation, colonies were counted to determine CFU/mL. This calibration allows us to translate OD readings into reliable estimates of bacterial concentration for use in later experiments.

Team members involved in these experiments: Guglielmo, Daniel

Experiments

We grew ***B. subtilis* 168** overnight in 5 mL of LB broth. The next day, we prepared a **500 mL autoclaved flask with grooves** on the bottom to grow a larger culture.

Estimating initial concentration

We measured the concentration of the overnight culture by making a **1:10 dilution** and measuring its OD600, which was **0.3**. This means the original culture had an approximate OD600 of **3.0**.

Preparing the large culture

We poured **50 mL of LB broth** into the autoclaved flask and added **1 mL of the overnight culture**.

Measuring OD600

1. Take a clean cuvette from the drawer under the large centrifuge.
2. Add **900 µL of LB broth** and place it in the OD600 machine ().
3. Match the arrow on the cuvette with the arrow on the machine, insert it, and press the **left button** to set zero (0.000).
4. Discard the LB broth from the cuvette and add **900 µL of your culture dilution**.
5. Place it in the same orientation and press the **right button** to read the OD600 value displayed on the screen.

Growth check

We monitored the culture to reach OD600 values **0.2 and 0.8**:

- 0th minute → OD600 = **0.05**
- After 20 minutes → OD600 = **0.06** (a bit odd but fluctuations are normal at this stage) (40 min total)
- After 40 more minutes → OD600 = **0.18** (80 min total)
- After 30 minutes ->=0.45 (110 min total)
- After 15 minutes ->= 0.57 (125 min total)
- After 15 minutes->= 0.7 (140 min total)
- After 10 minutes->=0.83 (150 min total)

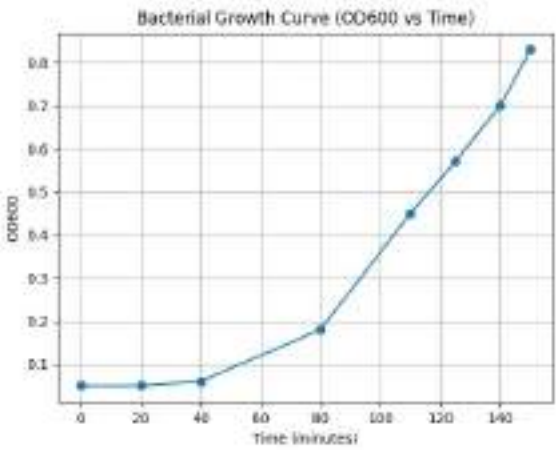
Dilution and plating

From this culture, we made a **10-fold dilution series up to 10⁻6** and plated 50 microliters for dilutions 10-6 to 10-4 at 0.18 and at 0.8 (2 plates each).

RESULTS:

Figure 1: The growth of B.subtilis (OD600 vs time)

OD.png



As expected, the bacteria start in the lag phase and after 1 hour start the exponential growth phase.

CFU_OD.png

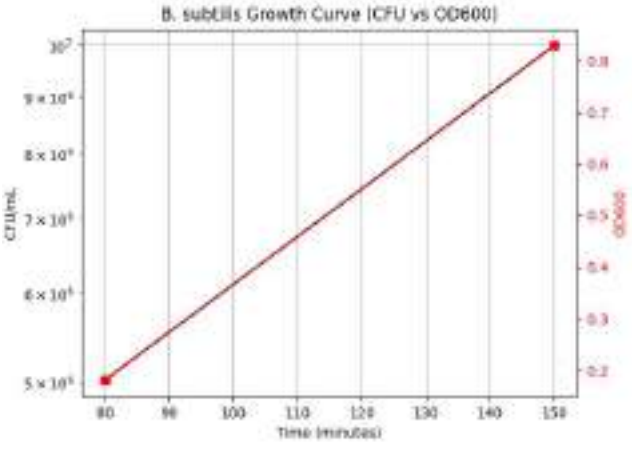


Figure 2: Exponential growth of *Bacillus subtilis* represented by colony-forming units per milliliter (CFU/mL, black, left axis) and optical density at 600 nm (OD600, red, right axis) measured between 80 and 150 minutes. Both parameters increase proportionally during this time window, indicating active exponential growth. The strong correlation between CFU counts and OD600 supports the use of OD600 as a reliable proxy for cell density under our experimental conditions.

In this graph we have correlated the OD600 with the CFU/ml in order to accurately predict the number of colonies we can expect in a precise condition. This will allow us to accurately estimate the efficacy of each nutrient in the benchmark asssay.

Graph

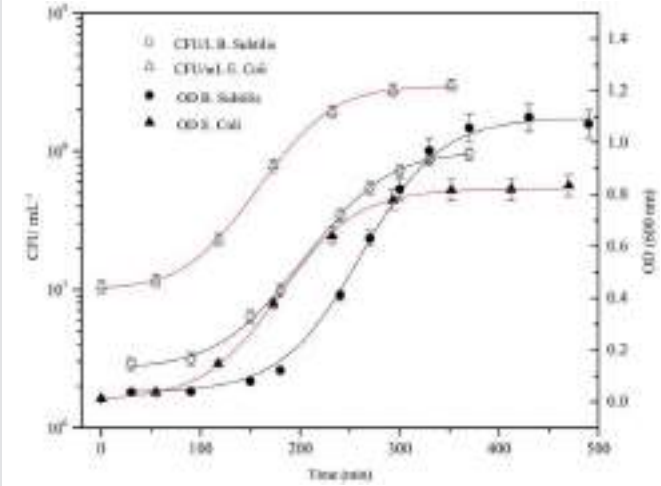


Figure 3. Growth curve of *Bacillus subtilis* monitored over the exponential phase (80–150 min). Cell density was quantified as colony-forming units per milliliter (CFU/mL, black, left axis) and optical density at 600 nm (OD_{600}, red, right axis). Both measurements show a proportional increase with time, highlighting the characteristic exponential growth behavior of *B. subtilis*. The agreement between CFU counts and OD_{600} indicates that OD_{600} is a reliable proxy for bacterial growth under these experimental conditions. Data are shown as mean values with error bars representing the standard deviation of biological replicates. *Adapted from* Massabò, D., Danelli, S. G., Brotto, P., & Prati, P. (2018). *ChAMBRe: A new atmospheric simulation chamber for aerosol modelling and bio-aerosol research. Atmospheric Measurement Techniques, 11*(10), 5885–5900. https://doi.org/10.5194/amt-11-5885-2018

CONCLUSION:
The growth curve obtained for *Bacillus subtilis* demonstrates a clear exponential phase in which both CFU/mL and OD600 increase proportionally with time. When compared to published literature (Figure 3), our data follow the same characteristic trend: a near-linear relationship between CFU counts and OD600 during exponential growth. Although differences in absolute values and time ranges are expected due to variations in strain, culture medium, and experimental conditions, the qualitative agreement confirms that our measurements are consistent with established growth dynamics. This comparability indicates that the experimental setup and methodology were successful in capturing the expected growth behavior of B. subtilis which is sufficient for our purpose.

MONDAY, 7/28/2025

We repeated this experiment again today, same exact setup only this time we used the nano star instead of the other OD measurement device.

We grew **B. subtilis 168** overnight in 5 mL of LB broth. The next day, we prepared a **500 mL autoclaved flask with grooves** on the bottom to grow a larger culture.

Estimating initial concentration

We measured the concentration of the overnight culture by making a **1:10 dilution** and measuring its OD600, which was **0.1**. This means the original culture had an approximate OD600 of 1**.0**.

Preparing the large culture

We poured 100 **mL of LB broth** into the autoclaved flask and added **1 mL of the overnight culture**.

Measuring OD600

1. Take a clean cuvette from the drawer under the large centrifuge.
2. Add **900 µL of LB broth** and place it in the OD600 machine ().
3. Match the arrow on the cuvette with the arrow in the nanostar, then press the blank measurement button on the screen.
4. Discard the LB broth from the cuvette and add **900 µL of your culture dilution**.
5. Place it in the same orientation and press the measure cuvette button on the screen.

Growth check

We monitored the culture every 30 min and aimed for the stationary phase

- Initially -> OD600 = 0,01
- After 30 minutes → OD600 = **0.017**
- After 60 minutes → OD600 = **0.019**
- After 90 minutes → OD600 = **0.048**
- After 120 minutes ->=0.129
- After 150 minutes ->= 0.347
- After 180 minutes->= 0.955
- After 210 minutes->= 1.657
- After 240 minutes -> OD = 2.659

Dilution and plating

Each time we measured OD we diluted until 10^{-5} and plated this dilution, all these plates were placed in the 37°C to incubate overnight and count colonies the next day.

RESULTS:

Table 1: This table presents the growth dynamics of a bacterial culture monitored over 240 minutes using both optical density (OD) and colony-forming units (CFU).

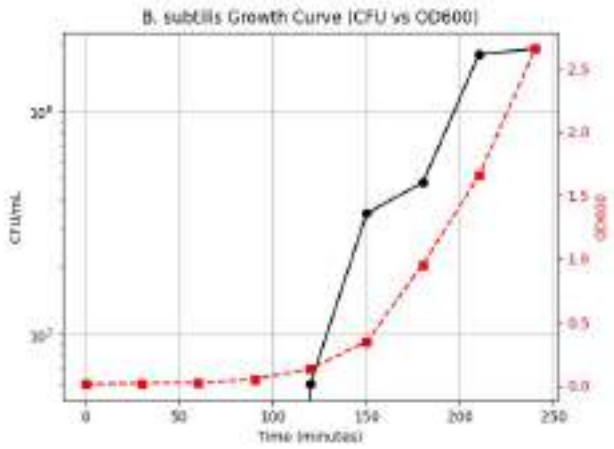
Table1

	Time (m)	OD	CFU (dilution)	CFU (original)
1	0	0.01	0	0
2	30	0.017	0	0
3	60	0.019	0	0
4	90	0.048	0	0
5	120	0.129	6	6.0e+6
6	150	0.347	35	3.5e+7
7	180	0.955	48	4.8e+7
8	210	1.657	180	1.8e+8
9	240	2.659	191	1.91e+8

At the start (0–90 minutes), OD values remain low (0.01–0.048) and no colonies are detected, indicating a lag phase where cells adapt to the environment. From 120 minutes onward, both OD and CFU increase sharply, marking the onset of exponential growth. OD rises from 0.129 at 120 minutes to 2.659 at 240 minutes, while CFU (diluition) increases from 6 to 191 over the same period. This correlation confirms that OD measurements reliably reflect bacterial proliferation, with a clear transition from adaptation to rapid cell division.

Figure 4: Graph showing the correlation between time, OD600, and CFU/mL.

Figure_1.png



CONCLUSION:

As shown in figure 4 both OD600 and CFU measurements in our experiment displayed the expected growth dynamics of *B. subtilis*, closely resembling those reported in the literature (Figure 3). In our data, OD600 remained low during the first 90–120 minutes, consistent with the lag phase where CFU values also stayed nearly constant at ~10^6 CFU/mL. After this period, both parameters increased sharply, with OD600 rising from ~0.1 at 120 minutes to ~2.6 at 240 minutes, while CFU expanded by nearly three orders of magnitude, reaching close to 10^9 CFU/mL. This coordinated rise demonstrates the classic exponential growth phase and mirrors the trends seen in published curves, where OD and CFU increase in parallel after a similar lag phase. The main difference lies in the experimental timeframe: the literature extends to ~500 minutes and captures the stationary phase plateau, while our dataset stops at 240 minutes, just before such stabilization occurs. Overall, the coherence between OD and CFU trends in both datasets confirms that our measurements align well with established growth behavior of *B. subtilis*. Moreover, Figure 4 and Figure 2 have similiar values showing that there is reproducibility in the B. Subtilis growth.

WP3.3 Phytophthora revival

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Author: Daniel Steukers
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WEDNESDAY, 8/20/2025

Introduction

Wednesday 20/8 we received P. capsici & P.palmivora in glass tubes form UC Louvain. We tried various methods of propagating this species for further use and easier handling.
Team members involved in these experiments: Daniel

Methods

1. From the tubes we received from UC Louvain we tried 3 methods to 'revive' the phytophthora in our lab
    a. Streaking multiple times in a zigzag motion along the full plate
    b. Streaking twice in parallell lines
    c. Cutting a piece of mycelium

Extra info: By streaking multiple times we mean the method used for culturing bacteria, we dipped a sterile loop inside the UCL tube and streaked it out - not the best method since mycelium will start to grow from everywhere.
When streaking twice we dipped the loop in the tube and streaked two lines parallell to each other - Results in normally lower mycelium propagating places.

Incubation

- Plates incubated at 27°C in RESHAPE biotech incubator.
- Imaging set to every 120min for 4 days.

Results

image.png

a)The top 4 are the ones we streaked a lot (like bacteria), b)The second row are the mycelium blocks and c) the third row is the one we streaked twice

Since the streaking twice method did not work for both phytophthora species we used the middle row to make a better starting plate for further experiments.

WP3.4 Benchmark growths

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MONDAY, 8/25/2025

Rationale

Our aim is to find out which agar is best for both *B. subtilis*- and *Phytophthora* mycelium growth. These experiments will thus determine which agar to use during our competition assays.
Team members involved in these experiments: Guglielmo, Daniel, Aditya, Josh

Preparation:

Four days before experiment: Plated mycelium plugs of *P. capsici* and *P. palmivora* on PDA plates. These serve as stock cultures for benchmark growth experiments.
One day before the experiment: Started overnight culture of *B. subtilis* from glycerol stock in LB broth.

Experimental details:

Agar prepared according to protocol:

- Carrot agar (CA)
- V8 juice agar
- Pea sucrose agar (PSA)
- Potato dextrose agar (PDA)

Supplementation: 3 g xylose dissolved in 20 mL distilled water, filter sterilized (0.22 µm), added to each agar, after the agar was able to cool off to a temperature comfortable to pour.
Agar poured into SBS 6-well plates, filled to approx. half the well.
Inoculation:

- *Phytophthora (Palmivora and Capsisci)*: Mycelium plugs (5 mm) obtained by using cork borers placed in the center.
- *B. subtilis*: 50 µL of 10^{-5} dilution from OD600 = 0.4 culture, spotted onto beads which spread the bacteria around.

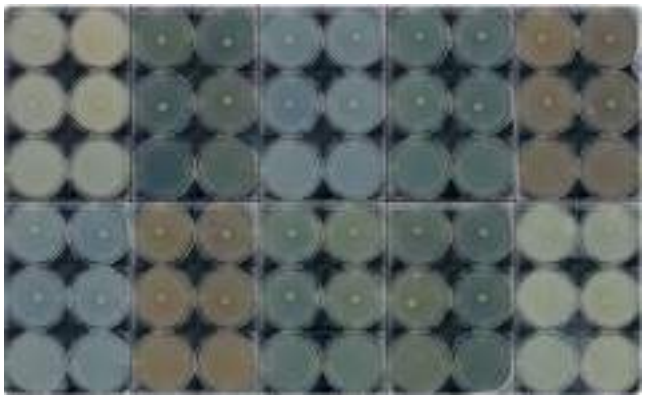
Replicates: 2 plates per condition, having each 2 wells per condition. 4 wells per condition in total.
Incubation

- Plates incubated at 27°C in RESHAPE biotech incubator.
- Imaging set to every 30 min for 4 days.

Results

- Raw images:

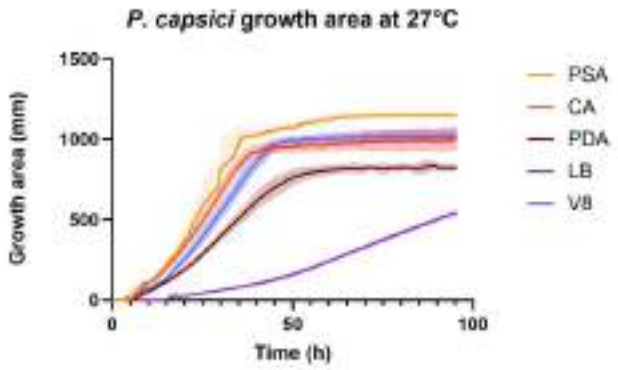
image.png



This is the visual of the machine at the start of measuring. Each plate contains at the top rows: *P. palmivora,* in the middle rows: *P. capsici* and in the bottom rows: *B. subtilis*

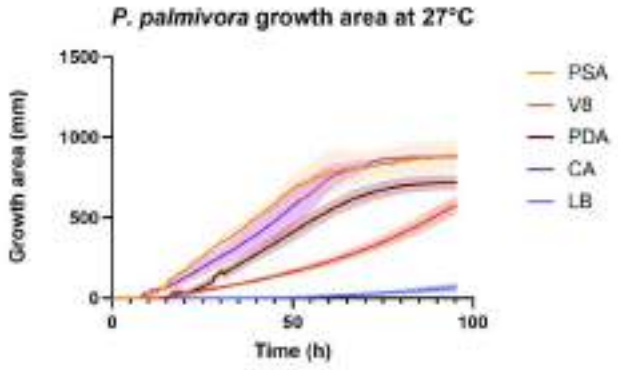
- Colony growth measurements:

PC (27) with CI.jpg



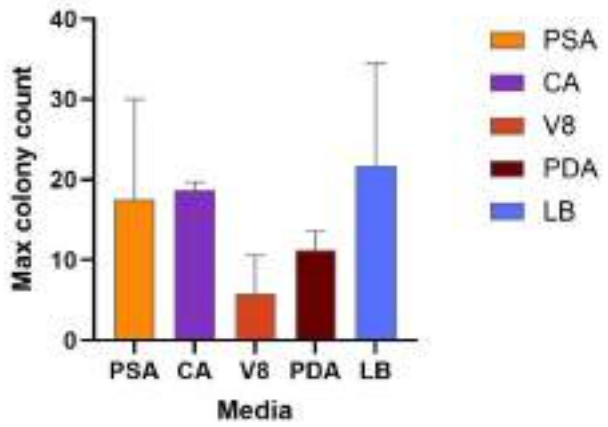
Growth of *P. capsici* on different culture media. Mean colony diameter is shown with 95% confidence intervals (CI). *P. capsici* exhibited the highest growth on PSA agar compared to other tested media.

PP (27) with CI.jpg



Growth of *P. palmivora* on different culture media. Mean colony diameter is shown with 95% confidence intervals (CI). It is less clear what media *P. palmivora* prefers growth on, but it is very similar between CA- & PSA agar

B. subtilis max colony count at 27°C.jpg



Growth of *B. subtilis* on different culture media. Mean colony count is shown with error bars. A expected count between 20~50 was expected, this would be our measure of how well the media is able to perform.

THURSDAY, 8/28/2025

Rationale

To determine the effect of agar type on *B. subtilis- and Phytophthora* mycelium growth at a higher incubation temperature (30°C).

This experiment is a repeat of **EXP-7-30-2025**, with the same setup, media preparation, and inoculation procedure.

Changes Compared to Previous Experiment

- **Incubation temperature increased to 30°C** (instead of 27°C).
- All other procedures, materials, and volumes remain the same as in **EXP-7-30-2025**.

Experimental Details

- **Inoculum**: *B. subtilis*, 50 µL of 10^{-5} dilution from OD600 = 0.4 overnight culture.
- **Media**: PDA agar + xylose, LB agar + xylose, V8 agar + xylose, PSA agar + xylose and CA agar + xylose
- **Plate format**: SBS 6-well plates, filled to ~half volume.
- **Replicates**: 2 plates per condition, having each 2 wells per condition. 4 wells per condition in total.

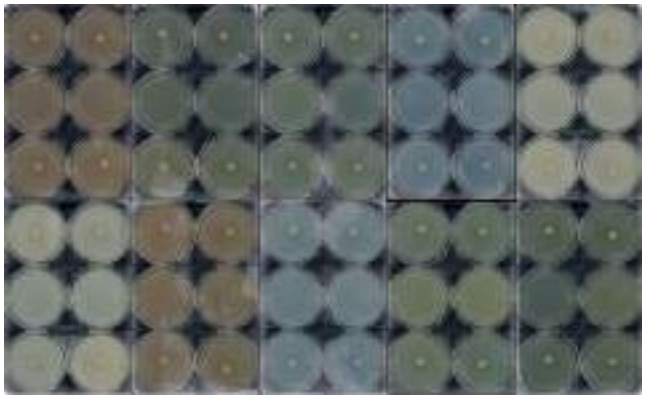
Incubation

- Plates incubated at **30°C** in RESHAPE biotech incubator.
- Images taken every 30 min for 4 days.

Results

- Raw images:

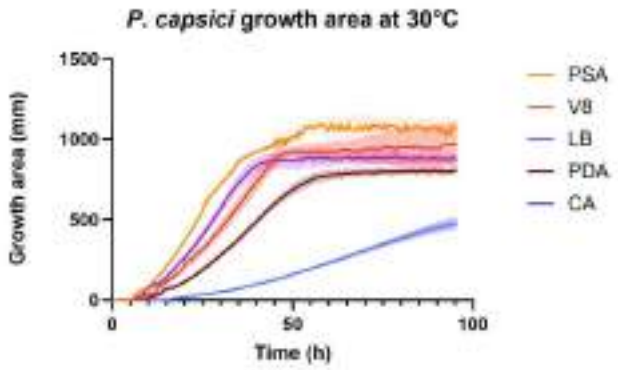
image.png



This is the visual of the machine at the start of measuring. Each plate contains at the top rows: *P. capsici,* in the middle rows: *B. subtilis* and in the bottom rows: *P. palmivora*

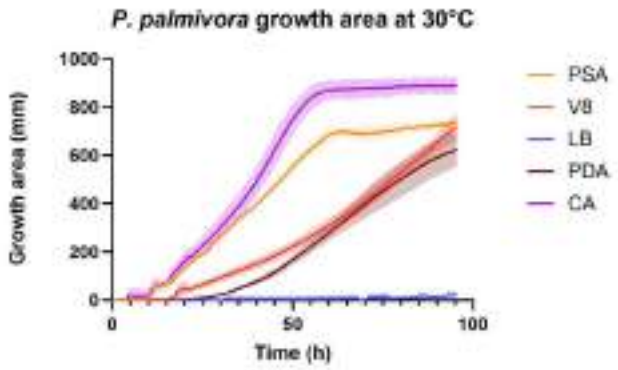
- Colony growth measurements:

PC (30) with CI.jpg



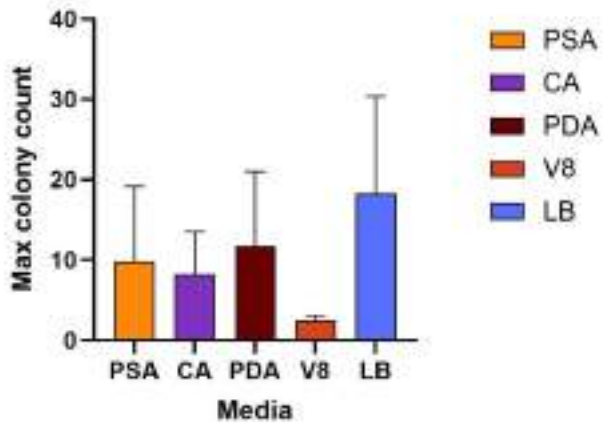
Growth of *P. capsici* on different culture media. Mean colony diameter is shown with 95% confidence intervals (CI). *P. capsici* exhibited the highest growth on PSA agar compared to other tested media.

PP (30) with CI.jpg



Growth of *P. palmivora* on different culture media. Mean colony diameter is shown with 95% confidence intervals (CI). *P. palmivora* exhibited the highest growth on CA agar compared to other tested media

B. subtilis max colony count at 30°C.jpg



Growth of *B. subtilis* on different culture media. Mean colony count is shown with error bars. A expected count between 20~50 was expected, this would be our measure of how well the media is able to perform.

MONDAY, 9/1/2025

Rationale

This is a replicate experiment of **EXP-7-30-2025** with the same setup, media preparation, and inoculation procedure. This to make sure we have robust data

Reference to Previous Protocol

This experiment is a repeat of **EXP-7-30-2025**, with the same setup, media preparation, and inoculation procedure.

Changes Compared to Previous Experiment

- No changes this is a repeat of **EXP-7-30-2025**.

Experimental Details

- **Inoculum**: *B. subtilis*, 50 µL of 10^{-5} dilution from OD600 = 0.4 overnight culture.
- **Media**: PDA agar + xylose, LB agar + xylose, V8 agar + xylose, PSA agar + xylose and CA agar + xylose
- **Plate format**: SBS 6-well plates, filled to ~half volume.
- **Replicates**: 2 plates per condition, having each 2 wells per condition. 4 wells per condition in total.

Incubation

- Plates incubated at 27°C in RESHAPE biotech incubator.
- Images taken every 30 min for 4 days.

Results

No data generated, incubator got interrupted due to loss of internet connection

Conclusion

Across our benchmark growth assays, both Phytophthora species reached their maximal growth area at 27°C. P. capsici showed a clear preference for PSA agar, whereas P. palmivora exhibited similar growth on CA and PSA. For Bacillus subtilis, colony counts were also higher at 27°C, and growth on PSA was closer to the LB control compared to the other media. Based on these observations, PSA was selected for subsequent experiments.

Carrot agar (CA)

Introduction

Get started by giving your protocol a name and editing this introduction.

Materials

- 12g potatoes (diced)
- 12g carrots (diced)
- 9g agar
- 600mL distilled water

Procedure

Prepare extract

- [ ] 1. simmer potatoes and carrots in ~300-400mL distilled water for 30 min
- [ ] 2. Mash during or after boiling
- [ ] 3. Strain through cheesecloth to remove solids

Combine

- [ ] 4. Add firltrate to a flask, add agar and bring to 600mL volume with distilled water

Sterilize

- [ ] 5. Autoclave at 121°C for 15 min

Potato dextrose agar (PDA)

Introduction

Get started by giving your protocol a name and editing this introduction.

Materials

- 39g of Merck PDA powder
  -

Procedure

Weigh

Dissolve

- [ ] 1. Add the powder to ~900mL of distilled water in a flask or beaker
- [ ] 2. Stir and heat gently until fully dissolved **(no clumps!!)**

Adjust volume

- [ ] 3. Bring the total volume to 1L by adding distilled water

Sterilize

- [ ] 4. Autoclave at 121°C for 15min
- [ ] 5.

Pea sucrose agar (PSA)

Introduction

Get started by giving your protocol a name and editing this introduction.

Materials

- 96g frozen garden peas **(no salt!!)**
- 3g sucrose
- 9g agar
-
  -

Procedure

Pea extract

- [ ] 1. Blend the peas with ~400mL-300 distilled water until very smooth
- [ ] 2. Boil gently for 15-20min
- [ ] 3. filter through cheesecloth

Combine

- [ ] 4. Add pea filtrate, 3g sucrose, agar
- [ ] 5. make up the volume of 600mL

Sterilize

- [ ] 6. Autoclave 121°C for 15 min
- [ ] 7.

V8 juice agar

Introduction

Get started by giving your protocol a name and editing this introduction.

Materials

- 120mL V8 juice (unfiltered)
- 5g $CaCO_3$
- 9g Agar
- 480mL tap water

Procedure

20% clarified V8-medium

1. Add 5 g of CaCO3 to 340 mL V8-juice in a beaker containing a stir bar.
2. Stir for at least 15 minutes.
3. Aliquot into 50 mL Falcon tubes, making sure the tubes will be balanced in centrifuge - either by weighing or using gradations of tubes
4. Spin tubes in the benchton centrifuge at 10,000 rpm for 15 minutes.
5. Transfer clarified V8-medium to 50 mL aliguots for storage at -20 ℃ (+ label!).
6. Place in -20 °C.

Making of 20% CV8-medium

7. 60mL clarified V8
8. 240 mL distilled water
9. 4,g agar for 300mL.
10. Mix and autoclave (15 min at 121 ℃).