Wet & Dry Lab Notebook

Extraction of Piperamides: Maceration
February 3, 2024

This procedure is based on the Piperamide Extraction protocol.

The extraction began by grinding 500g of black pepper. Initially, a mortar was used, but for practical reasons, it was decided to use a blender instead. The blender was thoroughly cleaned using soap, detergent, and chlorine solutions, with the help of a sponge and brush.

The grinding was done in two batches due to the blender's capacity. In both cases, the pepper was ground until a relatively fine powder was obtained, although there was some heterogeneity in the milling due to the blade grinding method.

Maceration Containers with Freshly Ground Pepper

The ground pepper was transferred to two maceration containers. Initially, only one container was intended to be used, but its volume was insufficient.

Approximately 680 ml of 96% ethanol was added (the volume was less than the planned 750 ml, as the bottles of alcohol held slightly less than 500 ml, and a small amount of alcohol was lost due to spills). The mixture was then stirred with a disposable PP spoon that had been previously washed.

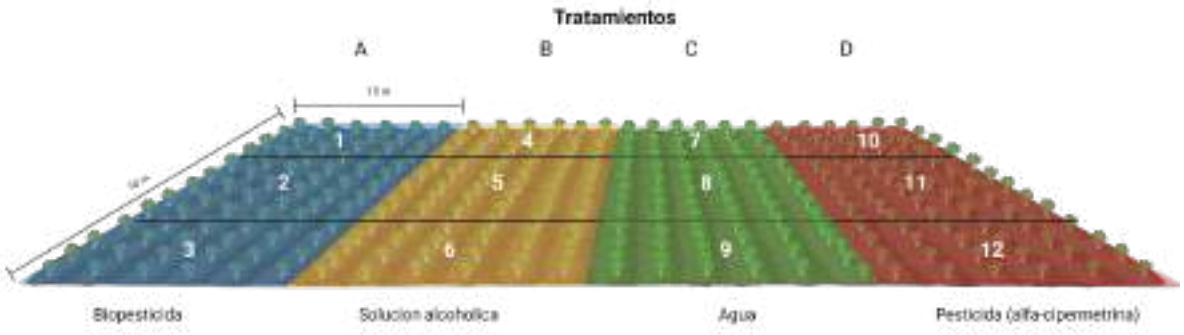
The two maceration containers were stored in a dark place and occasionally stirred.

Field Tests: Planting
February 5, 2024

On February 4, 2024, the corn planting for the field tests of the biopesticide was carried out. The process began with plowing the land using a tractor, followed by manual planting. This was done using a traditional method, where 2 to 3 corn seeds are placed in the ground and then covered with a small amount of soil. The distance between the seeds was measured using the foot of the person planting as a reference. After planting, XXX-type fertilizer was applied.

In total, an area of approximately 3,100 m^2 was planted.

Additionally, four quadrants were marked in section 1, corresponding to the four treatments. For this, stakes were placed every 15 meters, resulting in a total of 60 meters divided into sections of approximately 10 meters in width. The same section was also marked with colored ribbons, arranged as shown in the following diagram.



In section two, four quadrants were marked with a width of 5 meters and a length of approximately 20 meters. However, no colored tags were used in this case (because I had run out).

Extraction of Piperamides: Filtration
February 7, 2024

To continue with the extraction, NaOH (caustic soda flakes, brand Kitta Products), a granary scale (brand Truper), and a funnel were acquired. The NaOH was purchased at the hardware store "Casa Ray" and the scale and funnel at "Conrado's" hardware store.

First, the funnel, a PP beaker, and a spoon used for weighing the NaOH were thoroughly cleaned and dried.

The amount of NaOH was adjusted according to the molarity, as the molar mass of NaOH (40 g/mol) is lower than that of KOH (56 g/mol).

Based on the ratio of 1 g of KOH for every 100 ml of alcoholic pepper extract, 0.714 g of NaOH would be used. The calculation for the total amount of NaOH required is as follows:

((680×2)/100)× (0.714) =9.7104

It was rounded to 9.71 g.

9.7 g of sodium hydroxide was weighed.

The contents of the maceration jars were filtered using a funnel with two superimposed cellulose filters. The extract was added gradually to avoid exceeding the filter and funnel's capacity, a process that took over two hours.

After filtration, the jar containing the filtered liquid was placed in a water bath. However, due to the lack of instruments to maintain the water at exactly 60°C, hot water was added whenever the water in the pot, where the jar was placed, approached room temperature.

Piperamide Extraction: Filtration
February 9, 2024

This procedure is based on LW – Piperamide Extraction.

The filtered liquid from the previous day was divided into two containers, and water was added until no apparent precipitation of piperamides was observed. The containers were then sealed and left to rest.

The table above is to determine how much ethanol needs to be acquired to finish the first batch and begin the second extraction.

Four 9.2 L bottles of 96% ethanol, brand LAS CANITAS, were purchased from the hardware store "Casa Ray."

1L Biopesticide 2%

Ingredient	Amount
Black Pepper	500 g
Extraction Ethanol	1500 ml
Diluent Ethanol	1000 ml
NaOH	9.71 g
Total Alcohol	2500 ml

Piperamide Extraction: Isolation
February 10, 2024

This procedure is based on LW – Piperamide Extraction.

The filtered liquid from the previous day was divided into two containers:

A: 660 ml alcohol
B: X ml alcohol
Regarding this extraction, which I will refer to as Batch 2 (with Batch 1 being the first extraction), the process was carried out up to the step of precipitating piperamides with water. However, no precipitation was observed during this step.

Piperamide Extraction: Isolation
February 10, 2024

This procedure is based on Piperamide Extraction.

Previously, the process had reached the step of dissolving the piperamide paste in ethanol. However, the paste could not be dissolved in alcohol, even after heating or adding more ethanol while stirring. Part of the piperine paste remained insoluble in the ethanol container, while some dried in the beaker. When treated with acetone, the piperine in the beaker was successfully dissolved at 100%.

I then added the piperine-acetone solution to the bottle containing ethanol and undissolved piperine, with the aim of increasing the solubility of the piperine in ethanol by adding a solvent in which it is more soluble. However, what happened was that the piperine dissolved in acetone formed an emulsion with the ethanol-undissolved piperine solution.

I recovered part of the insoluble piperine paste floating in the previous solution, which represented the majority of the extracted piperine, and placed it in the beaker, where I then dried it using air.

Once dry, I added acetone to this piperine paste and dissolved it, resulting in a probably oversaturated solution.

To this beaker, I added acetone that I had used to rinse a container that had piperine paste on its walls to recover as much as possible.

The piperine began to precipitate as the solution cooled, so I reheated it, stirred, and placed the beaker on a shelf where it would experience minimal movement to allow crystallization.

—

Additionally, I tried to measure the amount of piperine in the piperine-acetone-ethanol emulsion to estimate how much piperine I had in total. I did this by placing 10 ml, measured with a syringe, into an Erlenmeyer flask and heating it in the microwave in 30-second intervals. After each interval, I took the flask out, shook it, and allowed the flask to vent. This took about 5 intervals of 30 seconds.

Prior to adding the solution, the Erlenmeyer flask was weighed, with a dry weight of 123.8 grams.

The contents of 10 ml of the solution were approximately 0.1 grams.

Therefore, the maximum estimated concentration of piperine in the emulsion is 1%.

Although the emulsion initially seemed like an inconvenience, it turned out to be a very useful discovery, as it can be mixed with water, making application easier and reducing costs if we want to apply it over large areas where alcoholic solutions would be too expensive (and would have a greater environmental impact).

The previous hypothesis turned out to be incorrect, as part of what was observed in the emulsion were pepper resins, which were later mostly separated. As a result, the piperine remained dissolved in the alcoholic solution. (02/15/2024)

Piperamide Extraction: Biopesticide Prototype
February 19, 2024

This procedure is based on LW – Piperamide Extraction.

For the biopesticide production, all the piperamides extracted in the previous days were used. They were mixed in a beaker with acetone, where some crystals were observed in the mixture.

The acetone-piperamide solution was then added to the container holding the piperine-acetone-ethanol emulsion, which caused the resins present in the crystals to precipitate, so the solution was filtered to separate them.

When the resin mass was observed in the filter, some piperine crystals were still attached to the resin, so the mass was placed in a beaker with 100 ml of ethanol and microwaved for 1 minute. The mixture was stirred continuously and filtered again.

Additionally, the filters containing precipitated piperine residues were rinsed with acetone, and this acetone was added to the solution in the beaker mentioned earlier.

The mixture was heated to dissolve any remaining undissolved piperine, then allowed to cool. The solution was later transferred to the bottles containing alcohol and sealed for transport.

Piperamide Extraction: Biopesticide Prototype
February 19, 2024

At the plot, the sprayer, which had been rinsed with 96% ethanol, was filled and the biopesticide was sprayed onto the maize plants, which were approximately 30 cm tall.

Each plant was sprayed between 1 and 3 times, depending on its size.

There were signs of fall armyworm damage on some of the plants, and in certain cases, *Spodoptera frugiperda* individuals could be observed on the damaged plants, as the application was done around 7 pm, a time when they tend to emerge.

It had rained earlier, and some leaves were still damp, but their surface was dry, so the hydrophobic nature of the biopesticide didn't pose any issues when the piperine dried on the leaf surfaces. However, further monitoring is needed to assess whether the rain caused any runoff or dilution of the biopesticide.

Field Testing: Sampling

February 19, 2024

Sampling began at around 4:30 pm, after it had rained earlier in the area. By that time, the weather was sunny and clear.

Sampling started in the experimental section, moving row by row, with each plant being thoroughly inspected.

The process involved first examining the central part of the plant for worms or adult individuals. If none were observed in this area, the lateral leaves were inspected. For larger plants, a flashlight was used to aid in observing hard-to-reach areas.

In most cases, plants that had worms also showed signs of leaf damage. However, in younger plants with no apparent damage, a higher presence of younger individuals was observed.

It was noted—though not confirmed, as this aspect was not formally measured—that individuals in treatment section A were generally larger compared to those in section B. Although the overall population was smaller, it seemed to consist of more adult individuals. This doesn't necessarily mean there were more adults on average in section A than in section B, but it suggests that larger individuals at the time of biopesticide application may not have been affected, which aligns with the observation (also not formally measured or recorded) that there were more very young individuals in treatment section B.

Field Testing: Analysis

February 29, 2024

A total of 2,174 plants were observed, distributed across 20 plots, with 10 receiving treatment A, 10 receiving treatment B, and 10 receiving treatment C.

Treatment	Description
A	Biopesticide prototype applied via spraying. The application involved spraying each plant 1 to 3 times, depending on its size, with a total of 1 liter of biopesticide used across the 10 plots. When the initial biopesticide solution was running low, alcohol was added to refill the sprayer, resulting in an approximate 50% v/v dilution. This diluted solution was then sprayed on the plants, each of which had already received the first application after the initial solution had dried. This time, only one spray per plant was applied.
B	This treatment served as the control, with no additional manipulation or intervention.
C	This treatment involved alcohol, without any additives, applied in the same manner as the biopesticide.

Field Tests: Analysis and Writing

March 4, 2024

Today, progress was made in data analysis, beginning with drafting a discussion to further systematize the analysis.

On Friday, August 2nd, I had a meeting with Lilia to show her how I was approaching the analysis and to get her feedback. Her observations were as follows:

Use the Sf/P graph in space as a visual introduction to the results, but not as a result in itself.

Compare Sf/Plant vs. Plant instead of Sf vs. Plant in the linear regression analysis.

Explicitly name each treatment in the discussion.

*She mentioned that I wasn't presenting results in the most conventional way, (which isn't necessarily a bad thing).

Important Dates for the Field Test:

Application: February 18

Sampling 1: February 21

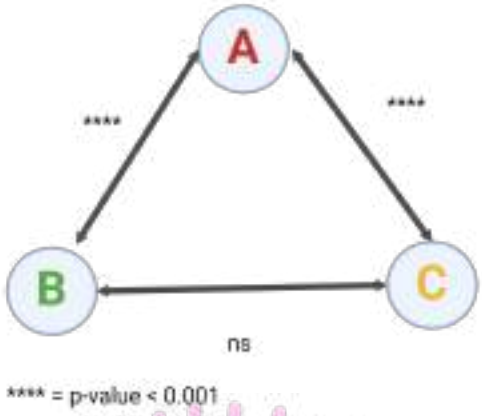
Sampling 2: February 24

Field Tests: Analysis and Writing

March 11, 2024

The writing of the results continued, and data from the control treatment C plots was added to the analysis. We have data for 10 plots from the first sampling, but due to terrain conditions (rain washed away some plants and soil, altering the number of plants and the layout of the plots), the irregular sections were removed. The analysis was conducted using only the unaffected parts of the terrain.

Outline of the t-test results



Assembly: Design of Peptide 2A Compatible with OYC

May 5, 2024

This page allows us to see how well the ligase will work with our overhangs.

NEBridge® Ligase Fidelity version 1.0
Tools for Golden Gate Assembly

In the 2A peptides, one end of peptide #2 is complementary to an end of peptide #3.

2A	OYC ends	Ligation	Compatibles	

			OYC	
P2A	GGTC			
	AGGT			
T2A	GAGG			
	TCCT			
E2A	AATT			
	AGGA			

Green sections are complementary 3’ ends, so they could not be used in the same assembly. The part marked in green is the codon, and I need to modify the sequence of one of the two to avoid complementarity, without altering the codon.

CCT codes for proline → CCG codes for proline

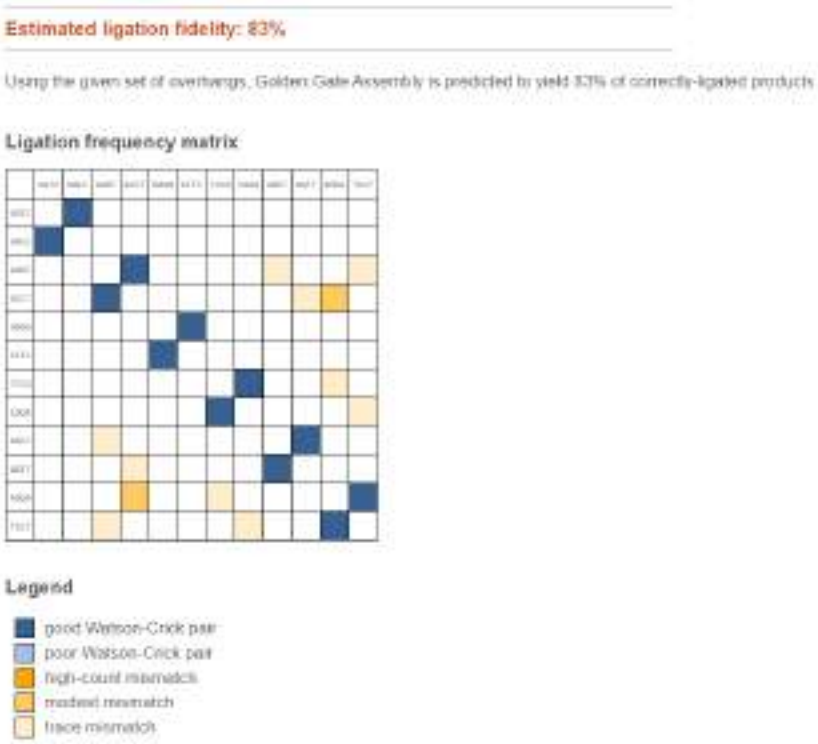
GGA codes for glycine → Not modified

AAT codes for asparagine → AAC codes for asparagine

Light blue sections are 5’ ends that may have problems assembling. The underlined part is the codon, and I need to modify the sequence of one of the two to avoid adhesion issues.

Fixed codons version

2A	OYC Ends	Ligation	Compatibles OYC	Modified on benchling
P2A	GGTC	good Watson-Crick pair	yes	NA
	AGGT	good Watson-Crick pair	yes	NA
T2A	GAGG	good Watson-Crick pair	yes	NA
	TCCG	good Watson-Crick pair	yes	yes
E2A	AACT	good Watson-Crick pair	yes	yes
	AGGA	good Watson-Crick pair	yes	NA



Type	End 5’	End 3’
Terminador	GCTT	CGCT
CDS	AATG	GCTT
Promotor	GGAG	AATG TACT
Plamid backbone	GCAA	AAAA
left-conector	ATGA	GGAG
right-conector	CGCT	AGAC
Homology region	AAAA	AAGG

	AGAC	CGAA
Origin	AAAA ACTA	AAGG AAAA
marker	AAGG GCAA	ATGA ACTA
bridge	AGAC	CGAA GCAA
transfer-origin	CGAA	GCAA
tag	CCAT TACT	GTCA AATG
signaling	TACT	CCAT AATG
intein	GTCA	TCCA
linker	CCAT GTCA	GTCA AATG
protease cleavaje domain	TCCA	AATG

Once the compatibility with RFC10 and the OYC overhangs was reviewed, it turned out that there was no incompatibility with any of the pieces.

The prefix and suffix were then added to convert it into a BioBrick.

There is a second prefix designed for protein coding regions (parts start with ATG, see RBS-CDS issues for more info):

```
              Prefix                                          Suffix
5' - GAATTC GCGGCCGC T TCTAG ...part... T ACTAGT A GCGGCCG CTGCAG - 3'
     EcoRI    NotI      XbaI               SpeI     NotI   PstI
```

A special prefix was used for protein-coding regions.

Adding the RFC10 ends prevents us from having to insert the sequence into a plasmid beforehand, as we can do this in any plasmid that allows the insertion of BioBricks (for cloning).

Linear schematic of the final piece (Benchling).

Piperamide Extraction 2.0

Sept 10, 2024

To proceed with the isolation step, the alcoholic extract of piperamides was first weighed:

608 g (Bottle 1) 778 g (Bottle 2) Total: 1386 g

Then, the volume was calculated using the density of ethanol:

Volume = (Density) / (Mass) = (0.789 g/ml) / (1386 g) ≈ 1757.28 ml

The bottles weighed 40 g, so the total weight was estimated to be 1300 g, adjusting the previous calculation to:

Volume = (0.789 g/ml) / (1300 g) ≈ 1647.15 ml

This was rounded to 1650 ml.

1 g of KOH per 23 g of pepper used in the extraction.

1/23 = 0.043

0.043 g of KOH per gram of pepper.

0.043 (KOH/Pepper) (0.043 KOH/Pepper) * (400 g Pepper) = 17.2 g KOH

It's better to use this ratio to determine how much KOH to use, as it provides more flexibility in the calculations and is independent of the volume and concentration of the alcoholic extract of piperamides.

17.2 g of KOH

That day, the KOH was weighed, but it was used the following day. However, it partially dissolved, likely due to humidity. For future extractions, it is recommended not to take it out of its container until ready to use or to keep it in an airtight, moisture-free container.

Piperamide Extraction 2.0
Sept 16, 2024

The piperamide paste was filtered during the previous week, washed with distilled water, and left to dry with a direct airflow in the dark. Later, it was dissolved in acetone and left to crystallize for two days.

Medium Preparation
September 13, 2024

On this day, the preparation of YPD (Yeast Peptone Dextrose) culture medium for yeast was carried out. The standard recipe and protocol were followed as outlined below, using the appropriate materials and equipment to ensure sterility and the quality of the prepared medium.

Consumables

Millipore water and/or MilliQ water
Yeast extract
Tryptone
Glucose
Bacteriological agar (for solid media)
Equipment used

Autoclave
Sterile 1 L flasks
Pipette tips, pipettes
Test tubes
Magnetic stirrer
Magnetic fly
Petri dishes 100x15mm (for solid media)
Ingredients for 1 L of medium

1% Yeast extract (10 g)

2% Tryptone (20 g)
2% Glucose (20 g)
2% Agar (20 g) before autoclaving for solid media

The ingredients were added in the following order to a sterile flask with water, which was already being stirred. For 1 L of medium, the ingredients were first dissolved in 750 mL of water, and the final volume was adjusted to 1 L. Sterilization was carried out at 120°C for 20 minutes.

We make the calculation to prepare 600 mL:

Glucose 12g
Tryptone 12g
Yeast Extract 6g
H2O to complete 600 mL

For solid media, 2% agar (20 g/L) was added before autoclaving. After sterilization, 20 mL of the medium was poured into each petri dish. We prepared 10 petri dishes

We make the calculation to prepare 200 mL:

Glucose 4g
Tryptone 4g
Yeast Extract 2g

Agar 4g
H2O to complete 200 mL

***To ensure sterile working conditions, all preparations were performed in the downflow cabinet/bench.

Yeast Strain Inoculation from Glycerol Stock
September 16, 2024

We utilized a petri dish (previously prepared) to retrieve our yeast strain from a glycerol stock. The 1.5 mL Eppendorf tube containing the frozen glycerol stock of *Saccharomyces cerevisiae* Cla WT had been stored at -70°C.

To begin the process, we briefly removed the tube from the freezer for about a minute. Using a sterile pipette tip, we carefully scraped off some ice and collected approximately two scoops of the frozen glycerol. We streaked these onto the Petri dish, allowing it to grow overnight at 30°C.

Upon inspection the following day, we observed growth; however, it was recommended to allow the cultures to grow for a longer period to achieve more robust colonies. This would enhance our subsequent experiments with the yeast strain.

Overnight Culture Media for Yeast
September 17, 2024

Today, we prepared the overnight culture media for our yeast strains. We worked under sterile conditions in the downflow cabinet to avoid contamination.

First, we took a Petri dish containing colonies of our yeast grown on YPD Agar and selected a single colony using a sterile pipette tip. The colony was inoculated into a Falcon tube of 50mL, with 5 mL of YPD culture and placed in the incubator at 30°C to grow overnight.

In preparation for the next steps, we planned to perform a dilution. We will take 50 µL of the overnight culture and add it to 5 mL of fresh YPD medium for a 1/100 dilution. The goal is to culture the diluted sample until it reaches an optical density (OD) of approximately 0.6, ensuring sufficient growth for subsequent experiments.

Cytotoxicity test, first test
September 18, 2024

Procedure:
With previously growing S. cerevisiae in YPD Liquid Medium
We began by adjusting the concentration of S. cerevisiae to 1 x 10^6 cells/mL in each tube. To do so, we prepared 9 tubes with 1 mL of culture medium in each.

Treatments Applied

The following table (Table 1) outlines the specific treatments applied to each tube:

Treatment	Description	Concentration in Culture Medium	Solution Applied (50µL)
NC_1	Negative control 1	NA	H_2O
NC_2	Negative control 2	20%	Ethanol 100%
PC	Positive control	6%	Hydrogen peroxide 30%
PS-1	Piperamides	0.02%	Piperamides solution 0.1%
PS-2	Piperamides	0.2%	Piperamides solution 1%
PS-3	Piperamides	2%	Piperamides solution 10%
FAS-1	Ferulic acid	0.004%	Ferulic acid solution 0.02%
FAS-2	Ferulic acid	0.04%	Ferulic acid solution 0.2%
FAS-3	Ferulic acid	0.4%	Ferulic acid solution 2%

Table 1

Each treatment sample was added to its corresponding 1 mL yeast culture, maintaining a 1:1 ratio. This step was performed under sterile conditions to ensure accurate results.

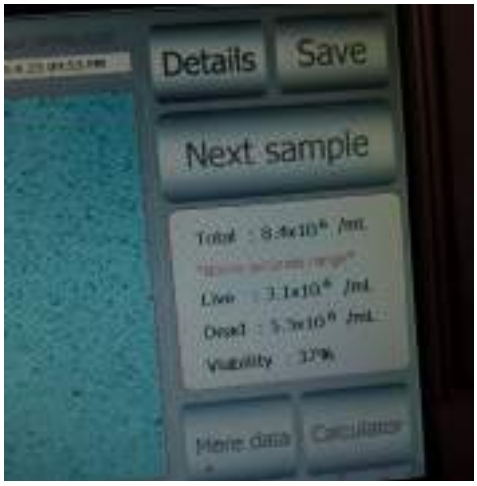
Incubation

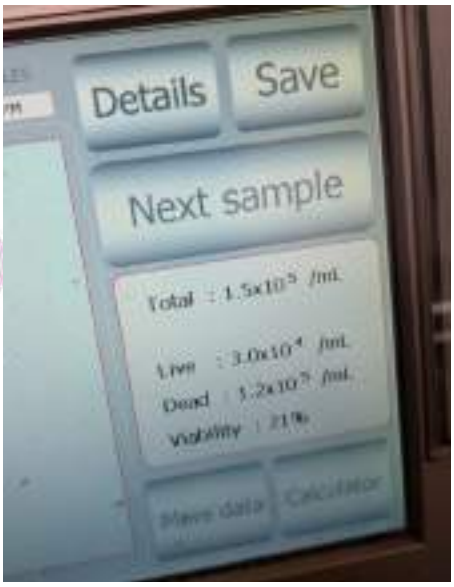
After adding the respective treatments (Table 1) to each tube, the samples were incubated at 28°C for 45 minutes.

Cell Viability Measurement

Following the incubation period, we took 10 µL of the cell culture solution from each tube and added 10 µL of Trypan Blue. After thoroughly mixing, we placed the mixture on a cell counter slide to measure cell viability for each treatment.

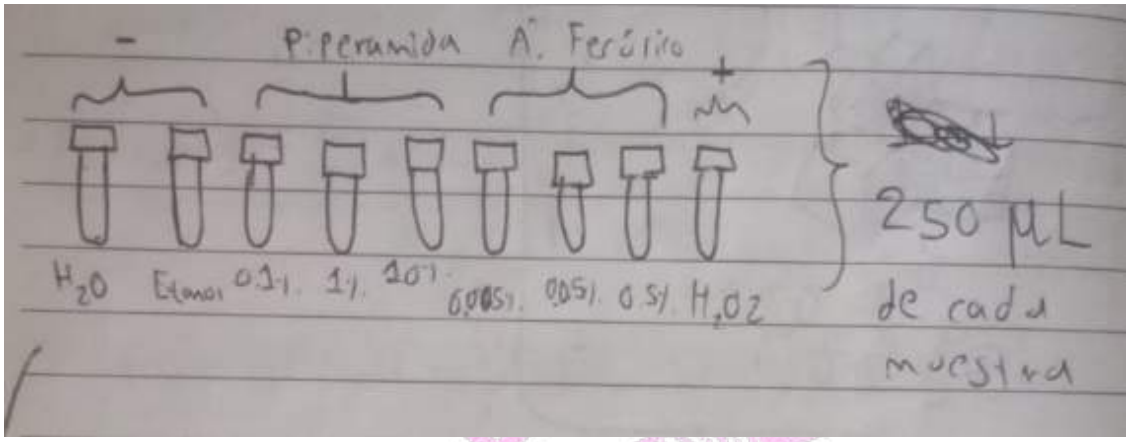
The viability results for all tubes were recorded. The next step will be to analyze and compare the viability across the different treatment conditions.

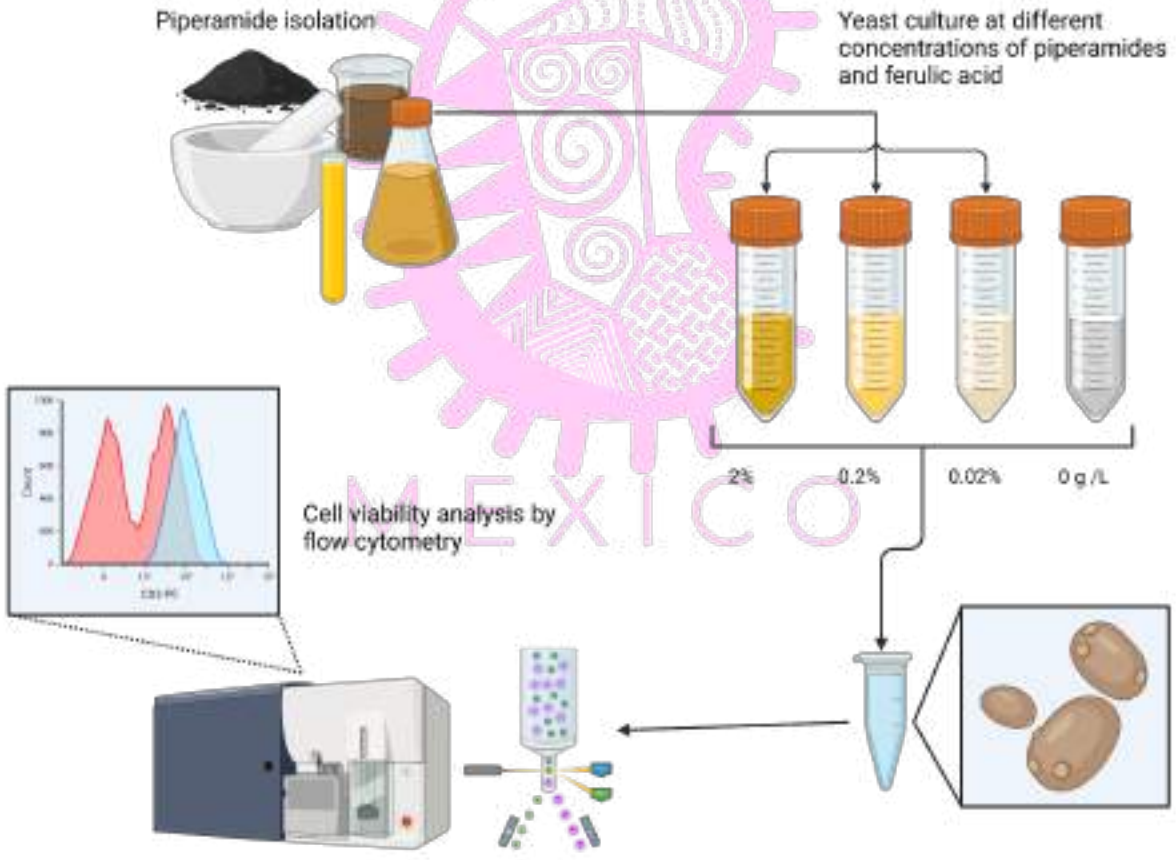




**Important note: The yeast culture mixed with H_2O_2 should not be sealed, as this can lead to gas buildup and potentially cause the

container to explode. It's essential to keep the flask loosely covered or use a vented cap to avoid any pressure-related issues.





Flux cytometry, Second trial: new piperamide and ferulic acid concentrations
September 23, 2024

Today, we performed a cytotoxicity assay using flow cytometry to assess the effects of piperamides and ferulic acid on S. cerevisiae cells. The protocol was adapted to incorporate flow cytometry for a more detailed analysis of cell viability.

Materials
S. cerevisiae culture
10 Flow Cytometry tubes
Zombie NIR™ viability dye
Paraformaldehyde (PFA)
Phosphate-buffered saline (PBS)
Culture media (YPD)

We adjusted the cell concentration to 1 x 10^6 cells/mL in every tube, resulting in 9 tubes total, each containing 1 mL of culture media.

Treatments Applied:
The treatments and their respective concentrations are detailed in the table below. 250 µL of each solution was added to the corresponding tube, and the tubes were incubated at 28°C for 45 minutes.

Treatment	Description	Concentration in Culture Medium	Solution Applied (250 µL)
H_2O	Negative control 1	NA	H_2O
ET	Negative control 2	20%	Ethanol 100%
PE	Positive control	6%	Hydrogen peroxide 30%
P1	Piperamides	0.6%	Piperamides solution 3%
P2	Piperamides	1.2%	Piperamides solution 6%
P3	Piperamides	1.8%	Piperamides solution 9%
F1	Ferulic acid	0.000004%	Ferulic acid solution 0.00002%
F2	Ferulic acid	0.00004%	Ferulic acid solution 0.0002%
F3	Ferulic acid	0.0004%	Ferulic acid solution 0.002%

Table 2

Staining with Zombie NIR™:
After incubation, 1 µL of Zombie NIR™ was added to each tube to stain dead cells. The tubes were incubated for an additional 15 minutes at room temperature in the dark to ensure proper staining.

Fixation:
100 µL of 4% Paraformaldehyde (PFA) was added to each tube to fix the cells. The samples were incubated for 20 minutes at room temperature.

Washing:
The cells were washed twice with PBS to remove any excess dye and PFA.

Final Preparation:
The samples were resuspended in PBS and stored at 4°C until flow cytometry analysis could be performed.

**Next Steps: We will run the prepared samples through the flow cytometer at INER with Heriberto, to quantify cell viability and analyze the effects of the different piperamide and ferulic acid concentrations on S. cerevisiae cells. Results will be recorded and compared across treatments.

Flow Cytometry Analysis: FACS Discover S8 Becton Dickinson
September 24, 2024

Today, we analyzed the samples from the previous day's experiment using the FACS Discover S8 Becton Dickinson Flow Cytometer at INER.

This analysis was aimed at quantifying cell viability after treatment with varying concentrations of piperamides and ferulic acid.

Procedure
Sample Preparation

Each of the 9 tubes prepared the previous day was retrieved from storage at 4°C.
The samples were gently mixed to ensure even distribution of the cells before loading them onto the flow cytometer.

The data from the flow cytometry analysis will be processed to visualize the effects of the different treatments on cell viability. Comparisons will be made between the piperamide and ferulic acid concentrations, as well as the controls.

Phytotoxicity test
Date: September 17, 2024

To evaluate the effects of piperamides on maize seed germination we are going to apply different concentrations of piperamide solutions to vermiculite, and observe the germination changes over a 7-day period.

Procedure:
Piperamide Treatment Application:
Prepare piperamide solutions at varying concentrations (see Table below). Each solution is going to be applied to vermiculite, ensuring an even spread across the substrate.

Drying:
Treated vermiculite has to be left to dry under direct ventilation for three days. Drying conditions have to be consistent across all samples to ensure uniform treatment.

Hydration and Seed Placement:
Once the vermiculite is fully dried, add water to simulate natural growing conditions.

Pre-hydrated maize seeds are going to be placed onto the treated substrate within sterile Petri dishes.
Seed placement had to be randomized to avoid bias.

Treatment	Description	Estimated piperamides concentration in substrate (g/kg)*
Control	Deionized water	0
PS-1	Piperamides 0.02% in absolute ethanol	0.02
PS-2	Piperamides 0.02% in absolute ethanol	0.2
PS-3	Piperamides 0.02% in absolute ethanol	2

Table 1

Monitoring Germination:
Germination is going to be observed and recorded on the following days:

Day 1: Early seed response post-treatment.
Day 4: Mid-term evaluation of germination progress.
Day 7: Final assessment of germination rates

*Notes:
Ensure consistent hydration levels across all Petri dishes post-placement.
Monitor room temperature to maintain consistency across all treatments.

Preparation of piperamide treatments and vermiculite setup
September 17, 2024

Today, the piperamide treatments were prepared by diluting the 10% stock solution to the required concentrations for the experiment. The solutions were made according to Table 1, and for each treatment, 25 mL of the corresponding solution was measured.

Materials used:
10% piperamide stock solution
Water (for dilution)
Measuring cylinders (for accurate volume measurement)
30 grams of vermiculite for each treatment
Stirring rods for mixing
Ventilated dark area for drying
Each 25 mL of piperamide solution was added to 30 grams of vermiculite in separate containers.
The mixture was stirred thoroughly to ensure the solution was evenly distributed throughout the substrate.

Once prepared, the treated vermiculite was spread out in shallow trays and left uncovered in a dark, well-ventilated space. The trays will remain there for three days to allow complete evaporation of the alcohol in the solution.

Observations will be recorded once the vermiculite is fully dried and ready for the next stage of seed placement.

Preparation of Maize seeds
September 19, 2024

The maize seeds used in the experiment were purchased from a local supermarket, branded as Verde Valle, and specifically of the “Maíz Palomero” variety.

Materials used:
Maize seeds (“Maíz Palomero”)
Beaker
Tap water

The seeds were washed thoroughly with plain water to remove any dust or debris. After the initial cleaning, they were transferred to a beaker filled with fresh water for hydration. The seeds were left to soak for 24 hours, which is necessary to promote uniform hydration across all experimental groups and to encourage germination.

The hydrated seeds will be ready for the next step of distribution into the treated vermiculite tomorrow.

Seed distribution, incubation, and monitoring setup
September 20, 2024

After 24 hours of soaking, the maize seeds were evenly hydrated and ready for placement in the experimental setup.

Materials used:

Pre-hydrated maize seeds
Petri dishes with treated vermiculite (from September 17)
Incubator set to 25°C
Thermometer
Light source (for photoperiod control)
For each treatment group, 90 maize seeds were distributed across three Petri dishes, each containing the treated vermiculite substrate prepared earlier. The Petri dishes were arranged inside the incubator, which was set to a controlled temperature of 25°C, with a 12-hour light/12-hour dark photoperiod to simulate natural day-night cycles.

Monitoring Protocol: Monitoring for seed germination will be conducted on days 1, 4, and 7. Each Petri dish will be examined, and the number of germinated seeds will be counted and recorded for each dish individually. This data will be compared across different treatments to assess the impact of piperamide concentrations on seed germination rates.

Monitoring for seed germination, data from day 1, 4 & 7

September 20 to 27, 2024

We counted the germinated seeds for each treatment, and we filled the data in the excel sheet. Then we proceed to analyze the data after seven days of growth.

	Seeds germinated per day		
Treatment	**1**	**4**	**7**
Control	20	25	26
Control	22	25	25
Control	24	27	27
Piperamides 0.02%	0	0	13
Piperamides 0.02%	0	0	12
Piperamides 0.02%	0	0	13
Piperamides 0.2%	0	0	4
Piperamides 0.2%	0	0	10
Piperamides 0.2%	0	0	6
Piperamides 2%	0	0	4
Piperamides 2%	0	0	3
Piperamides 2%	0	0	4