



When life gives you cacao...

A perspective on an International Research Experience for Students (IRES) in the Era of COVID and Social Unrest.

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Acknowledgements



NSF – IRES Award # 2107424 : The Broad Impact of Flavor, A Research Experience on Metabolomics of Microbial Populations during Cacao Fermentation



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Objective

- Discuss how we turned many challenges into a powerful set of educational experiences, expanded collaborations, and the path forward for continued research and education



<https://www.nsf.gov/funding/opportunities/ires-international-research-experiences-students>

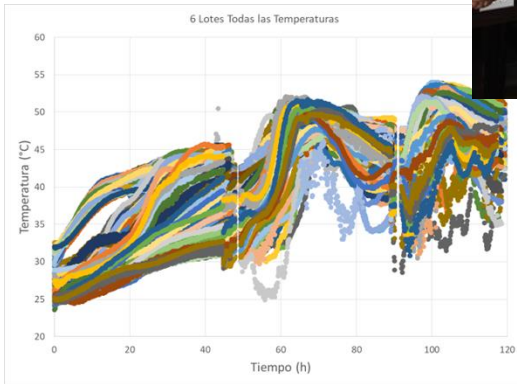
- The IRES program contributes to development of a diverse, globally engaged workforce with world-class skills. IRES focuses on active research participation by undergraduate and/or graduate students in high quality international research, education and professional development experiences in NSF-funded research areas.
- The overarching, long-term goals of the IRES program are to enhance U.S. leadership in science and engineering research and education and to strengthen economic competitiveness through training the next generation of science research leaders. IRES focuses on the development of a world-class U.S. STEM workforce through international research experiences for cohorts of U.S. students.

Some characteristics of the IRES program

- Participant-centered
- Great educational experience
- Great science
- Collaborative
- PI-supervised – International Collaborator-led



Goals of the proposal



- **STUDENT DEVELOPMENT:**

To strengthen the global scientific and social competency of US graduates in STEM disciplines.

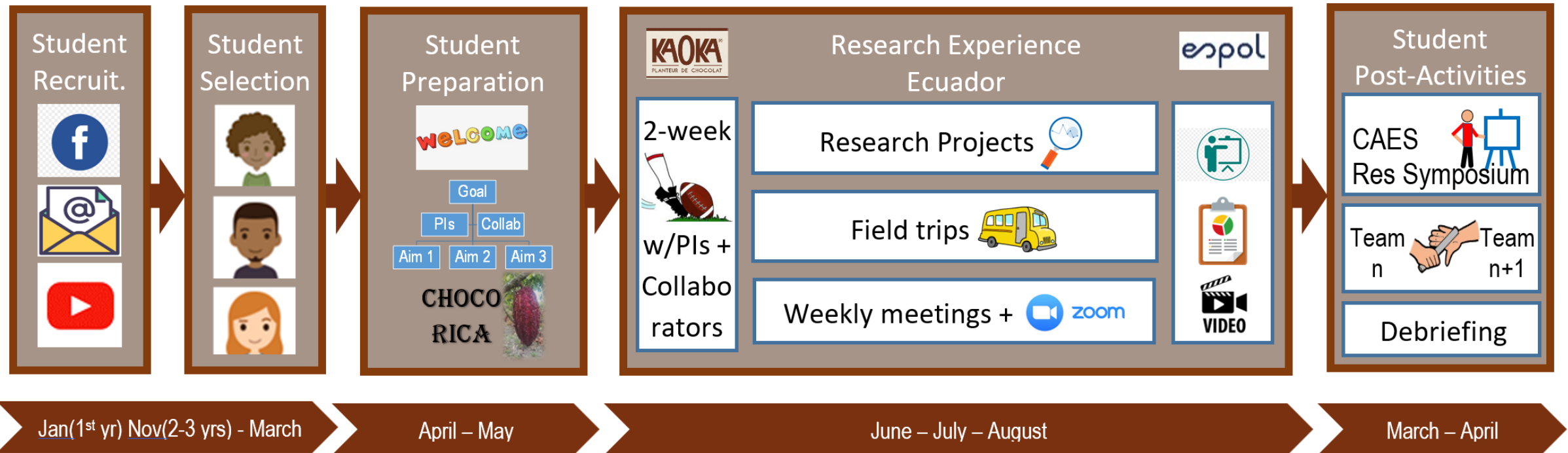
- **SCIENTIFIC PROBLEM**

To consistently produce high-quality cocoa products through controlled cocoa fermentations.

- **SOCIAL CHALLENGE**

To improve the livelihood of cacao smallholders in Latin America and support emerging craft-chocolate manufacturers in the U.S. by providing them with high quality fermented cocoa beans.

The logistics



First Cohort – Study Abroad

Study Abroad



First Cohort – Trip to Ecuador

Arrival to Quito



https://es.wikipedia.org/wiki/Paro_Nacional_de_Ecuador_de_2022

Arrival to Guayaquil



Cancel and Return



The News:
Water supply in Ecuador being poisoned
People may to be able to reach airports



Second Cohort – Many changes

Job Change



8/16/2022



Project Change

Study abroad replaced with predeparture Zoom sessions + 1 additional on-site week

Re-budget:

- An additional cohort
- Meet the goal of having 12 participants
- Broaden regional participation

Week before Pre-departure Kickoff Meeting

Travel Advisory Levels

- 1 Exercise normal precautions
- 2 Exercise increased caution
- 3 Reconsider travel
- 4 Do not travel

Travel Advisory
April 15, 2024

Ecuador - Level 2: Exercise Increased Caution

C U K

Updated with information about land border travel restrictions.

Exercise increased caution in Ecuador due to **civil unrest, crime, and kidnapping**. Some areas have increased risk. Read the entire Travel Advisory.

Do not travel

4 Do not travel

- Guayaquil, **south** of Portete de Tarqui Avenue, due to **crime**.
- The cities of Huaquillas and Arenillas in the province of El Oro, due to **crime**.
- The cities of Quevedo, Quinsaloma, and Pueblo Viejo in the province of Los Rios, due to **crime**.
- The canton of Duran, in the province of Guayas, due to **crime**.
- Esmeraldas city and all areas north of Esmeraldas city in Esmeraldas province, due to **crime**.

Reconsider

3 Reconsider travel

- Guayaquil **north** of Portete de Tarqui Avenue due to **crime**.
- El Oro province outside the cities of Huaquillas and Arenillas, due to **crime**.
- Los Rios province outside the cities of Quevedo, Quinsaloma, and Pueblo Viejo, due to **crime**.
- All areas south of Esmeraldas city in Esmeraldas province, due to **crime**.
- The provinces of Sucumbios, Manabi, Santa Elena, and Santo Domingo due to **crime**.

Country Summary: Crime is a widespread problem in Ecuador. Violent crime, such as murder, assault, kidnapping, and armed robbery, is prevalent and widespread. The rate of violent crime is significantly higher in areas where transnational criminal organizations are concentrated.

Second Cohort – Move program to Costa Rica

Arrive to San Jose



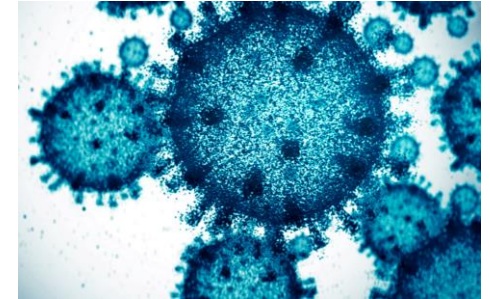
Build research infrastructure



Initiate research



COVID



Vacations

JULIO 2023

DOMINGO	LUNES	MARTES	MIÉRCOLES	JUEVES	VIERNES	SÁBADO
2	3	4	5	6	7	8
9	10	11	12	13	14	15
16	17	18	19	20	21	22
23	24	25	26	27	28	29
30	31					



Third Cohort

First Undergraduate Research Posters

Research @ The Heredia Campus and Experimental Farm



Optimizing Cacao Fermentation: The Impact of Yeast Starter Cultures on Microbial Diversity and Flavor Development

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INTRODUCTION

Cacao fermentation is a critical step in chocolate production, influencing the final products' flavor, aroma, and overall quality. Traditional fermentation relies on natural microbial communities, resulting in high variability and inconsistent flavor profiles, particularly for small-scale farmers. This study explores the use of yeast starter cultures to enhance and control fermentation, with the goal of improving quality and efficiency for small-scale cacao producers.

Objectives:

- Investigate the impact of yeast starter cultures (YSC) on microbial diversity, particularly lactic acid bacteria (LAB) and acetic acid bacteria (AAB).
- Assess how controlled microbial activity influences flavor and aroma profiles during cacao fermentation.
- Use molecular and chemical analysis (PCR, DNA sequencing, GC-MS) to correlate microbial activity with volatile compound production.
- Provide insights to help small-scale farmers implement more controlled and effective fermentation techniques.

METHODS

Small-Scale Fermentation and Drying

- 2 kg of cacao beans fermented with/without YSC, LAB, AAB.
- 6-day fermentation samples collected at 24 & 48 hrs.
- Beans dried for 10 days with regular rotation.

Microbial Analysis (PCR & Sequencing)

- DNA extracted at 24 hrs (Pre-fermentation).
- PCR (Polymerase Chain Reaction) used to amplify 16S rRNA for bacteria and ITS region for fungi, targeting specific, generic primers for microbial identification.
- High-throughput sequencing used to assess LAB & AAB diversity.

GC-MS (Volatile Analysis)

- Volatiles extracted via Solid Phase Microextraction - Gas Chromatography-Mass Spectrometry (SPME-GC-MS) from frozen beans and pulp.
- Headspace solid phase microextraction (HS-SPME) used for volatile analysis.
- Analyzed for key aroma compounds.

RESULTS

Volatile Compound Analysis (GC-MS)

High-throughput GC-MS revealed the presence of key desirable fermentation aroma compounds associated with high-quality cocoa, including:

- 4-Ethyl acetate** (fruity, sweet): Detected at high abundance (502% increase YSC vs. AAB).
- 2-Phenylacetaldehyde** (rosy, floral): Consistently high abundance (50% increase YSC vs. AAB).
- 4-Ethyl acetate** (fruity, sweet): Detected at high abundance (502% increase YSC vs. AAB).
- 4-Ethyl acetate** (fruity, sweet): Detected at high abundance (502% increase YSC vs. AAB).

These compounds are widely recognized as positive fermentation markers due to their fruity and floral aroma contributions. Drug compounds like, diethylene glycol and diethylene glycol were excluded as contaminants or unrelated to fermentation.

Microbial Analysis (PCR & Sequencing)

DNA was analyzed at 48 hours using the PowerPac kit, and PCR amplification of 16S rRNA and ITS regions was successful, as shown by gel electrophoresis. These regions were selected to target bacteria (16S) and fungal diversity. All samples have been successfully sequenced using high-throughput methods.

Future Steps

The project team is in the next research program on multiple aims, all relevant key steps remaining to complete the analysis and address the initial research objectives.

Dissemination Analysis

YSCs that are used at all PCR products have been successfully sequenced, indicating a high degree of consistency in LAB and AAB populations. This will be analyzed for species diversity and abundance across fermentation stages and under various conditions, including the use of YSCs. The results will be used to further inform small-scale farmers on the importance of microbial diversity in cacao production.

Integrated Microbial-Chemical Correlation

PCR results will be compared to gene sequencing data to explore correlations between microbial diversity and volatile compound production. This integrated approach will help determine how microbial composition influences flavor development during cacao fermentation.

These steps will help determine whether yeast starter cultures can be used to enhance microbial fermentation and improve desirable aroma development in a controlled, repeatable way.

Figure 1. Small-scale fermentation trials, by PCR instrument. CH-PCR vs. PCR instrument.

Figure 2. Gas chromatograms of A) YSC 0 h, B) YSC 12 h, C) YSC 24 h, and D) YSC 48 h.

Cacao Fermentation: Effect of Antibiotics on Polyphenols & Aroma Compounds

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INTRODUCTION

Microbial influence on cacao fermentation. Cacao fermentation is a critical step in chocolate production, impacting both the phenolic content and aroma profile. Microorganisms, particularly yeast and bacteria, play a role in breaking down complex compounds, releasing both flavor and aromatic compounds.

Methods

Antibiotics and their role in fermentation. Antibiotics, such as penicillin, contribute to the microbial profile and influence of cacao fermentation. During fermentation, microbial metabolism can lead to the degradation or transformation of these compounds, affecting the final phenolic concentration. Understanding the role of YSC and the influence of antibiotics on cacao fermentation is key to improving fermentation for high-quality chocolate.

Results

Antibiotic development in fermentation. Antibiotic compounds are essential to cacao production, with their role in breaking down complex compounds and releasing aromatic compounds. The interaction between microbial and antibiotic production is complex, involving various metabolic pathways and the release of volatile compounds. The interaction between microbial and antibiotic production is complex, involving various metabolic pathways and the release of volatile compounds.

Conclusion

Yeast starter cultures influence polyphenol retention and aroma formation in cacao fermentation. While polyphenols are traditionally considered to be degraded during fermentation, our results suggest a positive impact of YSC on desirable flavor compounds. Further analytical analysis is required to confirm these findings. Future work includes refining GC-MS sample preparation and expanding large-scale fermentation.

Future Direction

Future research will focus on analyzing the GC-MS samples to further investigate the role of YSC and the influence of antibiotics on microbial activity. Additionally, bacterial and yeast sequencing will be conducted to gain deeper insights into the microbial community structure during fermentation. Expanding large-scale fermentation studies with varied YSC and LAB conditions will help assess scalability and optimize fermentation parameters. Integrating molecular and chemical data could provide a broader understanding of their role in fermentation and flavor development. Applying these findings to chocolate production and creating sensory evaluations will be essential in understanding the impact of microbial modulation on final chocolate quality.

References

Adhikari, K., & Salano, F. (2020). The Role of Yeast in Cacao Fermentation: A Review. *Journal of Food Science*, 91(1), 1-10.

Acknowledgements

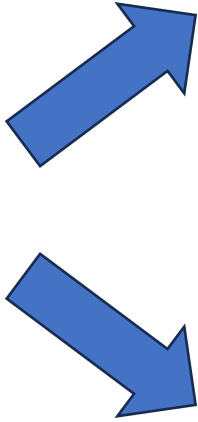
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Figure 1. Small-scale fermentation trials, by PCR instrument. CH-PCR vs. PCR instrument.

Figure 2. Gas chromatograms of A) YSC 0 h, B) YSC 12 h, C) YSC 24 h, and D) YSC 48 h.

Insufficient data for refereed publication

Last Cohort – Effect of adding selected amino acids to the started cultures



Sensors Failed



Automatic titr

Lack of lab sup.

...to be continued...

Goal 1: Student development

- 16 students expose the to world of cacao and chocolate
- Enriching International experience
- 12 carried out research including experiments on
 - Cacao fermentation
 - Gas chromatography – mass spectroscopy
 - DNA extraction
 - Data analysis (limited)
- Leveraged the CV and ability to pursue graduate studies
 - Of the 12 undergraduates at least 7 are pursuing or intend to pursue graduate studies
- Use the learning and experience to teach “The science and engineering of chocolate for a more just society.

Goal 2: Scientific Problem

- Modest scientific progress
 - 8 weeks of research was not enough for stand-alone projects
 - Delays caused by change of country and lack of alignment with on-going project to ensure local continuity
 - Calendar and instrumentation challenges
 - Cacao supply logistic challenges

Goal 3: Social Challenge

- No progress

When life gives you cacao...



...make chocolate!

