Microbes Associated with Spontaneous Cacao Fermentations - A Systematic Review and Meta-Analysis

Authors: Alexander J. Taylor<sup>1,a</sup>, Eduardo Cardenas-Torres<sup>2</sup>, Michael J. Miller<sup>1</sup>, Sihai Dave Zhao<sup>2</sup>, and Nicki J. Engeseth<sup>1</sup>

- <sup>1</sup> Department of Food Science and Human Nutrition, University of Illinois at Urbana-Champaign
- <sup>2</sup> Department of Statistics, University of Illinois at Urbana-Champaign
- a Corresponding author, <u>ait3@illinois.edu</u>

#### **Abstract:**

## **Introduction:**

Chocolate is a product of the fermentation of cacao beans. Typically, these fermentations are carried out on-farm or at local cooperatives and are not influenced by starter cultures. This leads to various microbes being associated with spontaneous cacao fermentations (SCFs). To better understand the microbiome associated with SCFs, this study sought to identify the associated microbes, their relationships, and other key parameters that influence fermentation. This is important because differences in fermentation may/can have an impact on final product quality.

#### **Methods:**

In this study, a systematic data extraction was performed, searching for literature that identified microbes from SCFs. Each unique microbe, whether by location or by fermentation material, was extracted from the articles, along with parameters associated with fermentation (including origin, material, duration, identification methods, weight, and type of microbe). Data were collected and analyzed for three key interactions: microbe-to-geography, microbe-to-fermentation method, and microbe-to-microbe. The goal was to attribute microbes to geographical locations, fermentation materials, or to other microbes. Any statistically significant relationships within the three above interactions will reveal target areas for future research.

### **Results:**

Over 1,700 microbes were identified across 60 articles. The top three countries represented are Brazil (22 articles, n = 612 microbes), the Ivory Coast (14 articles, n = 237), and Ghana (10 articles, n = 257). Several countries were far less, or never represented, and should be considered for future research. No specific relationship was identified with microbes to either geographical location or fermentation method. Over 440 unique species of microbes were identified and then used to make a Presence-Absence chart. Analysis on this chart helped to identify 127 statistically significant microbe-to-microbe interactions across Yeasts, lactic acid bacteria (LAB), acetic acid bacteria (AAB), and Miscellaneous (Misc). While there were many interrelationships, Yeasts were the only sole interrelated ones. Misc were the most intrarelated with AAB and LAB, *Weissella fabria* was the most connected LAB, and *Gluconacetobacter hansenii* for the AAB. Misc bacteria overall were far more numerous and diverse than any other group.

# Significance:

Data extraction into SCF research has revealed major gaps of knowledge for the cacao microbiome. By better understanding the cacao microbiome, researchers will be able to identify key microbes and fermentation parameters to better influence the fermentation. These findings will lay the foundation for researchers and provides a guide as to which starter cultures or microbes are important, especially when combined with associated flavor profiles.