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Understanding Kombucha fermentations: dynamic follow-up of a lab scale fermentation

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Introduction

At a time when consumers are more and more concerned about their diet and health and with a constantly increasing consumer demand for more natural and organic products, Kombucha is becoming a very popular drink in Western countries and as an alternative to sodas. Moreover, producers highlight the potential beneficial properties associated to the tea itself or the metabolites produced by the Kombucha microbiome. Kombucha is a naturally fermented beverage made from sweetened tea and is characteristically acidic and naturally fizzy due to metabolic activities of its complex microbial ecosystem (Figure 1). During fermentation, a biofilm is formed by acetic acid bacteria that floats on the surface of the tea and is very rich in yeast and acetic and lactic acid bacterial species. However, Kombucha, and more particularly biofilm formation and the creation of the complex microbial networks needed for fermentation, has not yet been well studied. Moreover, this biofilm is used in a backslipping process to start the next fermentation batch, which can impact overall product quality if changes in microbial populations occur.

In this study, a complex but controlled microbiota was used to dynamically follow lab scale fermentations. During the fermentation, several key parameters were followed including microbial populations (counts and metagenetics), physico-chemical (pH and density) and biochemical (organic acids, sugars, volatile compounds) parameters and, for the first time, biofilm formation by scanning electron microscopy and confocal microscopy.

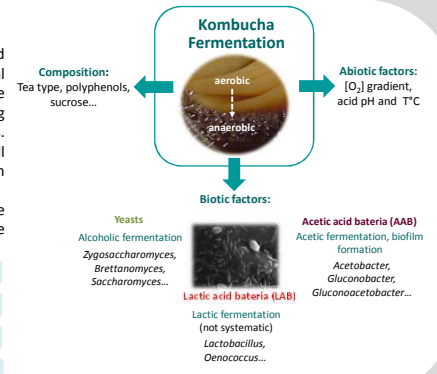
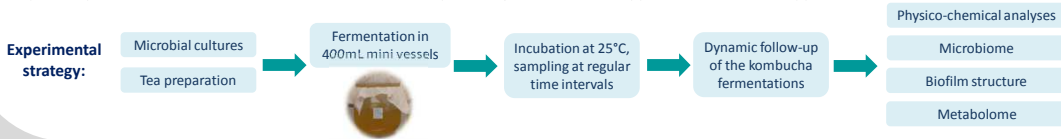


Figure 1: Different factors impacting Kombucha fermentation

Microbial and physico-chemical dynamics

Microbial counts monitored over 27 days:

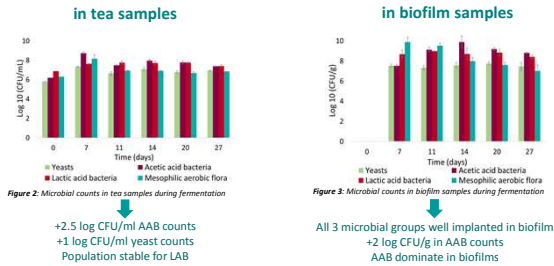


Figure 2: Microbial counts in tea samples during fermentation

Figure 3: Microbial counts in biofilm samples during fermentation

+2.5 log CFU/ml AAB counts
+1 log CFU/ml yeast counts
Population stable for LAB

All 3 microbial groups well implanted in biofilm
+2 log CFU/g in AAB counts
AAB dominate in biofilms

Microbial activities associated with:

biofilm formation at a rate of 0.34g/d for 7d then 0.15g/d up to 27d
rapid decrease in pH
rapid decrease in density
ethanol production (<1%)
metabolite production

Figure 4: Thick biofilm formed by 27 days

Kombucha metabolome elucidated

Volatile metabolites determined by GC-MS (headspace)

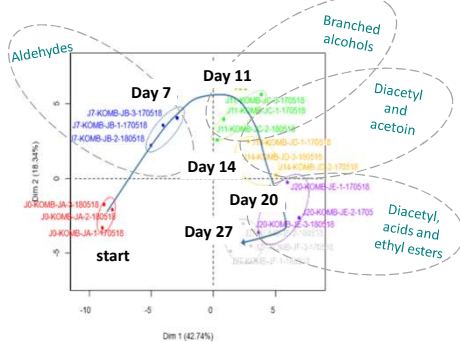


Figure 8: Principal component analysis on volatile metabolites during the lab scale kombucha fermentation

Good repeatability of biological triplicates
Distinct changes in volatolome directly linked to fermentation time
Upcoming metagenetics data will help link species to functions

Biofilm formation and microbial network

Biofilm observed by SEM and confocal microscopy at days 7, 11, 14, 21 & 27

Sample preparation: cells fixed with 1:1 PBS 1X ice-cold ethanol 96% before hybridization with specific yeast and AAB/LAB FISH probes, in green and red, respectively, blue represents biofilm autofluorescence.

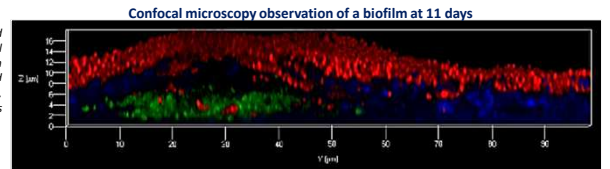


Figure 5: Confocal microscopy observation of a biofilm at 11 days fermentation

14 day biofilm sample:

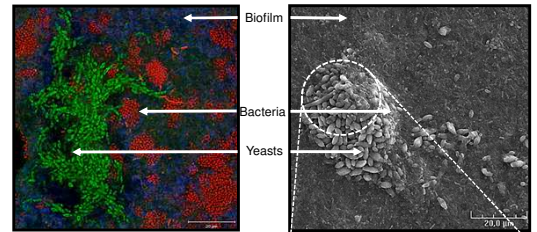


Figure 6: Confocal microscopy with FISH probes

Figure 7: Scanning electron microscopy (SEM) observations

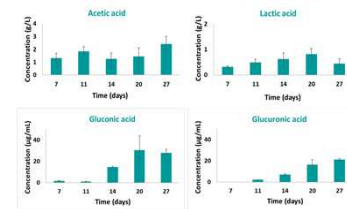
Sample preparation for SEM: biofilm samples were treated with a cacodylate & 2.5% glutaraldehyde fixation solution then dehydrated using an ethanol bath before coating with a thin layer of metal.

Complex network between bacteria and yeast:

- yeast in clusters
- bacterial biofilm network surrounds yeasts
- biofilm densifies over time

Biofilm potentially linked to their symbiotic relationship

Organic acids determined by QTOF LC-MS and enzymatic kits



High concentrations of acetic acid linked to metabolically active AAB

Other organic acids vary in concentration and are mainly linked to AAB/LAB metabolism

Upcoming metagenetics should link these data to microbial species

Conclusions and perspectives

- Acetic acid bacteria actively participate to biofilm formation which surrounds other bacteria yeast over time
- Clear symbiotic relationship between the different microbial groups exists
- Microbial species involved in Kombucha fermentations produce a specific metabolome over time
- Upcoming metagenomics data should lead to better understanding of kombucha fermentation and product quality
- Better understanding the role of each microbial species leads to better management of microbial resources

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