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▶ To cite this version:

Océane Savary, Jérôme Mounier, Anne Thierry, Elisabeth Poirier, Julie Jourdren, et al.. Understanding Kombucha fermentations: dynamic follow-up of a lab scale fermentation. FEMS 2019, Jul 2019, Glasgow, United Kingdom. hal-03279747

HAL Id: hal-03279747 https://hal.inrae.fr/hal-03279747

Submitted on 6 Jul 2021

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

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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 etened 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 backslopping 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.

in biofilm samples

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+2 log CFU/g in AAB counts

ate in biofilm

ed in biofiln

All 3 microbial groups

Branched

Diacetyl

acetoin

Diacetyl, acids and

ethyl esters

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%) etabolite producti

Volatile metabolites determined by GC-MS (headspace)

Kombucha metabolome elucidated

Dav 11

Day 14

Day 27 💢

Day 20

AAB domi







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

nple preparation: cells fixed h 1:1 PBS 1X:ice-cold ethanol 6 before hybridization with cfic yeast and AAB/LAB FISH bes, in green and red, pectively, blue represents film outfoll worscramers



ervation of a biofilm at 11 days



14 day biofilm sample:



Sample preparation for SEM: biofilm samples were treated with a cacadylate & 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

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

Upcoming metagenetics should link these data to microbial species

Conclusions and perspectives

e 8: Principal component analysis on volatile m the lab scale kombucha fermentation

Good repeatability of biological triplicates

- Acetic acid bacteria actively participate to biofilm formation which surrounds other bacteria yeast over time
- Clear symbiotic relationship between the different microbial groups exists

Distinct changes in volatilome directly linked to fermentation time

Upcoming metagenetics data will help link species to functions

Microbial and physico-chemical dynamics

Microbial counts monitored over 27 days:

in tea samples

Ļ

+2.5 log CFU/ml AAB counts +1 log CFU/ml yeast counts

Aldehyde

30

start

ble for LAB

Day 7

Microbial activities associated with:

- 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

Collaborations:

Acknowledgements: We would like to thank Mr.

Philippe Elies of the Microscopy platform at the UBO

Financial support:

for the microscopy acquisitions.



FEMS 2019 - 7 to 12 July - Glasgow, Scotland