A Kombucha Community Roll Call and Profile of Prebiotic Carbon Source Preference

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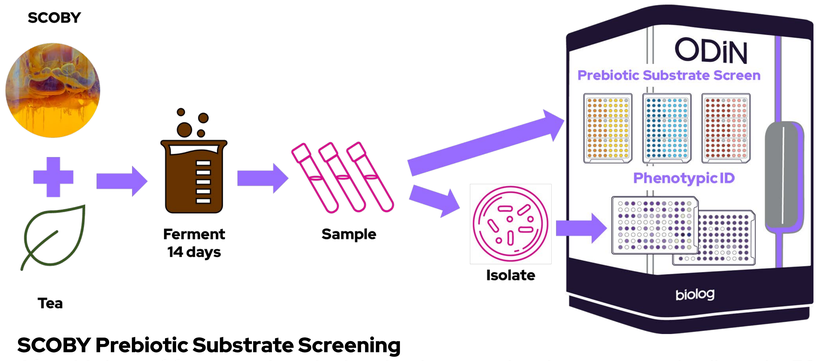
Introduction

Kombucha, a fermented tea beverage, has been produced and consumed beginning in China around 220 BCE using the naturally occurring bacterial and fungal microbes present on tea leaves and in the surrounding environment. Typically made with table sugar (sucrose) and green or black tea, the popular beverage is produced through fermentation carried out by the Symbiotic Community of Bacteria and Yeast, or SCOBY. The SCOBY microbes reside in both a thick cellulose biofilm layer produced by bacteria and in suspension dispersed throughout the tea where lactic acid and acetic acid bacteria and yeasts like *Schizosaccharomyces*, *Zygosaccharomyces*, and *Brettanomyces* break down complex sugars to feed different members of the community. Here, we utilized Biolog PreBioM™ plates and the Odin™ system to gain a holistic view of what substrates can be used by the SCOBY. The PreBioM line comprise a series of microplates containing a variety of known prebiotic carbon sources including mono- and di- saccharides (PreBioM 1) polysaccharides (PreBioM 2) and complex carbohydrates, starches, fibers, and artificial sweeteners (PreBioM 3). Community structure can be nebulous and difficult to ascertain; however, phenotypic ID, employed here, presents a flexible solution to identify key players in the SCOBY.

Methods

Kombucha fermentation

1. Boil 1200 mL sterile filtered water and add sterile solution of 150 g sucrose in 150 mL water
2. Brew commercial black tea bags (10 g total) for 10 minutes
3. Add commercial SCOBY
4. Loosely cover in sterile foil to allow air flow and ferment at room temp (~27° C) for 14 days
5. Passage kombucha SCOBY by repeating Steps 1 & 2, and then transfer the SCOBY cellulose biofilm and 150 mL kombucha to freshly brewed tea



SCOBY Prebiotic Substrate Screening

After 14 days of fermentation, the kombucha was stirred to resuspend cells and a 50 mL sample was pelleted at 4000 x g for 10 minutes. The cells were then washed twice in 1 X PBS and resuspended in IF-0a to a density of ~35% T. Following the standard Biolog Aerobic PreBioM plate protocol, Dye Mix D was added, and then the suspension was inoculated into PreBioM 1, 2, & 3 plates and placed in Odin for incubation and reading at 30° C for 96 hours with reads every 20 minutes.

Phenotypic ID

100 µL kombucha in a 1:100 dilution was plated on a variety of media selective for yeast, lactic and acetic acid fermenting bacteria at 26-30° C (M17, R2A, MRS +/- malic acid). Colonies were picked and transferred to BUG + Blood (bacteria) or BUY (yeast) agar before being identified using GENIII and YT MicroPlates respectively according to standard Biolog protocols

Phenotypic Data Analysis

PreBioM plates contain triplicates of 30 different carbon sources each, so replicates were aggregated by mean. Background subtraction was applied by subtracting the average negative control well values at each time point from each well. Maximum curve height (OD_{590}) was then calculated for each well and a cutoff of 0.2 applied to determine biological significance. Average Well Color Development, Shannon's Diversity Index, and Shannon's Evenness Index were calculated across all wells in PreBioM 1 to determine overall metabolic output, diversity, and evenness of substrates utilized over time respectively.

SCOBY prefers simple sugars (PreBioM 1) and can utilize complex carbs and fibers (PreBioM 3)

PreBioM 1	1,5,9	2,6,10	3,7,11	4,8,12
A	Negative Control	**L-Arabinose**	**D-Ribose**	**GlcNAc**
B	D-Xylose	NANA	D-Galacturonic acid	**D-Fructose**
C	D-Fucose	D-Psicose	D-Sorbitol	β-D-Allose
D	**α-D-Glucose**	**D-Galactose**	**D-Mannose**	**D-Mannitol**
E	Negative Control	**β-D-Methyl-D-Glucoside**	**D-Trehelose**	**D-Melibiose**
F	**D-Cellobiose**	**Turnaose**	**Gentibiose**	**Maltitol**
G	Chondroitin sulfate	α-D-Lactose	**Maltose**	Lactulose
H	**Sucrose**	3-O-β-D-Galactopyronosyl-D-Arabinose	**Palatinose**	**Sucralose**

	1,5,9	2,6,10	3,7,11	4,8,12
A	0.105	0.824	0.686	0.334
B	0.127	0.165	0.162	0.972
C	0.045	0.077	0.126	0.064
D	0.825	0.381	0.885	0.235
E	0.082	0.307	0.647	0.295
F	0.410	1.040	0.241	0.224
G	0.146	0.111	1.042	0.152
H	1.090	0.159	0.505	0.822

PreBioM 2	1,5,9	2,6,10	3,7,11	4,8,12
A	Negative Control	Stachyose	D-Raffinose	**D-Melezitose**
B	**Maltotriose**	FOS	GOS	**XOS**
C	MOS	**IMOS**	2``-Fucosyllactose	Arabinoxylan
D	Xyloglucan	GOS + β-Glucan	Lactitol	Amylose
E	Negative Control	β-Cyclodextrin	γ-Cyclodextrin	Amylopectin
F	Inulin	Pullulan	Polydextrose	Steviol glycosides
G	Lichen	Sodium carboxymethyl cellulose	Chitosan	Astragalus polysaccharide
H	Konjac glucomannan	Larch arabinogalactan	Lentinan	Scleroglucan

	1,5,9	2,6,10	3,7,11	4,8,12
A	0.062	0.074	0.103	0.431
B	0.598	0.048	0.065	0.206
C	0.093	0.479	0.000	0.000
D	0.121	0.028	0.000	0.012
E	0.034	0.000	0.000	0.054
F	0.049	0.029	0.079	0.000
G	0.078	0.000	0.280	0.184
H	0.000	0.000	0.135	0.026

PreBioM 3	1,5,9	2,6,10	3,7,11	4,8,12
A	Negative Control	Resistant Maltodextrin	Wheat Dextrin	Oat fiber
B	**Apple fiber**	full fat soya	Haricots bean fiber	Polydextrose
C	Maize dextrin	**Vegetable fiber**	**scFOS**	**Soluble veg. fiber + 0.1% glucose**
D	**scFOS + 0.1% glucose**	**Gln + plant extract**	Stevia extract	**Okra extract**
E	Negative Control	**Monk fruit extract**	**Mussel tissue**	**White cabbage**
F	Maize flour (deoxynivalenol)	**Monk fruit extract + 0.1% glucose**	**Mussel tissue + 0.1% glucose**	**Baobab**
G	Brussel sprouts	Baobab acacia	Quercetin	XOS maltodextrin + 0.1% glucose
H	β Sitosterol	Gum arabic	**XOS maltodextrin**	Erythritol

	1,5,9	2,6,10	3,7,11	4,8,12
A	0.017	0.107	0.022	0.047
B	0.297	0.108	0.126	0.081
C	0.134	0.531	0.477	0.313
D	0.584	0.216	0.078	0.299
E	0.051	0.254	0.216	0.256
F	0.078	0.252	0.216	0.214
G	0.121	0.060	0.046	0.191
H	0.016	0.038	0.257	0.000

Figure 1. Metabolic activity varies for Kombucha SCOBY on different substrates

Each curve represents metabolic activity (OD 590) of the SCOBY microbes over 96 hours. Values displayed in each well indicate the maximum OD reached after background subtraction; values in purple text were determined to be significantly above baseline (OD > 0.2). Plate maps (left) show substrates which met the OD > 0.2 cutoff in bold except for chitosan (PreBioM 2, wells G 3,7,11) which shows a high initial OD as the chitosan dissolves.

Phenotypic ID reveals some key players present in the kombucha SCOBY

Table 1. Enriched taxa isolated from Kombucha SCOBY

ID	Type	Notes
Streptococcus parasanguinis	Bacteria	Found in artisanal fermented foods[1]
Sporolactobacillus terrae	Bacteria	Lactic acid bacteria commonly found in soil[2]
Candida entomophila	Yeast	Found in fermented agave juice[3]

Community Analysis reveals 2-phase growth pattern showing substrate preference shifts

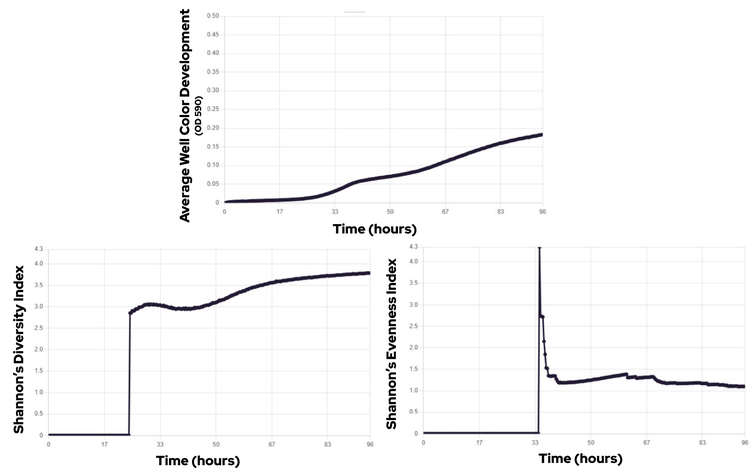


Figure 2. Kombucha SCOBY demonstrates flexibility in consumption of mono- and di-saccharides

The Average Well Color Development (AWCD) shows a slow start indicating an adjustment period, followed by entering the first of two growth periods driven primarily by early metabolism of fructose, glucose, mannose, and sucralose (Figure 1). These substrates also drove an initial spike in Diversity and Evenness indexes. At ~50 hours, AWCD, Diversity, and Evenness begin to trend upwards again driven in part by metabolism of sucrose, turanose, and maltose (Figure 1).

Conclusions and Future Directions

- Prebiotic substrate preference screening revealed that the SCOBY was able to rapidly metabolize simple sugars and sweeteners like fructose, glucose, mannose, and sucralose in a 2-phase process (Figure 1)
- Complex carbohydrates like vegetable and apple fiber along with okra and monk fruit extracts were more long-term energy sources being broken down steadily over time. This is likely due to the input of multiple community members (Figure 2)
- Key members of the SCOBY were enriched and identified, opening the door for future exhaustive studies of community structure (Table 1)
- In future studies, changing the carbon source during fermentation and monitoring subsequent changes in community and carbon source preferences could reveal more about the community's functional profile
- The combination of PreBioM and Odin is well-poised to make pairwise comparisons between communities that have been subject to various types of perturbations without wasting time making DIY screening plates

References

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