

The Culture of Culture: An Interdisciplinary Study on Kombucha, its Human Producers, and Their Microbially Entangled Relationship

A thesis submitted in total fulfilment of the degree of Master of Arts

Jacquelyn Jane Evans BSc/BA

Discipline of Anthropology

Department of Social Inquiry

College of Arts, Social Science, and Commerce

La Trobe University

Victoria, Australia

May 2021

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Abstract

The fermented tea drink kombucha has become increasingly popular in recent years in part due the increased focus on the importance of 'gut' health. Kombucha is made using a symbiotic community of bacteria and yeast, also known as a SCOBY. This microbial community is not well understood, with a limited number of studies having researched its community composition. From what is understood of kombucha's SCOBY it is a semi-open, tractable, and diverse microbiota whose composition differs depending on geographic origin and other unknown variables. In addition to being a microbiological beverage, kombucha has strong social facets when made domestically; its producers use social networks to share SCOBYs, methods, and information about production and health benefits. To investigate the biosociality of the relationship between kombucha and its producers a mixed methodological framework was created through which both the kombucha SCOBY and its human producers could be studied.

The ethnographic findings of this study discovered thematic trends which shaped the microbially entangled relationship of participants with their kombucha, and impacting SCOBY sharing and production practices. The molecular microbiological results as expected demonstrated the SCOBY has a conserved bacterial core community. However, each participants kombucha SCOBY had a unique collection of rare bacterial taxa differentiating them from the other communities. When both the contextual ethnographic and microbial community data were combined in a network analysis there was no correlation; although this was unexpected there were limiting factors that with further research could be addressed. Despite the lack of correlation in the network analysis this project successfully utilised a mixed methodological framework combining anthropology and microbiology to study the biosociality of kombucha and its producers, paving the way for future interdisciplinary collaboration between these disciplines.

Statement of Authorship

Except where reference is made in the text of the thesis, this thesis contains no material published elsewhere or extracted in whole or in part from a thesis accepted for the award of any other degree or diploma. No other person's work has been used without due acknowledgment in the main text of the thesis. This thesis has not been submitted for the award of any degree or diploma in any other tertiary institution.

Jacquelyn Jane Evans

14 May 2021

Additional information

This work was supported by an Australian Government Research Training Program Scholarship

The research undertaken in connection with this thesis was approved by the Arts, Social Sciences & Commerce College Low Risk Human Ethics Sub-Committee (Approval no: HEC19138).

Acknowledgements

Firstly, I would like to thank my supervisors Dr John 'Jack' Taylor, Dr Jen Wood, and Prof. Ashley Franks for their guidance and support. They have been there for me when the chips were down, battling the system to try and get me cross-college enrolled, taking a chance on a nut who wanted to do something that hadn't been done, and just generally being wonderful and supportive humans.

Thanks to Dr Anthony Moran who was a great help in honours and in masters as GRC, answering all my random questions and emails, and who attended almost all the weekly HDR check-ins with me last year.

A huge thanks to all my participants without which there would be no research. I appreciate your generosity in letting me into your homes, showing me how you make kombucha, sharing samples, feeding me, and the fantastic conversations that allowed this thesis to come into being.

Thank you and appreciation to the whole Applied and Environmental Microbiology Lab for their help and support throughout the years, I could not have done it without you. Dr Elizabeth Mathews and Dr Anya Schindler for their support even when they had much of their own stuff going on. Sarah Knowler for her help with all manner of things as well as the bacterial bioinformatics, and Rachele Gore for her practical advice about smashing biofilms. Joshua Vido for his endless work to get the sequencing runs for Matt and I done and a wealth of other help. Gene Drendel for the advice, as well as the Nintendo switch, photography, and Stardew Valley conversations. And Dr Howard Habtom for his endless optimism.

Thanks to Larry Liversage for wonderful conversations, books, and assistance at the beginning of 2019 in setting up my lab kombucha.

My fellow HDRs in Social Inquiry, many of whom I had only ever met on zoom last year. Thank you for attending my zoom check-ins, keeping me sane, offering chats and support. In particular, Adrienne, Suzannah, Kim, Bernardo, Gianmaria, Ben, and anyone else who came as regularly as they could.

The staff at Writers block, Sycamore Meadows, and Foreigner cafés who dealt gracefully with me camping out on their tables for hours on end, fed me, and caffeinated me, without which this thesis wouldn't have been written. Particular thanks to Jules, Kristina, Lou, and Josh.

And finally, thank you to my family and friends for their endless love and belief in me. Dad in particular for dealing with so many phone calls and providing the counsel that got me through. My mum who is enthusiastically supportive about whatever I do. Michael, my love, although he has only been present for some of this journey his stalwart belief in my capabilities and insistence that I am spectacular always gives me strength. To my friends and cheerleaders, Rowie, Yuhan, Antoinette, Sam, Ben, Matt, Rachel, Mick, and Megan <3.

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List of Abbreviations

ACM	Alternative consumption movement
bp	Base pair
BSE	Bovine spongiform encephalopathy
°C	Degrees Celsius
CAM	Contemporary and alternative medicine
DNA	Deoxyribonucleic acid
ESV	Exact sequence variance
gDNA	Genomic DNA
g	Grams
GMO	Genetically modified organism
ITS	Internal transcribed spacer
μl	Microlitre
ml	Millilitre
mins	Minutes
NMDS	Non-metric multidimensional scaling
PCoA	Principal co-ordinate analysis
PCR	Polymerase chain reaction
PICRUST	Phylogenetic Investigation of Communities by Reconstruction of Unobserved States
Sec	Seconds
SCFA	Short-chain fatty acid
SCOBY	Symbiotic community of bacteria and yeast
rRNA	Ribosomal ribonucleic acid
16S rRNA	A component of the 30S small subunit prokaryotic ribosome

A note to the reader

As a reader of this document, you might already be aware from the title, abstract, or other forewarning that this research is interdisciplinary. Therefore, it is important to emphasise the resulting text is not traditionally an anthropological ethnography or microbiological report. Instead, a hybridised structure was created in order to combine, clearly communicate, and do justice to the findings of this research. Furthermore, due to the diversity of readership, aid to understanding in the form of disambiguation and/or glossary of terms can be seen in the additional materials at the beginning of this thesis. The appendixes too are structured to offer clarity through the addition of detail that could not be included in the body of this thesis. It is the author's earnest hope that you enjoy reading this thesis.

Glossary of Terms

Amplicon sequencing: The process of isolating a piece DNA from the genome in order to analyse its sequence variation (the code making up the two halves of a DNA double helix). For this project the 16S rRNA and ITS2 regions were examined to provide information on the taxa of respective bacterial and fungal communities.

Biosocial: Relating to “the interaction of biological and social factors” (*OED Online* 2018).

Cellulosic biofilm (biofilm used for short): A thick layer of cellulose created by the microorganisms living in the SCOBY that sits on the top of the liquid kombucha (Chakravorty et al. 2016; Jayabalan et al. 2014; Reiss 1994).

Continuous crock: This is a kombucha brewing set up that consists of a large (usually) earthenware crock which has a tap on the bottom and is often covered with a tea towel or fabric and secured with string. It is started like other kombucha brews by the addition of cold sweet tea and a SCOBY, however when it is ready the producer decants it from the tap and tops the crock up. Therefore, this system is considered continuous as there is no emptying, cleaning, and refilling, the tea is just replenished as required.

Exact sequence variance (ESV): A term that refers to a DNA sequence gained through amplicon sequencing. Functionally, ESVs from amplicon sequencing output are grouped together based on the similarity of their DNA, allowing for differences to be identified and taxonomic ranks to be assigned.

Founder effect: A phenomenon involving a reduced genetic diversity in a population due to their being descended from a “small number of colonizing ancestors” (*OED Online* 2018). In kombucha sharing, given the random sampling of the “parent” SCOBY, there is the potential that the rare members of a new SCOBY are a random subset of the old. This does not take into account any environmental effects, however, which could also influence the kombucha microbial community.

Functional pathway: This term is used in molecular biology to refer to a schematic representation of molecular physiological machinery within cells, tissue, or microorganisms. In this setting, pathway data provides an understanding of what functions microorganisms within a community may be performing, for example the utilisation of sugar by bacteria or fungi for cellular energy through different kinds of fermentation and resulting in by-products such as ethanol. This kind of data is obtained from a Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt).

Genera (plural): Taxonomic category that ranks below family and above species (*OED Online* 2018).

Genomic DNA (gDNA): The chromosomal genome of an organism; a term used to differentiate between extra-chromosomal DNA, which can exist in some microbes in the form of plasmids for example.

Gel electrophoresis: A method for visualising the presence of large molecules, such as DNA. The outcome of this will also give an estimated base pair size of a molecule, so as to test if the isolation of a piece of DNA has worked.

Inoculation: When a single microorganism or community are purposefully added to an organism, substance, or media, for them to grow in.

Kefir: A fermented milk drink similar to yoghurt that is made with a SCOBY. The microbial community forms large granules, colloquially known as kefir grains which are shared between makers and used as a starter.

Library preparation (for amplicon sequencing): The creation of a pool of DNA (from DNA that has been prepared through the addition of primers and PCR) that have specific adaptors attached allowing them to be sequenced using a particular sequencing machine. In this project the Illumina protocols and MiSeq machine were used.

Microbiota: A community of microorganisms that inhabit a particular environment or niche (e.g. the gut microbiota are the microorganisms that live in the gut of an animal or human).

Disambiguation of microbiome vs microbiota: These two terms often get used interchangeably. Microbiota specifically means the microorganisms of a particular community whereas microbiome can also be used to indicate the collective genetic material of those microorganisms.

Microorganism: Umbrella term used to encompass bacteria, viruses, and some fungi and parasites.

Pipeline (in bioinformatics): A pipeline in this setting is a series of steps used to prepare data for analysis. It can also be used to describe the steps taken to analyse such data, often including a number of algorithms that are performed one after another.

PCR (Polymerase chain reaction): A method used to copy a specific section of DNA, yielding a much larger volume so the amplified region can be used in subsequent methods and analysis. There often needs to be a cleaning process after a PCR for by-products that will interfere with further use.

Primers (for amplicon sequencing): Primers are used in order to bind to the DNA section of interest, enabling it to be amplified with PCR and then sequenced. There are usually 'forward' and 'reverse' primers allowing for the for the two halves of the DNA double helix to be read (see 'reads' below).

Phylogenetic tree or phylogeny: This is a branching diagram that shows the evolutionary relationship between different taxa. For the purposes of this research the phylogenies and taxonomic assignment were based on the genetic differences present in the ESVs, and the utilisation of reference databases.

Reads - forward/reverse (in sequencing and bioinformatics): A read is the inferred sequence corresponding to one half (the forward or reverse) of a piece of DNA. In a bioinformatic pipeline and in preparation for data analysis, the sequence read of the two halves of a DNA need to be assessed for quality and merged.

Richness (or species richness): In ecology this term describes the number of different species in a community.

Disambiguation richness vs diversity: Richness and diversity often get used interchangeably, however technically diversity takes into account both richness and evenness of species. Evenness refers to the number of individuals belonging to a species within a community, and how similar those species populations are.

Supernatant: The liquid above a solid residue or pellet; formed when particles suspended in the liquid have been separated through centrifugation or other method (*OED Online* 2018).

Taxa (plural)/taxon (singular): A group or rank within the biological taxonomic system, indicating a ranked hierarchy from kingdom through to species and sub-species. The highest taxa discussed in this research will be phylum, being one step below kingdom, with kingdom indicating groupings such as animals, bacteria, and fungi.

Chapter 1: Introduction

In social settings, when people ask me, “so what do you do?”, I often begin with the following words; “the short answer is I study kombucha.” These words are often greeted with puzzled laughter. However, more often than not such amusement turns to curiosity, and then active interest. Once I explain that what I do is go to people’s houses, investigate their perspectives on kombucha, explore their sharing and production practices, take samples of their SCOBYs and examine the microorganisms living in them, and on this basis analyse the relationships that form and adhere between these human and microbial communities, it makes sense to them. Overwhelmingly, the people I talk to about my research respond with words to the effect that, “of course there is a relationship between the two”, and agree that it just makes sense for environmental microbial communities to become entangled together with humans through practices involving fermentation and fermented food. These interactions reflect widespread assumptions and “common knowledge” regarding the entanglement of humans and microbes, particularly in consideration of domestic production, exchange, and consumption of fermented foods, very little in-depth research has been conducted to explore such biosocial phenomena. Taking the first steps to fill this gap, this thesis explores the biosocial relationship between kombucha and the humans that produce it in domestic settings. In investigation of this topic an interdisciplinary mixed methodological framework was used, combining ethnographic methods from anthropology, as well as molecular and ecological methods from microbiology. Through the successful conduct of the project, it was demonstrated there is a microbially entangled relationship present intricately linked across a range of scales from the microbial and individual to the social and political.

In recent years there has been a resurgence of interest in fermentation and fermented foods. Having played an important role in preservation and nutrient acquisition in times of food scarcity for millennia in many parts of the world, fermented foods are also prized for their unique flavour and sensory properties which can be directly attributed to the microorganisms that characterise them (Davis 2017; Gibbons & Rinker 2015; Hutkins 2019; Katz 2006, 2012; Paxson 2008; Spackman 2018; Wolfe & Dutton 2015). This contemporary resurgence however is driven by discoveries in the biological sciences relating to the importance of ‘gut’ microbiota and other human microbial

communities to the bodily composition and health (Katz 2006, 2012; Paxson 2008; Spackman 2018). In large scale commercial production the microbial content of fermented foods like bread, beer, or wine are stabilised through the use of isolated and purified strains of yeasts and bacteria (Hutkins 2019). However, there is a wide range of foods whose production depends on undefined microbial communities (Hutkins 2019; Wolfe & Dutton 2015). The tea-based drink kombucha is one such example; given its increased popularity and relatively widespread domestic production within the state of Victoria, Australia—the location of this study—and its potential as a model microbial community used in everyday domestic food production, it has been chosen as the focus of this research.

Microbial communities like those present in kombucha are commonly referred to in domestic settings as ‘starters’, ‘mothers’, and ‘symbiotic communities of bacteria and yeast’ (SCOBYs) (Davis 2017; Paxson 2008). Increased international popularity accompanying the rise of alternative food movements, including paleo dieting, organic, super- and slow food, and the significance now placed on ‘gut health’ has led to an increasing number of people populating their kitchens with these microbial communities (Germov, Williams & Freij 2011; Gressier 2018; Rebrovick 2015). SCOBYs are also shared between makers, or bought from small artisanal suppliers, and are also used to make other foods including sourdough and kefir. Fermented food microbial communities, along with other reservoirs of microorganisms, such as household pets, are important to the ecological, microbial, and social landscape of the home and kitchen, making them intrinsically biosocial actors in a range of different food practices (Baschali et al. 2017; Carbonetto et al. 2018; Chakravorty et al. 2016; Flachs & Orkin 2019; Gilbert & Stephens 2018; Marsh et al. 2014; Wolfe & Dutton 2015).

Before describing the research methods employed in this research, as well as presenting primary results and analysis, the following introduction provides a brief overview of microorganisms, including especially kombucha, and their history, as well as a review of academic literature relevant to this study.

A brief history of microorganisms

A brief history of microorganisms in the context of western biomedical, dietetic, and food safety discourses is an important place to start as many of these contextual circumstances continue to influence contemporary understandings of and interactions with food and microorganisms (Pernick 2002). Ideas of contagion, miasma, disease, and illness are core to the story of microorganisms in this context (Blay-Palmer 2008; Douglas 1966; Pernick 2002; Spackman 2018). Negative moral and health-related associations are often discursively linked to these concepts and have dominated popular meanings assigned to microorganisms in Western settler nations, like Australia and the U.S., despite their ongoing use in fermentation and food preservation for thousands of years (Blay-Palmer 2008; Pernick 2002).

Miasma theory dominated Western medical thought in different forms for centuries, preceding the beginnings of modern microbiology and Robert Koch's germ theory of the late nineteenth century (Pernick 2002; Smeele 2016). Relating to the cause of contagion and disease, the notion of miasma has been present in medical texts since Hippocrates, however its specific meanings have shifted through time (Jouanna 2012). Prior to the mid-nineteenth century, the general meaning of the term miasma coupled the smell and emanations from certain infections, decaying organic matter, sewage, to various forms of pollutions to particular causes of pestilence and disease (Cole 2016; Jouanna 2012; Smeele 2016). Derived from the Greek word meaning "to stain," by the mid-nineteenth century, when it became heavily used in medicine, it also had significant legal and religious connotations (Jouanna 2012, p. 121). Due to such associations, miasma was framed as a "symptom of disintegrating boundaries", signifying sin, moral corruption, and a breakdown of biopolitical order (Cole 2016; Ellman 1990). The literal and social connotations of miasma created an awareness of how permeable and vulnerable the body was to disease (Cole 2016; Warin 2003). As a result, the moral and physical vulnerability of the human body heavily influenced how hygiene and the environment were constructed in the burgeoning Western European industrial societies of the nineteenth century (Blay-Palmer 2008; Cole 2016). With the advent of Koch's germ theory and Louis Pasteur's technique of pasteurisation in the late nineteenth century, understandings of the cause of diseases and illnesses rapidly changed from "invisible threat to manageable bacteria" (Latour 1988; Paxson 2008; Smeele 2016, p. 15). This situated microorganisms as germs, a target which through

the application science and technology could be predicted, ordered, and controlled (Latour 1988; Paxson 2008; Smeele 2016). Despite the shift away from miasma theory in western scientific thought, metaphorical and literal associations of miasma and contagion as related to sin, corruption, and defilement have persisted in popular discourse (Pernick 2002).

The dangers of uncontrolled microorganisms became more apparent in the late nineteenth and early twentieth centuries, with the increased industrialisation of food production causing cases of food poisoning and adulteration to grow (Blay-Palmer 2008). This brought food safety directly into the realm of biomedicine, with technology and science “applied enthusiastically” to production practices (Blay-Palmer 2008, p. 19). Approaching food safety in this way enabled “Pasteurianism” to flourish, a term coined by Bruno Latour (1988) after Louis Pasteur and his method of controlling microbial growth in food. Pasteurianism describes an approach to the formation and application of biopolitical methods to rationally order and predict microorganisms which became hegemonic over the twentieth century (Latour 1988). Heather Paxson (2008) develops these ideas further, drawing on Latour (1988) and Michel Foucault (1978) to introduce the term “microbiopolitics”. This concept works to understand and critique the “anthropocentric evaluation” of microorganisms, their categorisation, and the ways that human relations with regard to microorganisms are constructed by society (Paxson 2008, p. 17). According to this perspective, in the burgeoning global food system, through the legal and political mechanisms of Pasteurianism and microbiopolitics microorganisms were configured as “elements to be eliminated so that human polities might be cultivated” (Blay-Palmer 2008; Paxson 2008, p. 17; Latour 1988). Therefore, without the order and control of science and biomedicine, microorganisms collectively were considered dirty, dangerous, and as Mary Douglas puts it, “matter out of place” (Douglas 1966, p. 44).

At a similar time as Pasteurianism and microbiopolitics were contributing to the construction of how people ought to relate to microorganisms and each other in the early twentieth century, processed foods as products of the global industrial food system were being positioned moralistically as pure, healthy, and virtuous (Blay-Palmer 2008; Paxson 2008; Rebrovick 2015). Throughout its history western biomedical discourse has been inseparable from morality, with social values being

aligned with bodily health and moral personhood to such an extent that health has become indicative of “a virtuous life” (Rebrovick 2015, p. 680). The nutritionist dietetic discourse which has dominated much of the last two centuries is no exception, foregrounding connections between virtue and diet (Rebrovick 2015, p. 680). While previously it had been common for an individual to tailor their own diet in order to achieve a humoral balance, drawing on the medical knowledge of the time and personal experience, the nutritionist dietetic instead conceived of “the human body” as universally uniform and reduced food to a “quantifiable and calculable abstraction” (Rebrovick 2015, p. 682). Eating well became intimately tied into biopolitics, the capitalist ideal of producing an effective and healthy workforce, and being a good citizen. As such it was established as an essential focus and component of state projects related to the economy and public health (Blay-Palmer 2008; Paxson 2008; Rebrovick 2015).

Throughout much of the twentieth century processed microorganism-free foods made within the western industrial food production system continued to be associated with moral virtue (Blay-Palmer 2008; Latour 1988; Paxson 2008). Over this period, the increased size and complexity of this global system also worked to separate the means and knowledge of food production from the general public (Blay-Palmer 2008; Carolan 2011). Overall, the elaborate scrutiny of microorganisms in food science and the industrialisation of western food cultures, including the continued predominant perception of microorganisms as germs to be eliminated from food, has profoundly shaped contemporary understandings of what constitutes healthy food and consumption practices (Blay-Palmer 2008; Latour 1988; Paxson 2008; Rebrovick 2015).

Despite the industrialisation of western food cultures continuing to shape contemporary understandings of food and health, from the nineteenth and early twentieth century numerous alternative consumption movements (ACMs) have worked to critique and counter dominant discourses (Belasco 2007; Ross 2013). Features of these health food movements have included vegetarianism, critiques of industrial processed food, organic foods, and ecological campaigns, often accompanied by grass roots political struggles (Belasco 2007; Ross 2013). For much of the twentieth century however many of these ACMs remained marginal, in large

part due to their being associated with contemporary and alternative medicines (CAMs) which, deviating from hegemonic biomedical traditions, were often dismissed by authorities as the health beliefs and practices of “crackpots and cranks” (Belasco 2007, p. 16; Ross 2013). During the latter part of the century however ACMs and CAMs gained traction and recognition, including and especially through the countercultural movements in the 1960s (Belasco 2007; Rebrovick 2015; Ross 2013). Many of these movements, such as in the “slow food” movement of the 1980s, centred around environmentalism but also the celebration of food for pleasure (Belasco 2007; Rebrovick 2015; Ross 2013).

Into the twenty-first century the environmental and ecological effects of food consumption and critiques of industrial processed food have continued to increase (Blay-Palmer 2008; Brown 2007; Orlando 2018; Rebrovick 2015). These critiques have escalated to such an extent that Tripp Rebrovick (2015) has coined the term eco-dietetics to describe what they argue represents a new social and dietetic discourse. Rebrovick (2015) outlines dietetic discourses as having four axes, including distinct ideas of healthy food, moral implications of eating practices, a pre-supposed composition of a human body, and particular sites as sources of authoritative knowledge on food. As mentioned previously, the dominant discourse of western thought prior to the nineteenth century was humoral (Rebrovick 2015). The ensuing nutritionist dietetic discourse which followed the Enlightenment and early western biomedical reductionism, dominated well into the twentieth century (Rebrovick 2015). Eco-dietetics, according to Rebrovick (2015), is describes a collection of social movements that have gained traction over the last sixty years forming a new dietetic discourse, gradually taking over from nutritionism. Within this discourse the human body is understood as intimately linked to and inseparable from its environment (Rebrovick 2015). The focus is shifted to how a person eats rather than simply what they eat, meaning that the ethics of food, and the effect a person’s consumption has on the environment, are considered more important than its nutrient or calorific content (Rebrovick 2015). Additionally, the agrarian aspects of eco-dietetics seek to “overcome and replace the dominance of industrial agriculture” by focusing on local, ethical, and pleasurable food rather than profitable (Rebrovick 2015, p. 686). Current social movements that fit within eco-dietetics include, for example, slow food, sustainable agriculture, organics, and locavores (Rebrovick 2015).

Post-Pasteurianism is another facet of contemporary food discourse that benefits from this exploration of the history of microorganisms and food (Paxson 2008, 2013). Post-Pasteurianism is a term coined by Paxson (2008) to describe the changing discourse around how people and microorganisms ought to relate and interact with one another. Pasteurianism, defined previously, was a means to order, predict and control microorganisms and their interactions with people through biopolitics and microbiopolitics throughout the nineteenth and twentieth centuries (Latour 1988; Paxson 2008). According to Paxson (2008), central to the development of post-Pasteurianism over the last twenty years has been a determined move away from the pure and separate relations envisioned by hygienists and Pasteurians. There is of course an acknowledgement of the importance of safe food within post-Pasteurianism, and the dangers of pathogens are not disregarded (Paxson 2008). There is, however, an increased focus on the importance of microorganisms to human health, food, and the environment, embracing the collaborative potentials of “mould and bacteria as allies”, and recognising them to be “ubiquitous, necessary, and, indeed, tasty” (Paxson 2008, pp. 17–18). Health too plays a role within the post-Pasteurian discourse with the cultivation of ‘good’ or healthy microorganisms in food being a way to care for oneself (Paxson 2008). Within this discourse there is also a questioning of the “motives and logics underpinning” the hegemonic “Pasteurian regime”, and critiquing the industrial food system for its focus on the medicalisation of food and eating (Paxson 2008, p. 17). The critique of the industrial food system within post-Pasteurianism goes further, drawing on food scares involving microorganisms to question whether state regulators only have the interests of “citizen-consumers” in mind (Paxson 2008, p. 18).

Kombucha, its history, and an overview of relevant academic research

The fermented tea drink kombucha, in a domestic setting, is made predominantly from the cooled tea of steeped *Camellia sinensis* (“tea plant”) leaves and some form of sugar fermented by a SCOBY (Chakravorty et al. 2016; Jayabalan et al. 2014; Reiss 1994). The proportions of sugar, tea, and SCOBY are all variable based on tradition, recipe, experience, and desired flavour outcome (Chakravorty et al. 2016; Jayabalan et al. 2014; Reiss 1994). The kombucha SCOBY is made of two parts, the liquid kombucha and a cellulosic biofilm (hereafter referred to as a biofilm, see glossary), with both parts widely considered necessary for inoculation (see glossary) of the next batch (Reiss

1994; Villarreal-Soto et al. 2018). Once the tea and sugar mixture has been inoculated it is covered with a cloth, secured, and left to ferment for an average of seven to ten days (Chakravorty et al. 2016; Hasseltine 1965; Reiss 1994). Throughout the fermentation process the brewer will typically taste the kombucha to check how sweet or acidic their batch has become. Taste testing assists the brewer in determining when they wish to begin consuming the kombucha (Elliot 2018; Hasseltine 1965). At this stage a secondary ferment, which may involve the addition of flavours through fruit or other sugar additions, can be carried out if desired (Elliot 2018; Hasseltine 1965). The finished product without secondary fermentation will usually be a pale to deep golden-brown colour, or a yellow/green depending on the tea used, it will often be slightly effervescent and have a sweet and acidic smell. Kombucha at this stage can differ significantly in taste, generally it will be a little sweet and fruity, with pronounced acidity, and a characteristically fermented flavour. With secondary fermentation, the effervescence is usually more notable and the kombucha will have also taken on some of the colour, taste, and scent of whatever fruit has been added.

The sparse information available on the history of kombucha, as well as the depth of its history, shroud its origins in mystery. Similar to tea it has a number of origin stories and indeed could have arisen independently in multiple locations (Saber 2010). Kombucha is thought to have originated in mainland China some time in the last approximately two thousand years, with the earliest records of tea cultivation dating to 53 BCE in Sichuan (Chakravorty et al. 2016; Elliot 2018; Hasseltine 1965; Saber 2010). It is not known when kombucha spread from Asia through Eurasian Russia into Eastern Europe and Germany, but records indicate its presence there as far back at the nineteenth century (Chakravorty et al. 2016; Elliot 2018; Hasseltine 1965).¹ Wherever kombucha has gone it appears to have been thought of as a health drink (Chakravorty et al. 2016; Elliot 2018; Jasarevic 2015; Paxson & Helmreich 2014; Reiss 1994). Indeed, contemporary popular interest in kombucha arises primarily due to its long list of reputed health benefits which have accumulated over centuries (Paxson & Helmreich 2014; Spackman 2018; Villarreal-Soto et al. 2018).

¹ Origins unknown: Many sources claim to have knowledge of the provenance of kombucha (Dufresne & Farnsworth 2000). Although no assertions found in the course of this research were based on reputable academic sources. Furthermore, searching for kombucha's history in a wide range of databases yielded no papers that could trace it back further than the 19th century.

Similar to its documented history, there is limited academic literature on kombucha. This is changing however with the increased popularity of fermented foods more generally (Flachs & Orkin 2019; Kapp & Sumner 2019; Paxson 2008; Paxson & Helmreich 2014; Spackman 2018; Wolfe & Dutton 2015). Within the scientific literature progress has been made in understanding kombucha's action against a range of human pathogenic organisms including *Bacillus cereus*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Helicobacter pylori* and *Salmonella typhimurium* (Battikh, Bakhrouf & Ammar 2012; Greenwalt, Ledford & Steinkraus 1998; Jayabalan et al. 2014). Authors have attributed these effects to the acidic by-products made by acetic acid bacteria (AAB) (Greenwalt, Ledford & Steinkraus 1998; Reiss 1994; Steinkraus et al. 1996). A range of studies have also been conducted on animal models, for example investigating the effect of kombucha on liver and kidney function in hypercholesteraemic rats and indomethacin-induced gastric ulcers in mice (Bellassoued et al. 2015; Bhattacharya, Gachhui & Sil 2013). The results of animal studies so far indicate kombucha consumption has tangible health benefits, however to date there have been no clinical studies on humans (Chakravorty et al. 2016; Jayabalan et al. 2014; Kapp & Sumner 2019; Katz 2006; Spackman 2018; Villarreal-Soto et al. 2018).

Studies into the community composition of SCOBYs have found a range of bacteria and yeast living in kombucha (Chakravorty et al. 2016; Coton et al. 2017; De Filippis et al. 2018; Marsh et al. 2014; Reva et al. 2015). There appears to be some core genera including fungi like *Saccharomyces* and *Brettanomyces/Dekkera*, and the bacterial genera *Komagataebacter* and *Gluconacetobacter* (Chakravorty et al. 2016; Coton et al. 2017; De Filippis et al. 2018; Marsh et al. 2014; Reva et al. 2015; Teoh, Heard & Cox 2004). However, other community members differ widely between SCOBY source location, with a sample size of five being the largest investigation into kombucha microbial community variation thus far (Marsh et al. 2014). This leaves a broad area of investigation open to study, as kombucha is a small, semi-open, and tractable microbial community that can be studied more readily than other much larger, complex, and diverse environmental microbiota (Wolfe & Dutton 2015). The kombucha SCOBY is also embedded in a biosocial environment and presents a potential model

for investigating human impact on microbial communities (Lax et al. 2014; Marsh et al. 2014; Wolfe & Dutton 2015).

The microbial and scientific aspect of kombucha is only one facet of this biosocial beverage. It has, like all foods, embedded social and cultural meanings which, depending on context, dictates how it is produced, shared, and consumed. With the exception of Larisa Jasarevic (2015) and Christy Spackman's (2018) recent research, social scientific literature concentrating on kombucha specifically is limited. Jasarevic (2015) discusses kombucha in terms of a medical remedy in Post-Yugoslavia and Bosnia, exploring themes of agency and ontology, whilst Spackman (2018) uses the lens of microbiological citizenship to analyse the recent U.S. regulatory crackdowns on alcohol content in commercial kombucha production. In this work, Spackman (2018) introduces the important theoretical tool of microbiological citizenship to examine how consumers and producers strive to maintain relationships with the beneficial microorganisms believed to be contained within kombucha.

Regardless of this relative lack of literature, due to kombucha's inclusion or relation to a range of different foodways and social trends such as superfoods and paleo dieting, consumption and production of this beverage can be seen to fall under the umbrella of a number of active academic discourses (Gressier 2018; Jasarevic 2015; Paxson 2008; Rebrovick 2015; Spackman 2018). Post-Pasteurianism, as outlined earlier in this chapter, is utilised by Jasarevic (2015) in discussion of kombucha. Spackman (2018), too, explores post-Pasteurian ideology in the context of small scale kombucha production and his their participants approach of embracing microbial uncertainty in favour of gut health (Paxson 2008, 2013; Spackman 2018). Post-Pasteurianism in Spackman's (2018) research is used to tackle concerns about the problematic increases of anti-biotic resistance and the desire to establish a working relationship with 'good' microorganisms (Paxson 2008, 2013). The concept of eco-dietetics, also outlined prior, compliments the ideals of post-Pasteurianism with regard to kombucha (Paxson 2008; Rebrovick 2015). Eco-dietetics as a discourse and ideology is rooted the understanding and awareness of the impact of food production and consumption on the environment, prompting people to be as involved and local as possible with food purchasing or production (Rebrovick 2015). As the intersection of post-Pasteurianism and eco-dietetics indicates, in making and consuming kombucha

people thoughtfully incorporate their personal ethics and politics into their food purchasing decisions and production methods (Paxson 2008, 2013; Paxson & Helmreich 2014; Rebrovick 2015; Spackman 2018).

Entangled themes and issues of scale

As the above discussion demonstrates, there are a number of different perspectives and levels of scale from which both the human body and kombucha SCOBYs can be viewed. Providing a useful framework to understand these differences of perspective and scale, drawing on the seminal work of Nancy Scheper-Hughes and Margaret Lock (1987) three primary categories for viewing the body as associated with kombucha may be identified; the phenomenologically experienced *individual* body/self, the *social* body as a symbol for thinking about relationships between nature, society, and culture, and the body *politic*, being an artifact of social and political control (Scheper-Hughes & Lock 1987). According to Scheper-Hughes and Lock (1987) these categories represent three separate but overlapping units of bodily analysis that are complexly entangled through being both “naturally and culturally produced, and securely anchored in a particular historical moment” (Scheper-Hughes & Lock 1987, p. 7). The entanglement of these units of analysis is particularly relevant to this research as themes arising from participants span across and move between them. People are only one part of this equation, however, in that the kombucha SCOBY themselves complicate things further through the addition of non-human, microbial entanglement. The “three bodies” approach remains useful, however, if we consider that the political and cultural units of social analysis can be applied just as readily to microorganisms and microbial “communities” as to human ones, some of which were explored above in the history of microorganisms (Scheper-Hughes & Lock 1987). Unlike the human individual, however, microorganisms cannot speak for themselves, and are predominantly understood through the application and interpretation of the quantitative techniques in microbiology or through the interpretation and phenomenology of human experience.

Introducing themes that are key to understanding current social and interdisciplinary research on fermentation, the idea of entanglement, and more specifically microbial entanglement is of particular importance to this study (Flachs & Orkin 2019). The first theme, neo-cultural ecology, approaches human-microorganism interactions as an

iterative feedback loop, moving between the “scale of the human body to the scale of anthropogenic landscapes,” looking at how malleable microbial communities are at all scales (Flachs & Orkin 2019; Lorimer 2016; Paxson 2013). The second key theme is microbiopolitics, as coined by Paxson (2008, 2013). This addresses aspects of politics, how microorganisms are seen by people or states as threats or allies, and “food panics like *Escherichia coli* outbreaks reveal the fragility of state hygienic regulations” (Blay-Palmer 2008; Flachs & Orkin 2019, p. 36; Paxson 2013). Within this theme, home fermenters and small scale commercial producers argue that microbial entanglements can be positive, thereby questioning, and critiquing the motives of regulators and corporate agribusiness (Flachs & Orkin 2019; Katz 2006, 2012; Paxson 2013; Spackman 2018). The final theme is environmental humanities, which understands humans as plural through microbial entanglement (Flachs & Orkin 2019). Although ideas of plural and porousness personhood, and notions of the dividual, are not new in anthropology, they have been confined to predominantly symbolic and social discussions (Smith 2012). Environmental humanities complicates further the human bodily units of analysis mentioned prior as it destabilizes the notion of the human body as a self-contained natural artifact (Bordenstein & Theis 2015; Flachs & Orkin 2019). Moreover, this theme draws on the recent conception of people as ‘holobionts’, being multispecies assemblages which have ongoing relationships with microorganisms (Bordenstein & Theis 2015; Flachs & Orkin 2019). Under environmental humanities fermentation is imbued with social and biological meaning, signalling group identity with live cultures gifted and exchanged, and recipients of ferments invited to join in culturally significant taste as well as a shared ecological practice (Flachs & Orkin 2019; Jasarevic 2015; Katz 2012). The different bodily and microbial units of analysis in combination with these themes presents a complex multiscale picture of microbial communities, fermentation, and the humans who utilise, share and consume kombucha SCOBYs. Through the everyday domestic practice of fermentation, humans and microorganisms converge at “the nexus of local agroecological management, food practices, and human wellbeing” (Flachs & Orkin 2019, p. 37). Altogether these two approaches, the three bodies and microbial entanglement, provide points of reference and implicate a range of themes relevant to the research and analysis presented in this thesis. These references and themes provide a framework through which to engage meaningfully with the data being

presented and discussed while preserving complexity and scholarly efficacy at different scales.

Conclusion

The biosocial relationship between kombucha and its human producers in the context of this project presents unique opportunities to address gaps in current research. As discussed in this chapter there is meagre research on kombucha as either a social phenomenon or within the biological sciences as environmental microbiota with the potential to impact human health (Arikan et al. 2020; Jasarevic 2015; Kapp & Sumner 2019; May et al. 2019; Spackman 2018; Wolfe & Dutton 2015). This is changing, with increased popular interest in kombucha has come renewed interest in conducting research to grow the body of knowledge, but kombucha and its biosocial relationship with humans is still not a well understood phenomenon. The biosociality of kombucha also presents an opportunity to engage in interdisciplinary research spanning the biological and social sciences, with the complexities of the relationship between humans and microorganisms being investigated through taking a mixed methodological approach. As well as its primary objective of examining the biosocial entanglement of kombucha, in taking up the opportunity for interdisciplinarity this research responds to the increasing number of calls for productive research between the biological and social sciences (Benezra, DeStefano & Gordon 2012; Frost 2018; Goodman 2013; Meloni 2014; Meloni et al. 2018; Rees, Bosch & Douglas 2018; Roberts & Sanz 2018).

In order to take advantage of these opportunities, this thesis develops and implements an interdisciplinary methodological framework and mixed methods approach, combining qualitative ethnographic techniques from anthropology and quantitative ecological and molecular techniques from microbiology, to explore the shared biosocial space of kombucha and its producers. Through close examination of the relationship between kombucha's microbial communities and the people who make it in domestic settings, including and especially household kitchens, this thesis contributes to the growing understanding of the culture of kombucha from both social and biological perspectives.

This thesis is broken into two parts. In the first, chapter two introduces a methodological framework which brings together two distinct disciplinary approaches. Converging on the theme of microbial entanglement, these approaches are together employed to create an interdisciplinary platform from which to conduct this research. Chapter three describes the ethnographic methodologies and molecular microbiological materials and methods utilised in this project, the results from which are examined and analysed in the remainder of this thesis.

Part two presents the results, discussion, and analysis, approaching the ethnographic first, followed by the molecular microbiological, and finishing with the interdisciplinary findings and conclusion. Chapters four and five examine the ethnographic findings of this research, the former focuses on the practical aspects of the survey and interviews, including discussions of demographics trends, production methods, sharing practices, and the motivations for taking up kombucha brewing. Chapter five draws on the themes of health and well-being as motivation for the uptake of kombucha production in the previous chapter, analysing the phenomenologically affective experiences of participants to explore their shifting relationships with their SCOBYs which link to broader theoretical and ideological themes. Chapter six discusses the molecular microbiological results and analysis, this is comprised of the background and factors impacting results, followed by the bacterial community relative abundance and functional pathway data, finishing with the fungal community relative abundance data. This thesis is brought to a close with chapter seven, which explores the interdisciplinary findings, provides future directions for research, and a summary of conclusions.

Part 1 – Microbial entanglements and practical considerations

Chapter 2: Proposing a methodological framework

In this chapter a mixed methodological framework is proposed, purposefully bringing together two distinctly different foci and disciplinary approaches and, it is hoped, providing a platform from which further research may be conducted. An overarching methodological aim of this study is to test the efficacy of taking such a radically interdisciplinary approach, tested in the successful conduct and outcomes of this research. Establishing a constructive dialogue between microbiological and anthropological approaches, this chapter first re-examines the idea of microbial entanglement as a theoretical point at which both the biological and social sciences converge (Flachs & Orkin 2019). In order to approach and theorise co-ordination and contexts of scale in the creation of interdisciplinary knowledge from microbial, human, social, and microbiopolitical standpoints, two methodological tools used by Elizabeth Roberts and Camilo Sanz (2018) in their bioethnographic research platform will be utilised. Finally, this chapter will outline the specific data collection and analysis methods employed, including qualitative and quantitative surveying, semi-structured interviews, participant observation, 16S ribosomal ribonucleic acid (rRNA) and internal transcribed spacer (ITS) next-generation amplicon sequencing, and network analysis.

The idea that humans, their environment and associated microbiota are permeable and entangled is gaining increasing attention in interdisciplinary scholarship (Bosch & McFall-Ngai 2011; Flachs & Orkin 2019; Lax et al. 2014; Niewohner & Lock 2018; Rees, Bosch & Douglas 2018). Drawing on the themes discussed in the introduction, the concept of microbial entanglement encompasses the idea that human and microbial individuals and communities are intimately intertwined and subject to a host of contextual social and environmental variables. Despite the recent increased attention, the intertwined relationship between humans and their environment described by microbial entanglement has been long established in one form or another in both the biological and social sciences (Bosch & McFall-Ngai 2011; Lock 1993; Niewohner & Lock 2018). The discipline of ecology has, since the late nineteenth century, recognised “inter-species interdependence” of ecological systems (Bosch &

McFall-Ngai 2011, p. 186). Scholars of the humanities, too, have discussed personhood in the terms of porosity and the individual versus dividual since the mid twentieth century (Smith 2012). Indeed, on these terms entanglement and porosity provide a theoretical point of agreement between both areas of scholarship.

Although social scientists have predominantly focused on human-environment entanglement from a social or symbolic perspective, the idea of “local biologies” described by Lock (1993) represents an early attempt to conceptualise the material entanglement of humans and their local environments, highlighting the importance of understanding both the biological and social circumstances of embodied experience (Frost 2018). Recent scholarship has built on this perspective, investigating identity and the plurality of human existence through entanglements with microorganisms (Benezra, DeStefano & Gordon 2012; Bordenstein & Theis 2015; Helmreich 2015). Other explorations into human-microbial entanglements from the social sciences have examined relationships in the form of human hookworms, raw milk cheese production, and kombucha (Lorimer 2018; Paxson 2008, 2013; Spackman 2018). Altogether, discussion has shifted towards understanding and advocating for the need to understand human biology in terms of being a “collection of dynamic processes” sensitive to cultural and social factors, with biosocial phenomena best understood when situated contextually (Frost 2018; Niewohner & Lock 2018; Rees, Bosch & Douglas 2018).

As has been established, the understanding that ecological systems include complex interdependence between species has been present within ecology for approximately a century (Bosch & McFall-Ngai 2011). Challenging preconceived ideas about the human as an autonomous bounded individual, these understandings are also being incorporated into other scientific fields, predominantly due to improvements in technology, as well as significant decreases in the costs associated with next-generation sequencing. (Borghol et al. 2012; Bosch & McFall-Ngai 2011; Dill-McFarland et al. 2019; Rees, Bosch & Douglas 2018). With humans, animals, and plants now being understood through their varied microbial communities to be multispecies holobionts, whose “symbiotic microbes are fundamental to nearly every aspect of host form, function, and fitness” (Bordenstein & Theis 2015, p. 2). Particularly relevant to this study is the well-established understanding that the human microbiota

is shaped, for example, by living with other people, eating food, and having pets (Borghol et al. 2012; Dill-McFarland et al. 2019; Gilbert & Stephens 2018; Rees, Bosch & Douglas 2018; Rothschild et al. 2018; Sandoval-Motta et al. 2017; Vangay et al. 2018). In return, humans through the creation of built environments encourage the growth of particular microbiota and will, for example, noticeably shift the microbial composition of a new home within days of moving in (Lax et al. 2014; Reva et al. 2015). This means the permeable and “dynamic processes” of biology and sociality contribute to the creation and perpetuation of environmental and human microbiota (Frost 2018). Neo-cultural ecology is particularly relevant to this discussion as it approaches “human-microbe interactions” as an “iterative feedback loop” being continually shaped by one another, in addition to influences from larger social and political forces (Flachs & Orkin 2019, p. 35).

The theoretical discussion thus far has established a shared understanding of microbial entanglement between the social and biological sciences. Fermentation also intersects at this shared interdisciplinary meeting point (Flachs & Orkin 2019). As Andrew Flachs and Joseph Orkin (2019, p.36) argue, fermentation “with its complex impacts on microbial ecologies inside and outside human bodies” has an marked ability to reveal “how biological and sociopolitical mechanisms become entangled when they are shaped by larger environments”. For this reason, comprising as it does a semi-open microbiota in a shared domestic environment, and direct human interaction being common to its production, kombucha presents a perfect biosocial phenomenon through which to apply and further explore the approaches outlined above (Katz 2006; Marsh et al. 2014; Reva et al. 2015; Wolfe & Dutton 2015). In sum, and as proposed here, studying and understanding the relationship between kombucha and its producers was best approached from the perspective of the people making it and through the analysis of kombucha as an environmental microbial community and as a cultural object.

That being so, and building on the foundations laid thus far, this research in its methodological approach drew on the developing bioethnographic research platform of Roberts and Sanz (2018). This platform similarly attempts to combine biological and ethnographic data, but does so specifically in the context of biomedicine, health and disadvantage in Mexico, with the Early Life Exposure in Mexico to Environmental

Toxicants project (Roberts & Sanz 2018). For the purposes of this project, useful concepts from this platform include the strategy of co-ordination and the fractal approach. In defining a strategy of co-ordination, Roberts and Sanz (2018, p. 757) argue that in order to follow how phenomena are relationally enacted, logistical, epistemic, and temporal co-ordination across “differently positioned intellectual research environments” is required. Firstly, from a literal standpoint, this meant physically following kombucha through different research environments, in this case from participants’ homes where it holds specific relational meaning, through to the laboratory where it is transformed into samples to be understood in quantitative and statistical terms. Secondly, from an epistemic and theoretical standpoint, it meant following both the kombucha SCOBY and its producers in tandem through the application of both microbiological and anthropological research methods and subsequent analysis. Co-ordinating data production in this way throughout the process of research allowed for the “comparative production of knowledge” about kombucha as a microbial community but also about its producers, and helps us understand how their microbially entangled position is both relational and interdependent (Roberts & Sanz 2018, p. 758).

The fractal approach has been used in a number of other settings to help apprehend and describe complex issues of scale (Callon 1989; Jensen 2007; Roberts & Sanz 2018). The approach is based on the idea of geometric fractals, which demonstrate complexity at whatever level they are observed (Jensen 2007; Roberts & Sanz 2018). Such a perspective avoids the reification of hierarchical, often binary, orders of scale, instead viewing each “empirical situation” as having infinite detail regardless of its supposed place within a more traditional micro-macro perspective (Jensen 2007, p. 836). A framework for understanding differences in perspective and scale was established in the introduction, utilising the “three bodies” approach and the themes of microbial entanglement (Flachs & Orkin 2019; Scheper-Hughes & Lock 1987). The fractal approach works together with this framework as both recognise the complexities of multiscale relationships between kombucha microbial community and its human producers (Flachs & Orkin 2019; Jensen 2007; Roberts & Sanz 2018; Scheper-Hughes & Lock 1987). Applying fractals to this project entailed investigating the variable connections between kombucha producers and the SCOBY, and similarly to that of Roberts and Sanz (2018), the data about people’s everyday lives and the

microbiological data produced in this project took different qualitative and quantitative forms. The scales and perspectives of the “three bodies” and microbial entanglement approach identified prior were used to discuss and analyse the data, while the fractal approach aided in centring the “the complex relationships that produce each kind of data” as work was being done to combine them (Flachs & Orkin 2019; Roberts & Sanz 2018, p. 759; Scheper-Hughes & Lock 1987). Therefore, rather than preferencing a particular scale, be it environmental, structural, social, or microbial, this research sought to examine and understand the relationships forming across biosocially entangled scales.

In order to capture research participants’ diverse and shared experiences and perspectives this study incorporated the ethnographic approaches of an online survey, semi-structured interviews, and participant observations. Online surveys included both qualitative and quantitative components, and were dispersed through social media as a fast and cost-effective way to successfully recruit participants (Gobo 2008; King, O’Rourke & DeLongis 2014). In addition to this, semi-structured interviews and participant observation allowed for in-depth personal narratives to be obtained alongside description of specific contextual details. These ethnographic methods are described in more detail in the next chapter and were used in combination with biological forms of analysis.

The molecular biological methods and analysis adopted for this project were bacterial 16S rRNA and fungal ITS next-generation amplicon sequencing and microbial network analysis. This kind of sequencing is used in molecular microbiology to identify the respective bacterial or fungal taxonomic composition of a given community (Caporaso et al. 2011; Ihrmark et al. 2012). As such, it allows any differences or similarities between or changes across microbial communities to be identified. For the purposes of this project this approach was used to examine community structure of the SCOBYs, visualising and identifying differences and similarities between participants’ kombuchas. Microbial network analysis was then selected to combine the contextual details collected in interviews and the results of the next-generation sequencing. This technique allowed for the connectivity between microbial samples and how that related to or was mirrored in the contextual ethnographic data visualised.

As this chapter has demonstrated, the conceptualisation of microbial entanglement presents an important point of cross-over between biological and social scientific approaches to human-microbial relationships, such as is found in domestic kombucha production and consumption. This project is not alone in implementing a interdisciplinary mixed methodological approach to the research of such phenomena, and indeed draws on the approach to both co-ordination and problems of scale taken by Roberts and Sanz (2018). Nevertheless, the specific combination of ethnographic and molecular microbiological approaches proposed in this methodological framework is unique, as is the approach to testing the practical application on an unestablished model microbial community and its associated social conditions. Whatever the outcomes presented and discussed in the following chapters, this study has made important steps towards the practical application of this unique interdisciplinary combination, aimed to better understand the biosociality of human-microbial relations.

Chapter 3: Methodologies, materials, and methods

This chapter describes the specific ethnographic and microbiological methods combined in this study to understand the relationship between kombucha's microbiota and the humans that make it from an interdisciplinary perspective. These include a discussion of participant recruitment, survey deployment, interview, and participant observation methodologies, as well as an outline of the materials and methods used in the kombucha sample collection, genomic DNA (gDNA) extraction, 16S ribosomal ribonucleic acid (rRNA) and internal transcribed spacer (ITS) next-generation amplicon sequencing library preparation, bioinformatic and data analyses.

Ethnographic methodology

Survey

Domestic and artisanal kombucha producers were invited to complete an online survey on the social aspects of kombucha production and sharing.² To get as wide a variety of participants as possible the survey was circulated on Facebook, on both general public posts as well as a range of fermentation interest groups. The survey link was also emailed to a range of academic department mailing lists at La Trobe University, the host institution from which this project was conducted. In the interests of encouraging 'snowballing' throughout the community of practice in question, participants were also encouraged to share the link through personal networks.

Survey questions included closed-ended demographic questions as well as open-ended questions designed to illicit long form narrative and detailed answers. The closed-ended questions included options for participants to identify where they obtained their starter SCOBYs, and how many years they had been making kombucha. The open-ended questions centred around participants' motivations behind taking up kombucha making, who they have shared their SCOBYs with, the processes they have used to make kombucha, and their personal understandings of the microbes that live in their kombucha. The survey was created to compliment semi-structured in-depth interviews and provide an impression of the broader social

² See appendix 2 for survey schedule.

contours of the kombucha making community. It was also used as a means to obtain self-nominated expressions of interest from participants for those interviews, and for the sampling stage of the project.³

Interview and participant observation

The answers obtained from the survey informed the interview schedule, thus connecting the results from both these data collection methods.⁴ The interview questions were specifically chosen to be open-ended, allowing the participants enough conversational space to explore potentially unforeseen topics (Madden 2010). In choosing this interview style, participants' agency in providing specific points of detail and exploring potentially unexpected themes was encouraged, without imposing a direct line of questioning (O'Reilly 2005). Even so, a generally guiding interview schedule was followed in the interests of maintaining an essential conversational structure overall, allowing the researcher to guide conversation and re-focus when required, and ensuring essential information was both consistently obtained and remained comparable across the broader interview data.

The interviews were staged in participants homes, with their consent. This also gave rise to the opportunity to conduct participant observation of their kombucha making practices, and to collect SCOBY samples. In addition to gaining an understanding of participants' perspectives and contexts of practice and experience through the ethnographic methods, this project sought to examine the microbial composition of kombucha itself for the purposes of examining any potential correlations or other insights gathered across these typically disconnected contexts. Conducting interviews and participant observation together was also a conscious decision. It was thought that through the act of making the kombucha and discussing practice together, details in the embodied experience of making and interacting with kombucha *in situ* would arise that might otherwise be missed when decontextualised from that context. All interviews were audio recorded and later transcribed, and photos were taken throughout the process both to produce a visual document and to ensure accuracy of details was maintained. The photos taken throughout the interviews and participant

³ See appendix 1 for further details of ethics and data collection methods.

⁴ See appendix 2 for interview schedule.

observation excluded wide frame images of participants kitchens and faces. This choice was made deliberately, due to ethics and the wish to highlight physical interactions with and handling of the kombucha throughout the production process.

Most interview participants were self-selected as mentioned above, with the exception of a small number who were recruited at the encouragement of existing participants. The additional participants were recruited explicitly for the purposes of examining sharing practices and relationships, as well as obtaining samples of SCOBY that had been shared between friends and as a result were considered paired. Time and travel restraints dictated the limiting of participants chosen to within two to three hours' drive of Melbourne. Contact was made to participants who fitted this and other criteria, such as the ethical stipulation of being adults over the age of 18, via email to gauge interest and availability during the month of June 2019. As a result of this recruitment method, interview participants collectively reflected the wide range of demographics present in the survey, and as reflected in the discussion below the interviews themselves elicited a wide diversity of perspectives around practice, exchange, and ideology concerning kombucha and its social, political and health-related implications.

Microbiological materials and methods

In order to conduct next-generation amplicon sequencing which provided the microbial community data about participants kombucha SCOBYs, samples were taken, then DNA extracted, followed by a complex sequence of lab procedures involving the creation of gene amplicons, PCR amplification, sequencing library preparation, and finally sequencing. The following sections describe these procedures, and the subsequent processing and analysis of the data produced.

Kombucha sampling

When scheduling interviews participants were asked, for sampling purposes, to ensure that their kombucha was at the end of a fermentation cycle, ~7 to 21 days, which is the point before any flavours or fruit would normally be added. This was to make sure that all samples were as standardized as possible, with type of tea, and the added carbon source (e.g. white sugar, honey etc.) being the primary variables. Approximately 15 ml of liquid and 20-40 g of biofilm were collected in separate sterile Falcon tubes and transported at ambient temperature. Samples were stored at -18°C within two to five hours of collection, and then when practicable moved to -30 °C for storage until processing.

The participant kombucha samples were all collected in much the same way as they would have been had they been transferred to another person when sharing a starter SCOBY socially. This method resulted in a conscious decision aimed at maintaining the normal environmental conditions in which kombucha is produced and shared to preserve microbial transfer. For this reason also, those participants (rather than the researcher, for example) who would normally use their hands to break apart the biofilm portion of the SCOBY did so. All participants who handled the biofilm this way washed their hands beforehand, and some rinsed their hands in vinegar, as they believed this was less impactful on the microorganisms in the kombucha. Those who handled the biofilm either peeled a thin layer off, others with thicker biofilms cut a piece off with a clean knife or kitchen scissors, with both of these methods being authentic to the standard process of separating the SCOBY, and subsequently deposited it in a 50 ml sterile falcon tube. Participants who do not habitually handle the biofilm used clean utensils, like tongs, to lift the biofilm up out of the kombucha tea, similarly cutting or peeling the biofilm and similarly depositing it as per their preferred practice.

If a participant had a continuous crock set up (see glossary) with a tap on the bottom of the vessel, after the kombucha had been agitated a sample was taken from the tap. For those fermented in large glass jars, similarly after agitation, samples were taken using a clean spoon or ladle and poured carefully into the opened falcon tube. In this way, in collecting microbial samples as close an approximation of the participants' preferred methods for handling their kombucha and SCOBYs was maintained, including through this being carried out by the participants themselves.

Genomic DNA extraction

Tests were run with the extraction kit on both kombucha liquid and biofilm samples. Improved preparation strategies for the biofilms included submersion in liquid nitrogen followed by mechanical destruction on a vortex and applied blunt force with a sterile metal implement. Kombucha liquid samples were vortexed for ten seconds and inverted two to three times prior to gDNA extraction. 1 ml of the kombucha was then centrifuged at 10,000 rpm for two minutes to form a pellet. The supernatant was removed, and the pellet resuspended with 60 µl C1 solution from the DNeasy® Powersoil extraction kit (Qiagen). The entire volume was transferred into the extraction kit supplied PowerBead tubes.

The gDNA was extracted from the kombucha liquid samples using the DNeasy® Powersoil kit (Qiagen) as per the manufacturer's instructions.⁵ The following modifications were made to the extraction kit protocol to optimise gDNA yield: At step 4 the horizontal vortex time was increased to 20 mins. The volume of solution C6 at step 19 was reduced to 50 µl and incubated at ambient temperature for 5 mins. The gDNA was then stored in the collection tubes at 4°C until required.

16S rRNA and ITS metagenomic sequencing library preparation

The gDNA extracted from participants liquid kombucha samples was then prepared for amplicon sequencing on the Illumina MiSeq. The primers used are in Table 1 below, as described Ihrmark et al. (2012) for the fungal and Caporaso et al. (2011) for the bacterial. For both the bacterial 16S rRNA and fungal ITS2 gene amplicons this

⁵ For DNA extraction kit instructions see appendix 3

was done as per the library preparation protocols obtained from Illumina (Part # 15044223 Rev. B, Document # 1000000064940 v00). Followed by a PCR clean up using AMPure XP beads. A gel electrophoresis was conducted at each stage to confirm presence of DNA fragments which were expected 390 and 450 base pairs (bp) in size for the bacterial and fungal respectively.

Table 1 Forward and reverse primers for bacterial 16S rRNA V4 and fungal ITS2 amplicon PCR.

Primer name and direction	Sequence - Illumina adapter sequences here in blue and gene specific in orange	Region
Forward – 515f	5'- AATGATACGGCGACCACCGAGATCTACACGCT GTGYCAGCMGCCGCGGTAA-3	V4 (Caporaso et al. 2011)
Reverse – 806r	5'-CAAGCAGAAGACGGCATACGAGAT GGACTACNVGGGTWTCTAAT-3	V4 (Caporaso et al. 2011)
Forward – fITS7	5'- TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GTGARTCATCGAATCTTG-3	ITS2 (Ihrmark et al. 2012)
Reverse – ITS4	5'- GTCTCGTGGGCTCGGAGATGTGTATAAGAGACA G TCCTCCGCTTATTGATATGC-3	ITS2 (Ihrmark et al. 2012)

The indexing PCR stage of the fungal protocol was adjusted as seen in table 2 below. It was increased from eight cycles to ten due of the low volume of fungal DNA after amplicon PCR. Once library preparation was complete samples were loaded into the MiSeq as per the Illumina protocols.

Table 2 Illumina fungal indexing PCR program adjusted to ten cycles.

Cycles	Temperature	Time
1	95°C	3 mins
10	95°C	30 sec
	55°C	30 sec
	72°C	30 sec
1	72°C	5 mins
-	4°C	Hold

Bioinformatic pipeline: Applied to the bacterial 16S rRNA and fungal ITS sequencing output ⁶

The output from amplicon sequencing using the MiSeq provides a number of raw files. These need to be processed using a bioinformatic pipeline before analysis in the statistical computing environment R can be conducted (R Core Team 2014). Processing requires forward and reverse reads (see glossary) to be merged and primers trimmed, along with treatments that increase the reliability of downstream analyses. A key part of this process is taxonomically assigning bacterial and fungal sequences from the appropriate reference databases. In this case, Greengenes version 13.8 (<https://greengenes.secondgenome.com>) was used for bacterial sequences and UNITE version 8 set (<https://unite.ut.ee/repository.php>) was used for fungal sequences. In order to analyse the amplicon sequencing data in R, exact sequence variance (ESV) tables were created from the taxonomically assigned sequences which specifies for each kombucha sample how many unique ESVs and how many reads (sequences) there are of each. Finally, an additional bioinformatics software package (PICRUSt) was used to designate functional pathway information to the bacterial data. The bioinformatics were performed with the assistance of Dr Jen Wood.

Analysis of the bacterial 16S rRNA and fungal ITS sequencing data

Bacterial and fungal community data were analysed in the R statistical computing environment (version 3.6.1) (R Core Team 2014). All analyses were performed on ESV tables that had been total sum scaled, the fungal data was further logx+1 transformed prior to analysis to correct for the relative high abundance of some taxa. A filter selecting for the top 20% abundant taxa, present four or less samples was applied to the bacterial ESV table in order to visualise the rare community members. Exploratory bar charts for visualizing communities were performed using the R packages *ggplot2*, *microbiome*, and *phyloseq* (Lahti & Shetty 2019; McMurdie & Holmes 2013; Wickham et al. 2020). A non-metric multidimensional scaled (NMDS) ordination was created using the UniFrac distance matrix of the bacterial and fungal community data using the 'ordinate' function (package *phyloseq*). UniFrac distances

⁶ The following bioinformatic methods have been described to allow for accessibility by a range of audiences. For full detailed description see appendix 4. For definitions and explanations of technical or discipline specific terms that were unavoidable see the glossary of terms.

are based on phylogenetic similarity of two microbial communities. A principal coordinate analysis (PCoA) ordination was created using the Bray-Curtis dissimilarity matrix of the PICRUST functional pathway data. Bray-Curtis distances are based on compositional dissimilarity between two different communities. Networks were used to visualise the connectivity between kombucha microbial communities and their relationship to contextual data using the 'plot_network' function (package *phyloeq*). The networks were created such that nodes were represented by samples, with edges and their line weights representing the Jaccard distance connecting samples. The Jaccard distance values were calculated with the 'vegdist' function from the *vegan* R package. Jaccard distances represent the percentage of ESVs shared between two given samples, where 0% = complete sharing of ESVs and 100% = no ESVs shared. The overall performance of this sequence of lab procedures, data preparation and analysis resulted in a set of molecular microbiological data revealing the bacterial and fungal community composition of participants kombucha SCOBYs.

Part 2 – Results, discussion, & analysis

Chapter 4: Discussion of the survey and interview findings

The first part of this thesis outlined the mixed methodological framework, ethnographic methodologies, and the molecular microbiological methods employed in this project. This chapter presents the first set of resulting findings, being the general themes and everyday characteristics of kombucha production as revealed in the ethnographic survey and interviews. Following an outline of demographic details paying particular attention to gender, followed by broader trends including age, length of time producing, and the themes present in production and SCOBY sharing practices will be discussed. Finally, participant motivations behind taking up kombucha production are examined as they link to the larger ideological and theoretical exploration of the ethnographic findings in the chapter five.



Figure 1 *Oliver's kombucha all lined up on a shelf in his kitchen.*

Analysis of the demographic data collected in the online survey revealed an over representation in terms of gender, with women making up 82% of respondents. This is perhaps due to an increased likelihood for women to complete online surveys (Smith 2008). However, with a strong narrative around kombucha being a health providing beverage appearing in both the survey and interviews the difference could also be attributed institutionalised assumptions and realities of gender based inequality of caregiving and “domestic work” in Australia (Sayer et al. 2009). The interview responses provide further support for this argument. As Esme said, “if I can get my kids and me to eat a little bit of sauerkraut and a little bit of kombucha, I think that kind of microbial diversity is really important”. This view encapsulates a broader sentiment shared by many female participants with children or family who benefited from their kombucha production. With women being burdened by the “second shift” of housework and caregiving on top of any other employment they may be engaged in, the gendered difference in kombucha producers is not surprising given its status as a “home-made” means to provide a healthy low sugar drink for their families (Hochschild 1989).

In contrast to the over-representation of women, the age data showed an even distribution between 25 and 69 years old, with no participants appearing in the 18-24 age bracket or over 69 years. This data indicates kombucha has a wide appeal across adult generations. Furthermore, most participants had been making kombucha for approximately two to four years with none making it longer than ten. This does not account for those people who made it perhaps 20 to 30 years ago, discontinued and then picked it up again more recently as some interview participants had. Agnes and Susan are two such examples, with both participants recalling making kombucha in 1990s. Agnes in particular spoke of kombucha production as a part of a “hippy lifestyle” of “clean living” when she was young, and recalled housemates who made it, in addition to keeping chickens, vegetable gardens, and making sourdough. Both Agnes and Susan were prompted to make kombucha again after seeing it on supermarket shelves, particularly due to the prohibitive price, waste in the form of bottles, and distrust of commercial producers. Agnes described her stance as one of, “oh screw that, I’m going to start making it myself again”. Most participants were also prompted to start making kombucha when they started hearing about it and seeing it appear in cafes and supermarkets in the last three to five years. These results parallel the recent

growth in the commercial production of kombucha and public discussion of the importance of 'gut' health, which have been steadily becoming more mainstream over the last five to ten years (Elliot 2018; Jasarevic 2015; Paxson 2008; Spackman 2018).

In surveys and interviews participants were asked to provide either a description or demonstration of their kombucha production process. In response to the open-ended survey questions a range of answers were provided, from simple descriptions such as, "make sweet tea, add to SCOBY, and wait" to much more detailed answers that included specific volumes and weights. Approximation appeared to be a consistent theme in both the surveys and interviews. Many survey respondents noted a lax or blasé approach to quantities, and they just rinsed or washed bottles/utensils rather than sterilising as is common in other brewing practices. The vast majority of interview participants also had no qualms about reaching their hands into their kombucha to retrieve the SCOBY biofilm. All those who routinely touched the biofilm did wash their hands prior, with either soap and water or vinegar, with the vinegar being thought to have a lesser impact on the microbial community due in part to the acidic nature of kombucha. Similar perspectives are shared by popular fermentation books and are commonly taught at the fermentation workshops many of the participants reported to have attended (Davis 2017; Katz 2012). Caution and adherence to a recipe seemed to be indicative of people new to kombucha or fermentation.

Tea and sugar variation seen in survey and interview responses appeared to be due to a variety of reasons. Flavour outcome and other aspects of personal preference were important factors in these decisions. Using organic tea or not seemed to be a point of difference for interview participants. Kate, Samuel, and Esme all used an organic tea specifically; they all thought it was better for the SCOBY and those consuming the kombucha due in part to no pesticides having been used in its production. Others like William and Sacharissa, who participated in dumpster diving, made kombucha with only what they found. As a result, different teas and sugars were used all the time.



Figure 2 *Matthew's green tea and honey kombucha (jun) that lives in his bedroom.*

Decisions made by participants about what source of sugar they would use, and how much, often depended on what they had at hand and how sweet they wanted the resulting kombucha to be. Another factor in the tea and sugar variation seen between participants was what “lineage” the SCOBY was that they were using, a term that is used here to describe how some participants perceived kombucha SCOBYs made with different teas. It was common throughout the surveys and interviews for participants to mention that they thought a green tea and honey SCOBY, often called jun, was made up of a different microbial community to that of a kombucha made with black tea and some form of cane sugar. In terms of production methods, many participants expressed the opinion that you could take a jun SCOBY and use black tea, but it required an adjustment period in which a steadily larger amount of black tea was included in a kombucha batch. The procedure also went the other way, from taking a black tea kombucha to green tea. This contrasted to the practices of other participants, like Agnes and Susan, who changed between types of tea or sugar fairly easily without much adjustment needed. Susan had even tried making black coffee kombucha, after reading about it in the Foundations of Flavour fermentation book

(Redzepi & Zilber 2018). The experiment had worked and was delicious, apparently, however it killed her SCOBY in the process.

A secondary ferment is a common feature of kombucha brewing (Davis 2017; Katz 2012; Redzepi & Zilber 2018). This step is when the kombucha is decanted into a bottle after the producer thinks it has been fermented for long enough, achieving the right sweetness/acidic balance, at which point some fruit or additional sugar source is added for flavour and “food” for the microorganisms (Davis 2017; Katz 2012). The process of decanting kombucha into bottles for secondary fermentation can be seen in the picture below of Hamish’s kombucha. In this batch tea leaves were accidentally left in the brew, which in the photography are seen being strained out. Both survey and interview participants gave mixed responses about whether they conducted a secondary ferment or not. On the whole, personal preference dictated if they conducted a second ferment, or just drank it straight after the first. For those that did, home grown fruit or gifted fruit from friends was a common additive, many mentioning a preference for only using what was seasonal to add to their kombucha. Others like Polly, Matthew, and Samuel used fresh, frozen, or dried fruit depending on what was available to obtain the flavours they desired in their secondary ferments. Most interviewees who had been brewing for a number of years also commented that at first they were cautious and reserved about secondary fermenting, but once they had grown comfortable with maintaining it they started getting experimental with different flavours, teas, and sugars.



Figure 3 *Hamish's kombucha being decanted.*

Another consistent theme was information on who participants acquired their SCOBYs from. The vast majority of survey respondents received their SCOBYs from friends, while very few bought or obtained them from online interest or starter share groups. Similarly, in interviews, those participants who had existing social networks centred around fermentation knowledge and experience were less inclined to search online for communities to join or seek information from. By contrast, those who had no one in their social networks who shared their interest in kombucha actively sought out and engaged with online communities, with Lynne and Polly's story providing an example of this. These two participants met in a Facebook based online community for free kefir grain (see glossary) sharing. Polly, prior to migrating to Australia, drank kefir as a regular part of her diet. After arriving in Melbourne and having to resort to buying kefir grains that Polly had been accustomed to obtaining in a swap or share exchange, she started a free grain sharing group on Facebook. Lynne lived in a country town about a two-hour drive from Melbourne, she had an active interest in fermentation for both health and enjoyment reasons but had no friends or family who shared that interest. It was in Polly's Facebook group that the two met, when Polly posted about seeking a kombucha SCOBY despite it being a kefir group. Lynne responded to this

post, and they began a friendship which included Lynne gifting a SCOBY to Polly and their keeping in touch despite living hours away from one another.

Free sharing, like in Lynne and Polly's story, is a notable topic arising from both the survey and interviews. Participants were exceptionally willing to share kombucha SCOBYs with others, with the few exceptions being those who had businesses around teaching workshops and/or selling SCOBYs. A couple of participants differentiated between friends and strangers, gifting to the former and selling to the latter for nominal sums on websites like Gumtree or Facebook, but this was not the norm. Jasarevic (2015) explores a similar phenomenon in their work on kombucha in Post-Yugoslavia, commenting that "the gift is incorporated into the very nature of probiotic cultures", due in part to its health-giving properties but also to the common problem of over surplus as the SCOBY grows. Word of mouth or online gifting and sharing are further described as an extensive system, spreading the SCOBY and medicinal "fame" of kombucha (Jasarevic 2015). These are an apt portrayal of the gift giving and reciprocity coupled with the struggles of microbial abundance present in this Australian context also.



Figure 4 William and Sacharissa's kombucha living in the original jar that was gifted to them for Christmas.

When sharing almost all participants said they included some form of instruction with the SCOBY. This finding appears to link with the survey responses indicating that, overwhelmingly, people learnt how to make kombucha from the person who gave it to them. The Internet appeared to be a secondary information resource for most kombucha makers, and the place lone producers especially went for knowledge and support. Most interviewees when asked said they would have felt remiss if they did not provide some information on care and production methods. One participant, Lynne, also described her first attempt at kombucha as a failure because she had not been given instructions, being told to “google it”. Instructions appear therefore to be an important part of kombucha and its perpetuation in the Australian context (Jasarevic 2015).

In the survey, respondents were overwhelmingly motivated to make kombucha due to reasons of health, general fermentation interest, and enjoyment of the drink’s flavour. Ease of production was an important aspect of kombucha for interview participants as well, preferencing it over other fermented products which in their opinion were often more complicated and time consuming to make. Additionally, there were more significant drivers, including limiting sugar intake and the inclusion of fermented food in diet for health and well-being reasons, a do-it-yourself approach to food production, a conscious decision to reduce their impact on the environment, and an inclination towards what are often described as ‘alternative’ health or lifestyle practices.

Narratives of health and well-being emerging from both the survey and interviews overwhelmingly dominate any other reason for kombucha production. According to participants, kombucha is used as a way to reduce dietary sugar intake, to model good ‘gut’ health consumption behaviours in children, as a probiotic diet supplement, and as a treatment for food intolerances, general gastrointestinal problems and associated health concerns and ailments. These narratives suggest a pluralistic approach to medicine and health similar to that observed by Jasarevic (2015). However, rather than comprising a combination of Western biomedicine and what are known as ‘domaće’, or home foods and remedies born and produced out of survival and a deep sense of distrust in the soviet state food and medical care, in the context of this research the healthy reputation, influence of alternative health practices and anecdotal evidence preceding kombucha worked in combination with the biomedically

sanctioned consumption of probiotics as a general way to improve overall health (Davis 2017; Dufresne & Farnsworth 2000; Kapp & Sumner 2019; Katz 2003, 2012; Spackman 2018).

As this chapter has shown, the participants in this study were mostly women from a broad range of age groups, for whom kombucha production is an important part of either personal health or family care giving through the provision of a low sugar, microbially rich and therefore 'healthy' soft drink alternative. Approximation and a relaxed or blasé approach to production practices was another common theme in both the survey and interview participants. The type of tea and sugar used in used in making kombucha varied in response to availability, taste, and in some cases the green tea and honey, or black tea and sugar, 'lineage' of the SCOBY. Secondary ferments too, were conducted or not, and the additives used differed based on participants personal taste and preference. Sharing and gifting SCOBYs and knowledge between friends was most common amongst the participants in this study, only those who were solitary producers sought out online communities for advice and sharing opportunities. Finally, health and well-being dominated the motivations behind the uptake of kombucha production, with the health-giving reputation, increased popularity and availability of commercial kombucha primarily driving the uptake of kombucha brewing. This chapter provides the first part of the human perspective on kombucha, the everyday practical themes, and social context which informs participants' practice.

Chapter 5: Health, well-being, and the affective experience of the kombucha SCOBY

Many participants in my research described the horror they experienced, due to its appearance, in first encountering the kombucha SCOBY. Equally they spoke with relish of their friends' and families' disgust when they showed them their microbial communities. In one of the most visceral descriptions, the kombucha SCOBY was likened to a "foetus in a jar", a description invoking the grotesque and harkening back to nineteenth and twentieth century "freakshow" and circus sideshow spectacles (Ackerman 1960; Durbach 2014). These displays were renowned for exhibiting anatomical and natural science "deformities" and "medical specimens" including human fetuses stored in jars and pickled in formaldehyde, often called "pickled punks" (Ackerman 1960; Durbach 2014). This participant's quote and its associations highlights how strong the affective embodied experience can be for some people, especially when they encounter a SCOBY for the first time. Description and analysis of reactions such as this provide a basis from which to understand the historical and cultural dimensions that influence the phenomenological experience of microorganisms and food, as described by the individual participants of this research. It also allows for an examination of the larger conceptual and ideological themes present within this particular community of kombucha producers.

Drawing on approaches in symbolic anthropology this chapter argues that when the microbially entangled relationships my research participants form with their SCOBYs are examined, important categorical and ideological shifts can be observed. Explored through these lenses the phenomenological and affective experience of embracing jars of "alien" or "freakish" looking SCOBY in a domestic space, particularly kitchens, is indicative of both acknowledging and subsequently rejecting, or shifting, pre-existing culturally normative perceptions of and bodily orientations to microbial life. In positively changing their attitude and affective relationship to their SCOBYs my informants can be seen as exemplifying a more generalised cultural shift towards transformed understandings and discourses not just to microbial communities found in fermented food, but also more broadly to western biomedicine, the globalized food system, and the environment.

The ethnographic analysis in this chapter expands on the historic and contextual discussion provided in the introduction, as that context is central to understanding participants' present cultural milieu. It does so through reference to the three intertwined units of analysis proposed by Scheper-Hughes and Lock (1987)—the *individual* body, the *social* body, and the body *politic*—drawing these into relation with themes of microbial entanglement. The first section combines the symbolic and psychoanalytic approaches of Douglas (1966, p. 44) and Julia Kristeva (1982), utilising especially the concepts of “matter out of place” and “affect” (also see Warin 2003, 2010), to examine the phenomenologically experiencing *individual* body, and how emergent cultural and moral understandings of microorganisms, food, and sociality, have impacted participants' views of relationships with kombucha SCOBY. Building on these insights, the second section explores the *social* body, and the perspective of environmental humanities, including how the SCOBY become imbued with social meaning, signalling group identity and signifying health and wellbeing for kombucha producers. The final section focuses on the relationship between participants and larger social bodies, drawing on aspects of microbiopolitics to examine how environmental concerns are linked to ideas of health and well-being, and are central to ideas of political, ecological, and individual agency.

The phenomenologically experiencing *individual*

Describing a social, cultural and embodied phenomenon, the concept and phenomenon of affect provides a useful way to understand how ideas and values are maintained in connection with objects (Ahmed 2010). This phenomenon also helps to explore how subjective experiences are directly influenced by historic and societal perceptions (Ahmed 2010). Sara Ahmed (2010) argues that when someone is affected by an object, they are evaluating it, and these evaluations are expressed in how we react towards that object, whether positively or negatively, or with revulsion, disgust, desire, attraction or mere ambivalence. Affect has in this way been described as “sticky”, sustaining the connection between the values and ideas associated with objects (Ahmed 2010). Douglas (1966) similarly observes that the perception of objects is not passive. Rather it is shaped by an individuals' cultural milieu and past experiences, forming a patterned system to which a person is committed. Contextualised within the strong moral and social connotations discussed in previous chapters, food with signs of microbial growth are objects that often possess particularly

negative affective connotations. Here an understanding of affect as related to the notion of “matter out of place” provides a theoretical link between the visceral descriptions and experiences of the participants in this study, and established structural categories and symbolic systems, doing so to help illuminate how the perceptions of microorganisms have changed for the producers of kombucha in my research, creating new systems, discourses, and ways of relating to microbes (Douglas 1966, p. 44).

Of the many vignettes present in participant interviews which demonstrate visceral descriptions of affective embodied experiences in reaction to kombucha SCOBYs the accounts of Samuel and Gytha provide a particularly interesting contrast. Samuel had been making kombucha for many years at the time of interview and could not remember ever being disgusted by its appearance. He was well aware however of how disgusted and offput by its appearance others often are. Samuel described a number of encounters with people who drank commercial kombucha in which he would ask if the person knew how it was made. Usually they did not, and Samuel would proceed to show them a kombucha SCOBY, enjoying the highly emotive and often revolted reactions to its appearance would elicit. In this example, the fact that Samuel purposefully used kombucha SCOBY to evoke reactions of disgust demonstrates the extent to which these reactions are representative of most reactions from people outside of the kombucha and fermentation community and reflect other participants experiences.



Figure 5 Samuel holding up his SCOBY biofilm.

Gytha, on the other hand, had been making kombucha for about a year at the time of interview, however she had been drinking commercially available varieties for a number of years before that. While Gytha attributed continuing to drink the “commercial stuff” to being “grossed out” at the mere sight of a SCOBY, as she described it, the situation had reached a point where her household were drinking so much store bought kombucha a significant and to Gytha an unacceptable expense and waste in the form of bottles was taking place. At this point she decided, “this is ridiculous”, obtained a SCOBY and started making her own. It had helped that Gytha’s son who lived close by was making it, and she described how that once she got used to its presence “it wasn’t so hideous”. Interestingly, but not uncommonly, Gytha also reported that over time the SCOBY become so much a part of the household that it was talked to like one of her pet dogs or chickens. Thus, even though she was willing to drink it, Gytha’s strong affective and emotional response to the SCOBY initially prevented her from making kombucha, as she would have had to look at it and interact with it as a physical object. Such feelings however changed drastically when she finally took up kombucha production for herself, being the result of a growing awareness of the financial and ecological impact of consuming commercial varieties and through the

experience and encouragement of a close family member. In taking up kombucha production Gytha formed a radically transformed and intimately affective relationship with her SCOBY.

Given the historic associations of microorganisms to contamination, disease, and contagion, it is no wonder the sight of abundant microbial growth in a SCOBY affects many people, generating feelings of disgust and repulsion. Considering the dominant symbolic and categorical system that define my participants' worldviews, reacting with disgust on first sight of a SCOBY is completely "normal". As has been established, microbial growth has been considered symbolically dirty, and as "matter out of place," for a long time (Douglas 1966). To Douglas (1966, p. 44), "dirt the is a by-product of a systematic ordering and classification of matter" carrying often more symbolic than literal meaning. It is the perception of the discordant or ambiguous, that which falls outside the established system which elicits a strong affective reaction, ranging broadly in intensity (Douglas 1966; Kristeva 1982).

My participants, and others such as was demonstrated in Samuel's vignette above, are not alone in experiencing these kinds of feelings. One of the most colourful descriptions of the kombucha SCOBY in the literature, for example, states that it is "more disturbing than the buoyant remains of a large rodent, or an abandoned prosthesis" (Money 2018). This kind of disgust reaction resonates strongly with notions of the abject, a topic Kristeva (1982) has explored extensively, developing on Douglas' (1966) structuralist approach to the analysis of social and cultural symbolic systems. To Kristeva (1982, pp. 2, 4), like Douglas (1966), that which "disturbs identity, system, [and] order" gives rise to abjection, with "food loathing" comprising the "most elementary and archaic form". As described by many of my participants, witnessing the abundance of microbial growth exhibited by a SCOBY for the first time disturbed them, disrupting their established symbolic structural order. It appeared to them as "matter out of place," particularly within the context of kitchens where it was likely offered to them at the same time as a drink to be consumed and enjoyed (Douglas 1966, p. 44).

Kristeva (1982) also develops on Douglas' (1966) ideas about individual identity formation, arguing that a person develops a unique patterned system of understanding the world, based on both their cultural environment and lived experience. Drawing on psychoanalytic theory, Kristeva (1982) posits that identity is formed through opposition. Foods that are experienced and thought of as abject are placed in opposition to self, and as such are not to be consumed (Kristeva 1982; Warin 2003, 2010). Megan Warin's (2003, p. 78) ethnographic analysis of women living with anorexia develops these ideas further, arguing that beyond the structuralist and psychoanalytic readings offered by Douglas (1966) and Kristeva (1982), "at the heart of people's experiences" was "embodied sentience". Encounters with or discussions of abject foods by participants in this research were described in emotive language of disgust and as giving rise to visceral bodily reactions (Warin 2003, p. 78). For Warin (2003, p. 88), it was "the sensory experiences of food, visceral and corporeal experiences, and intersubjective relationships between people and objects" that developed new significance and understanding. Given that my participants were all enthusiastic kombucha producers at the time of interview, I recorded no reactions akin to Warin's (2003) descriptions. There were, however, numerous anecdotes like Lynne's, for example, who recalled attending a fermentation workshop where the other eight students could not bear to look at a SCOBY let alone touch it, as they were "actively recoiling" from it. Considered in light of Kristeva (1982) and Warin's (2003) research and analytic perspective bodily experiencing a SCOBY as abject combines emotional experiences and thought together, and "if food is not good to think, it is not good to eat" (Le Breton 2017, p. 249). This experience positions the kombucha and its microorganisms as Other to the individual human body, with the thought of consuming or even looking at such an object challenging the systemic structural worldview, identity, and physical emotionally experiencing human body it is external to (Scheper-Hughes & Lock 1987).

The *social* body, lay epidemiologies, and environmental humanities

In positioning kombucha and its SCOBY as "matter out of place" relative to the individual body in the last section, it is important to recognise the ability of these ideas to shape and define the social body, particularly considering its status in mainstream ideologies as an abject Other within the domestic space in which it is made (Douglas 1966, p. 44). Scheper-Hughes and Lock's (1987) conception of the social body

considers the body as a “natural symbol” for exploring relationships between nature, culture, and society. This unit of analysis becomes relevant at this point with the positioning of the kombucha SCOBY as Other, relative to the human body. Considered this way the SCOBY aligns with one of the most salient features of the abject for Kristeva (1982, p. 2), that being ambiguity; it is a signifier of a non-object, with things that are abject described as “beyond the set”. Those things that do not conform, the in-between or composite are often those that are identified as lying outside cultural categories and as such are considered inherently dangerous or bring into question the solidity of boundaries (Kristeva 1982).

These ideas echo those of Douglas (1966, p. 117) who argues ambiguity and disorder are destructive to existing patterns but also “symbolises danger and power”. The kombucha SCOBY is ambiguous in a literal sense by its very nature, as it is both solid and liquid, with the appearance of amorphous tendrils of yeast that have grown down from the biofilm that sits at the very top of the fluid. Gytha’s kombucha in figure 6 exemplifies this. This ambiguity also hearkens back to the concept of miasma discussed in the introductory chapter, with its inherent ability to cross and disintegrate boundaries (Cole 2016; Ellman 1990). The power of the SCOBYs ambiguity is also embodied by other features discussed by participants. A prime example of this is the ability for the microorganisms in the kombucha to spread beyond the vessel the SCOBY is kept in. It was mentioned throughout numerous interviews that kombucha was kept away from other sugar-based ferments as the vessel boundaries were not respected by the microorganisms, and small biofilms had been found growing in water kefir for example. Lynne’s kitchen layout is a reflection of this, as different ferments were kept in opposing corners. The kombucha was purposefully placed “four feet” from the water kefir, and the vegetable ferments a similar distance from everything else. Microbial movement outside the control of the producer adds to the enigmatic reputation of the kombucha SCOBY.



Figure 6 *Gytha's kombucha with a hand-made cover.*

The ambiguous nature and enigmatic reputation of the kombucha SCOBY imbues it with social meaning, something the members of this kombucha producing community either grew accustomed to after, or immediately embraced openly, when they started making kombucha. This appeared in stark contrast to how participants often described their interactions with people not involved with kombucha or fermentation more broadly. Historically fermentation was seen as a “volatile mixture of magic and menace”, and it appears that this kind of reputation still persists in some form or another (Le Breton 2017, p. 230). Relatives of one participant, Gytha, had even gone so far as to imply she was “a witch” on account of her fermentation and preservation practices. Upon seeing Gytha’s pantry and fermentation set up, one relative asked her, “where’s the cauldron?”. Another, when seeing the kombucha SCOBY, exclaimed, “get it away from me!”, leading Gytha to suspect the interaction to be the “last nail in the coffin” and cementing her status as a “frigging weirdo”. Thus, exemplifying the relationship between the individual and social bodies, this participant

themselves was posited as socially abject and “matter out of place”, as a “weirdo” or “witch”, through their association with the kombucha SCOBY.

Not all participants reported reactions as extreme as these. However, it was a distinct theme that they were often alone in their fermentation interest and sought fellow enthusiasts online or had specific friends who shared their passion. People outside of the kombucha and fermentation groups or communities tended not to “understand” the appeal of making things like kombucha, and were generally the ones who reacted with disgust and abjection when shown a SCOBY. Considering the analytic perspectives of Douglas (1966) and Kristeva (1982), this positions the kombucha producers as having developed a transformed system of relating to microorganisms in their food through their relationships with the kombucha SCOBY. Moreover, in adopting, gifting, and exchanging SCOBYs and continuing to produce kombucha in spite of the disinterest or hostility they may receive from friends or family, participants’ interests and relation to kombucha came to define aspects of their social relationships, and in doing so they engaged with the theme of environmental humanities. Through joining in shared ecological and production practices, as well as embracing the ambiguity of the SCOBY, participants can be seen to have rejected aspects of the pre-existing structural and symbolic perceptions and practices at the intersection of microbial and human social life and engaging in new discourses and social practices that better accorded with the kombucha social environment. The kombucha SCOBY, imbued with social meaning in this context, in addition to signalling group identity for producers also signifies and introduces themes of health and wellbeing. These themes are also important for understanding the social body as a unit of analysis in this research, as they represent an intersection nature, the body, and society.

There are a number of ideas and concerns relating to health and wellbeing that can be unpacked from within the narratives described by participants in this study. Firstly, present in all narratives there was an overwhelming concern about gut health, and an understanding that fermented food was good for you and as such should comprise a part of a healthy diet. For participants like Lynne, kombucha and fermented foods featured heavily in her health and wellness narrative, with gastrointestinal health being something she had struggled with her whole life. Lynne described her gut as “so out of balance”, and even after other diet and lifestyle changes which had improved her

quality of life, she was not where she wanted to be in terms of her health. However, within a short time of regularly drinking home-made kombucha and adding other ferments to her diet, these additions were described as the positive step that “took it to the next level” and to a point where she had no gastrointestinal symptoms unless she ate food she knew she “shouldn’t” eat. Others, like Samuel and Esme, did not emphasise stories of healing, but rather they consumed kombucha predominantly as a tasty alternative drink to sugary soft drinks, but also as a part of maintaining and improving their overall health. Samuel in particular started brewing kombucha as he had a general interest in making fermented foods like kvass, sauerkraut, and pickles, but also found he felt better when he ate and drank fermented products. These examples illustrate how gut health is always present within participants’ motivations and central to their kombucha production and consumption practices.

These ideas around gut health embedded within the production and consumption of kombucha are in part indicative of changes in how western biomedical science understands the human body and what constitutes a healthy body (Bordenstein & Theis 2015; Lorimer 2018; Ross 2013). Developments in scientific understandings, first within the Human Genome Project and then the Human Microbiome Project, have in the last twenty years instigated a “probiotic turn in western health care” (Lorimer 2018). This turn has taken the western biomedical certainty, that the human body is a discrete unit separate from its environment, and reconceptualised it as a supra-human or holobiont; a being composed of the host, its associated microorganisms and the outcome of interactions between the two (Bordenstein & Theis 2015; Rees, Bosch & Douglas 2018). As a result of this there has been a shift in what constitutes a healthy body, with findings in once disparate areas of biomedicine linking changes in gut microorganisms and dysbiosis to a wide range of diseases and illnesses (Blaser & Falkow 2009; Gentile & Weir 2018; Gordon 2012; Jameson & Hsiao 2018; Mu et al. 2017). Despite significant advances in this area, scientific research is still in its infancy regarding the question of what constitutes good gut health (Gentile & Weir 2018). Currently it appears that individuals’ biologies and microbiologies vary so greatly there is no one best approach, and recommendations for the best probiotics, prebiotics, and other food supplements remain contested (Gentile & Weir 2018).

Biomedical science continues to shape western cultural institutions through its achievements in germ theory, vaccinations, antibiotics, and more specifically here with discoveries about the significance of the gut microbiome (Blay-Palmer 2008; Lorimer 2018; Paxson 2008; Ross 2013). Throughout much of the twentieth century these achievements have created something of a hegemonic authority, dominating the medical system and popular understanding of what constitutes medicine and effective medical treatment in western societies like Australia (Blay-Palmer 2008; Paxson 2008; Ross 2013). That having been said, it is being increasingly recognised that practices of medical pluralism, the utilisation of more than one form of medical knowledge and practice, including in this instance “folk” and “alternative” approaches, are more common in western settings than previously thought (Leslie 1980; Ross 2013). As a similar and related area of knowledge and practice to medical pluralism, lay epidemiologies, was conceptualised and described by Davison, Smith & Frankel (1991) in context of their study on coronary disease in the UK. This term describes the way people construct their own explanations for the how and why of disease and illness (Davison, Smith & Frankel 1991). Lay epidemiologies are complex interactive systems of understanding which are deduced from a combination of knowledge gained from upbringing, wider society, their own observations, and advances in scientific understanding (Davison, Smith & Frankel 1991). Medical pluralism and lay epidemiologies reflect the dynamic and heterogeneous nature of the contemporary medical landscape, in which “people seek to maximise their chances for well-being and adapt to rapidly changing or unfavourable circumstances, drawing on multiple sources and resources of knowledge and authority” (Orlando 2018; Ross 2013, p. 10).

This idea of a contemporary medical landscape, where lay epidemiologies abound amid formal, hegemonic forms of knowledge and practice, is apparent throughout participants’ health and wellbeing narratives, as collected in the interviews examined here. Gytha for example described having problems with gastrointestinal health in the form of “leaky gut”, and resolution of this condition was predominantly attributed to the incorporation of kombucha and fermented foods to her diet. The term “leaky gut” is used predominantly outside of clinical settings to describe generalised intestinal barrier dysfunction (Camilleri 2019). In biomedical science it is mostly associated with gastrointestinal inflammatory conditions and autoimmune diseases such as Crohn’s disease (Camilleri 2019; Mu et al. 2017). Lay use of the term however is not

necessarily linked with specific gastrointestinal inflammatory diseases, and is considered by clinicians to often overreach current scientific understandings, and to be heavily based in ‘folklore’ (Camilleri 2019). This represents an example of what Brown (2007) describes as a contested illness.

Although scientific research has linked dysfunction of gut barriers with factors as diverse as stress, there are no clinical diagnostic tests and understanding is still limited (Camilleri 2019; Mu et al. 2017). Regardless of this, people form the how and why of illnesses both from science and other sources of knowledge and authority to form their own lay epidemiological understanding (Davison, Smith & Frankel 1991). So, to Gytha the symptoms of gut barrier dysfunction are indicative of her gut microbiome being out of balance and needing the addition of microbial diversity. To this end, her approach to treatment was one of “I want those bacteria in me”, turning Douglas’ (1966, p. 44) term of “matter out of place” and a stance more typically seen, into microorganisms as “matter in place”, and as a result Gytha was purposefully not washing her garden vegetables unless covered in obvious dirt. Another important part of treatment for Gytha was the consumption of kombucha and a variety of other fermented foods, as these foods signified health and well-being through the presence of microbial communities. Gytha’s understanding of her symptoms and the efficacy of the treatment confirms validity of her personal lay epidemiology.

Contested illnesses like leaky gut feature in a number of participant’s narratives about themselves, and others, in relation to health and wellbeing. They are also prominent in complementary and alternative medicines (CAMs) (Brown 2007; Orlando 2018; Ross 2013). CAMs deviated from the hegemonic biomedical tradition to varying degrees, persisting in smaller numbers throughout the earlier twentieth century but proliferating in the latter (Belasco 2007; Brown 2007; Orlando 2018; Ross 2013). This reflects Esme’s experience with kombucha production approximately 30 years ago, when it was only commonly known, made, and consumed by her friends, who were “hippies” as she put it, and into “clean eating” and what has been labelled by academics as the “alternative consumption movement” (ACM) or “alternative food movement” (Belasco 2007; Blay-Palmer 2008, p. 1; Ross 2013, p. 192). The visibility and acceptance of CAMs has increased however within the lay epidemiologies of wider society, and in the practice of medicine more broadly in western societies in the

twenty-first century (Ross 2013). This is due to the professionalisation of practitioners, increased consumption and commercialism, the impacts of the internet and globalisation more generally, and the integration of aspects of biomedicine into CAMs (Ross 2013). Increased visibility and acceptance have led to the scientific validation of some treatments, and lifestyle choices which were once considered 'alternative' and indeed 'quackery', for example the use of helminths as a therapeutic for inflammatory diseases, or even the use of probiotic supplements which are now commonplace (Elliott & Weinstock 2017; Lorimer 2018; Ross 2013).

Although there has been integration of western biomedical knowledge into, and an increase in mainstream appeal of CAMs and ACMs, there are remaining tensions. Themes arising in participant interviews included critiques of, and concerns or anxieties about the capitalist industrial global food system. An example of this is Esme's concern about the "absolute crap", implying industrial processed food, that her children were eating outside of the home. These perspectives reflect the heritage of CAMs and ACMs as being social movements, with active concern for peoples' impact on the environment, and being heavily critical of the growing global industrial food system of the nineteenth and twentieth centuries (Belasco 2007; Blay-Palmer 2008; Ross 2013). Many participants in this study identified as, or their values aligned with, one or a number of food movements which fall under the purview of ACMs, including slow food, locavores, freegans, organic, raw food and ethical vegetarians (Orlando 2018; Ross 2013). William and Sacharissa for instance participated in aspects of freeganism, including dumpster diving, using only the sugar and tea they find to make their kombucha. Similarly, a friend of theirs and interview participant, Oliver participated in dumpster diving and his household grew much of their own food in a large vegetable garden that covered both the front and back yards of their suburban home. Just as the embodied experience of abjection related to the kombucha SCOBY can be seen to have shaped participants social worlds, the broader social dynamics examined in this section link to the body politic (Scheper-Hughes & Lock 1987). This link relates especially to the political economy and practical efficacy of approaches to health and wellbeing, as seen in lay epidemiologies and as shall be seen in the broader contemporary environmental concerns.

The body *politic*

The aforementioned tensions, critiques, and anxieties in discussions of CAMs and ACMs introduce the final unit of analysis in this discussion, the body politic. This body concerns the relationships of individuals with social bodies, and are often about power and control (Scheper-Hughes & Lock 1987). Microbiopolitics as an aspect of microbial entanglement also comes into play in this section as it pays “attention to how people and states seek to live with microbes” as allies or threats, linking the scale of the global food system with the individual body through recognising participants encounters with microbially laden foods like kombucha as inherently political (Flachs & Orkin 2019, p. 36; Paxson 2008, 2013). This section predominantly focuses on the theme of environmental concerns present in participants narratives, providing a means to consider health, well-being, and alternative consumption at the level of the body politic as well as in the terms of political, ecological and individual agency.

Participants in this study expressed deep concerns about their direct impact on the environment. Relatedly, they described practicing various strategies in the minimisation of waste creation, leading to a ‘do-it-yourself’ approach to kombucha, rather than purchasing wasteful bottles for example. Some participants like William, Sacharissa, and Oliver worked even harder to reduce their environmental impact through practicing ‘freeganism’. However, the picture becomes more complex when considered in combination with other concerns, including the consumption of processed foods, sugar, antibiotics, food preservatives/additives and the impact of other aspects of modern life raised by numerous participants. These concerns, despite appearing perhaps unrelated to environmentalism, are in fact interconnected (Blay-Palmer 2008; Orlando 2018; Stassart & Whatmore 2003). In his research into organic food consumers in Palermo Italy, Giovanni Orlando (2018) argued that the push towards the consumption of organics and other ACMs is a reaction to politicised risk factors like environmental pollution, loss of biodiversity, chemical fertilisers, carbon dioxide emissions, and genetically modified foods (GMO). Similarly, participants like Esme described buying organic foods, while others like Tiffany, Oliver, and Agnes grew their own vegetables in order to be closer to the means of their food production, and to remove themselves as much as possible from the global industrial food system, the food from which is seen by them to be inherently less healthy. Orlando's (2018) informants similarly identified divisions between ‘healthy’ and ‘industrial’ foods, with

organic foods being healthy and mediating the effects of the heavily processed industrial or 'risky' foods. On top of organics, participants like Gytha and Esme spoke of utilising their kombucha and other fermented foods as a means of balancing and offsetting adverse effects of unhealthy foods and other aspects of contemporary life with its reliance on industrially produced food and other consumer items. Gytha commented on her relatives "bad diet" which in her opinion needed mediating with kombucha and other ferments to improve their health. Esme also used kombucha and homemade pickles to help model "good" food habits for her teenagers, and to help compensate for the "crap [food]" they ate outside of the home. Participants concerns over the environment therefore encompass the traditional ideas of environmentalism as well as the fears and anxieties of politicised risk factors polluting or contaminating themselves and the environment.



Figure 7 Polly pouring her fresh batch of kombucha into a reused soda bottle.

The views and critiques of the industrial food and agriculture system, and its impacts on the environment, maintained by both Orlando's (2018) organic food consumers and the participants in this research are not new. Chemicals, hormones, antibiotics and pathogens are "familiar interlopers" in modern industrial diets, with food safety scares having become endemic (Blay-Palmer 2008; Stassart & Whatmore 2003). Relentless industrialisation has caused significant environmental damage and public health issues over the last one-hundred and fifty years (Blay-Palmer 2008; Brown 2007). With examples like bovine spongiform encephalopathy (BSE) or mad cow disease, and GMO foods creating persistent fears about invisible and geographically pervasive pollution and disease (Blay-Palmer 2008). Consumer anxiety about food are fuelled in part by these scares, as well as conflicting risks and instructions about how to mitigate them (Fuentes & Fuentes 2015; Stassart & Whatmore 2003).

Complicating matters further, over the last two centuries there has been an increased separation of people from the social, physical, and intellectual origins of food (Blay-Palmer 2008). The divide between humans as "distanced consumers" from the dangerousness and unpredictability of nature is a necessary condition for the current capitalist food system where people are reliant on regulators and food processors to assure them of safety (Blay-Palmer 2008; Orlando 2018; Stassart & Whatmore 2003). Participants like Hamish, Lynne, Esme, Gytha, Tiffany and Samuel talked about actively resisting this separation through informed purchasing choices, growing their own food, and making as much food from scratch as possible. Reflected in their interviews were concerns about hormones, preservatives, fillers, and other contaminants in both food and the environment, demonstrating a belief that the industrial food system has more interacting and conflicting priorities than can be regulated or controlled meaningfully (Blay-Palmer 2008). These views indicate increasing "contemporary culinary anxieties" which have been magnified and transmitted in the "vernacular of food choice for over thirty-five years" (Stassart & Whatmore 2003; Orlando 2018, p. 45).

These contemporary anxieties about food, environmental contamination, and risk, are clearly linked to concepts of ambiguity, miasma, and of boundary crossing and abjection, as described above via Douglas (1966) and Kristeva (1982). The symbolic power and danger inherent within the potential destructiveness of ambiguity and

disorder means cracks in the industrial food landscape, through which pervasive and miasmatic pollution in the form of hormones, GMOs, and diseases like BSE emerge, revealing “the fragility of state hygienic regulations” (Blay-Palmer 2008; Douglas 1966; Flachs & Orkin 2019, p. 36). This fragility creates distrust in the global food system, a distrust that is clearly seen in the participant narratives discussed thus far (Blay-Palmer 2008; Flachs & Orkin 2019). Moreover, food’s status as a “ready messenger of connectedness and affectivity” means that despite the assurances from public health officials and scientists, food scares have encouraged individuals to seek other forms of “authoritative knowledge,” such as in CAMs and ACMs, incorporating them into their lay epidemiologies (Davison, Smith & Frankel 1991; Orlando 2018; Rebrovick 2015; Stassart & Whatmore 2003, p. 450). ACMs in particular are used in this way as a symbol and tool to “bypass the political-economy of industrial food systems” (Blay-Palmer 2008, p. 113). ACMs are an auto-regulatory strategy that help individuals, like those in the kombucha producing community, to manage risks as well as resolve tensions and anxieties over environmental pollution, and other issues related to the global industrial food system with which they have few other tools to be able to address (Blay-Palmer 2008; Orlando 2018).

Although many people outside of kombucha interest groups continue to view microbial life, such as is found in SCOBYs, as abject, along with increased appeal of ACMs there has been an additional rise in popular interest in fermented foods and beverages (Blay-Palmer 2008; Elliot 2018; Katz 2012; Orlando 2018). This suggests a shifting relationship with microorganisms in western societies, one that embraces them as scientific and biological knowledge about them has increased. Participants like Agnes, Samuel, Kate, and Susan, who have made kombucha either on and off, or continuously for many years, noted in interviews that they have witnessed a surge of interest in and availability of kombucha SCOBYs particularly in the last five to ten years, not to mention how widely available in supermarkets and cafes commercially produced purportedly probiotic drinks like kombucha are. It was this widespread commercial availability that sparked the uptake of home production for some informants who are newer to the practice. Moreover, Agnes and Susan both made kombucha years ago, up to 30 years in Agnes’ case when it was a niche interest, and she was inspired to re-obtain a SCOBY and start producing again with the appearance of the kombucha on supermarket shelves.

The increased popularity of kombucha, society's shifting relationship with microorganisms, and the wider appeal of ACMs re-introduces the discourses of eco-dietetics and post-Pasteurianism outlined in the introduction of this thesis. These discourses are pertinent to both the kombucha producing participants of this research and are indicative of shifts in wider society and individual's relationships with larger social bodies. Despite eco-dietetics and post-Pasteurianism being characterised as separate entities by Rebrovick (2015) and Paxson (2008), they are arguably two facets of the same discourse. Both these concepts concern the intersection of food, environmentalism, politics, health, and science, sharing many of the same conceptual roots, drawing and expanding on ACMs, environmentalism, and critiques of the industrial food system. They are in part a societal response to new understandings from biology and science, with the "probiotic turn" in western biomedicine acknowledging that microorganisms are all around us, being intricately and intimately tied up in our bodies and health (Gordon 2012; Greenhough et al. 2018; Gentile & Weir 2018; Paxson 2008; Paxson & Helmreich 2014; Lorimer 2018, p. 4). In addition, they are a social and political response by the wider public to anxieties about the current and future health of the planet, its animals, and now microorganisms (Paxson 2008; Rebrovick 2015). In post-Pasteurianism and eco-dietetics, humans are considered biologically and ecologically inseparable from their environments, where microorganisms are "part of the political, social, and experiential landscape" as well as a the biological, and food production and eating practices "materially reconfigure both individual bodies and social formations' (Paxson 2008, p. 39; Rebrovick 2015, p. 687). Throughout the participant narratives there were pronounced shared perspectives on food, production, and concern for the environment which indicated active engagement with these discourses. It is this engagement that link participants from the kombucha producing community together as an interest group and situates them at the forefront of increasingly popular food movements.

Conclusion

Through analysis of participants personal narratives this chapter has discussed how the phenomenological and affective experience of embracing the kombucha SCOBY indicates an acknowledgement, and subsequent rejection or adjustment to older perceptions of microbial life. This chapter started with a description of a SCOBY as an object of repulsion, disgust and abjection, representative of the visceral embodied

experience that many people have on first encountering homemade kombucha. In the domestic setting, food is a liminal substance that “constantly shifts register between matter and meaning” (Douglas 1966; Goodman & Sage 2014; Kristeva 1982; Orlando 2018; Stassart & Whatmore 2003, p. 450). Food as a potential carrier of the abject and its liminal transgressive abilities can through experience and embodiment implicate and affect everything from culture, discourses, and society to nature, the environment, and technology (Goodman & Sage 2014, p. 2; Stassart & Whatmore 2003, p. 450). The SCOBY, carrying abject connotations, highlights an example of how microorganisms have been major factors of fear with regards to human health. Even for those participants who first recoiled at seeing a SCOBY, reflecting more socially widespread reactions to them as abject “matter out of place”, the themes and ideological stances of post-Pasteurianism and eco-dietetics run strong. These stances were strong enough drivers for those initially disgusted to overcome their fear and embrace the in-betweenness of the kombucha microbial community, causing the previously maligned SCOBY to lose its abject designation as Other, and instead seeing it consumed as health-producing “matter in place”. Extending these ideas further, along with these transformed bodily reactions, a shared ideological stance rejecting persisting ideas of microbes as dangerous and unhealthy formed the participants in this research into a community, thus forming a social body. Instead of fearing microorganisms, this community embraced microbial entanglements by engaging in ACMs, eco-dietetics, post-Pasteurianism, using these discourses to create their own hybridized system of relating to not only the microbial communities in kombucha but also the body politic of the industrialised global food system, western biomedicine, and the environment.

Chapter 6: Molecular microbiological results and discussion

Having discussed the ethnographic findings of this research, the following chapter presents and discusses the molecular microbiological results that emerged from subjecting my participants' kombucha SCOBYs to quantitative analysis in a university laboratory. In order to establish context for this examination, the first section provides background information about the participants and their kombucha samples, as well as briefly outlines some of the factors that impacted the next-generation sequencing outcomes. Following this, the bacterial results are introduced and analysed, finding all participants kombucha shared a strong conserved bacterial core community, however each had a unique selection of rare taxa present. The functional analysis performed on the bacterial data also found most pathways present were commonly shared across all kombucha samples. Finally, the kombucha fungal community findings were outlined and discussed, showing a diverse but relatively small number of fungi were present in each kombucha compared to the bacteria, with no conserved core observed between SCOBYs.

Background and factors impacting the next-generation amplicon sequencing results

The first aim of the molecular microbiological component of this project was to examine the microbial community structure of participants SCOBYs for similarities and differences that could be identified between the kombuchas. The second aim was to use microbial network analysis to combine the contextual details from the ethnographic findings with the results of the next-generation sequencing to visualise connectivity between the participants' kombucha samples and how the microbiological data related to or is reflected in the contextual data. It was hypothesised that there would be similarities seen between the kombucha microbial communities as all SCOBYs were performing fermentation, despite this, it was thought there would be notable differences in composition between communities. Furthermore, it was hypothesised that the microbial network analysis would show distinct relationships between samples, based on either a relationship between samples, or on the contextual variables present in the ethnographic data.

The kombucha samples collected in this study all come from distinct households and different producers. There were sixteen participants in total, four of the sixteen had shared or gifted a SCOBY with another participant. An instance of sharing between paired participants for the purposes of this study, so the 'parent' and 'offspring' microbial communities can be compared for similarities (See table 3 below of paired and unpaired participants). The integration timeline of the kombucha SCOBY to the new household and producer ranged from approximately two months to over one year. Both biofilm and liquid samples of participants kombucha were collected for this study. To standardise extraction of genomic DNA (gDNA) the DNeasy® Powersoil kit was used for both sample types, with increased agitation time from 10 to 20 mins at the horizontal bead vortex stage, as this had been successfully used on other biofilms in this laboratory. However, when tested even with the increased vortex duration the kit was not successful at obtaining gDNA from the kombucha biofilm samples. Subsequent attempts to break apart the biofilms with submersion in liquid nitrogen and mechanical destruction on a vortex or applied blunt force were also unsuccessful. Due to time limitations biofilm gDNA extraction troubleshooting was suspended.

Table 3 *The participant pseudonyms, giver or receiver designation, and the labels used for the figures in this chapter.*

<u>Label</u>	<u>Participant</u>	<u>Giver (G)/receiver (R)</u>
Pair 1a	Polly	R
Pair 1b	Lynne	G
Pair 2a	Gytha	R
Pair 2b	Gytha's Son	G
Pair 3a	William & Sachrissa	R
Pair 3b	Oliver	G
Pair 4a	Tiffany	R
Pair 4b	Rob	G
Unpaired 1	Matthew	N/A
Unpaired 2	Samuel	N/A
Unpaired 3	Eskarina	N/A
Unpaired 4	Esme	N/A
Unpaired 5	Kate	N/A
Unpaired 6	Agnes	N/A
Unpaired 7	Susan	N/A
Unpaired 8	Hamish	N/A

The liquid kombucha gDNA extraction was successful, therefore 16S ribosomal ribonucleic acid (rRNA) and internal transcribed spacer (ITS) next-generation amplicon sequencing was conducted on the liquid alone resulting in a total of sixteen samples. While all bacterial communities were successfully sequenced, for fungal communities, three samples had a low sequencing read turnout. The average reads per sample across all sixteen was low however, and could indicate the comparative low abundance of fungi in the kombucha liquid versus the biofilm as reported in previous studies (Chakravorty et al. 2016; Villarreal-Soto et al. 2018). Of the three samples, two were retained as the read spread across all samples was dynamic, and although these contained low reads their read count was not indicative of the samples being outliers. The third sample was removed as it had extremely low reads in comparison to the other two, which was likely due to an error in sequencing.

Strong bacterial core community present across all kombucha microbiota

Exploratory bar charts visualising the relative abundance of taxa in the kombucha were created in the R statistical computing environment (R Core Team 2014). Core microbiome analyses were performed on exact sequence variances (ESVs) which had been total sum scaled as a pre-treatment to normalise for the differing numbers of reads between samples. The total ESVs across all samples was 7102. When the data was filtered for ESVs that appeared in 50% or more samples to visualise the overall community core, there were 1172 ESVs present. This demonstrates the presence of a strong core bacterial community. β -diversity, or community turnover, was also measured using Jaccard distance to investigate the percentage of dissimilarity between samples. This resulted in a range of dissimilarity distances from 35% to 60%, meaning inclusive of the core bacteria the samples ranged from relatively similar to somewhat dissimilar respectively.

Comparatively, when looking strictly at those ESVs that appeared in 100% of samples there were 273, demonstrating conserved core. The Jaccard distances carried out on the core bacterial data support this finding, with samples ranging in dissimilarity between 10% to 36%. These results contradict the sparse previous studies which report low richness, with core bacterial communities of one or two ESVs in kombucha and other fermented beverages (Arıkan et al. 2020; Chakravorty et al. 2016; Gomes et al. 2018; Marsh et al. 2014). Muzaffer Arıkan et al. (2020) for example reported that Proteobacteria was the dominant phylum comprising more than 99% of the communities in their two kombucha samples of Turkish origin. Furthermore, 99% of the Proteobacteria present were assigned to a single family, the *Acetobacteraceae* family (Arıkan et al. 2020). Alan Marsh et al. (2014) in their investigation into five geographically diverse kombucha samples described limited richness, with *Gluconacetobacter* dominating all samples down to the genus level. In contrast, figure 8 below shows a rich core bacterial community at family level across all 16 samples belonging to a range of phyla. The main methodological difference between Arıkan et al. (2020), Marsh et al. (2014), and this study is that both of the former used a food specific DNA extraction kits whereas a kit commonly used for soil and environmental microbiota was used in this study. The disparity between DNA extraction kits, due to procedural differences, would yield contrasting results as each kit is customised to

different kinds of microbial communities. It was considered more appropriate to use an environmental extraction kit for this project as the kombucha SCOBY has more in common with an environmental microbiota, and food kits are used more frequently to inspect samples for food based for pathogens. The difference between kits is perhaps reflected in the richness of kombucha communities seen the results of this research versus the other studies.

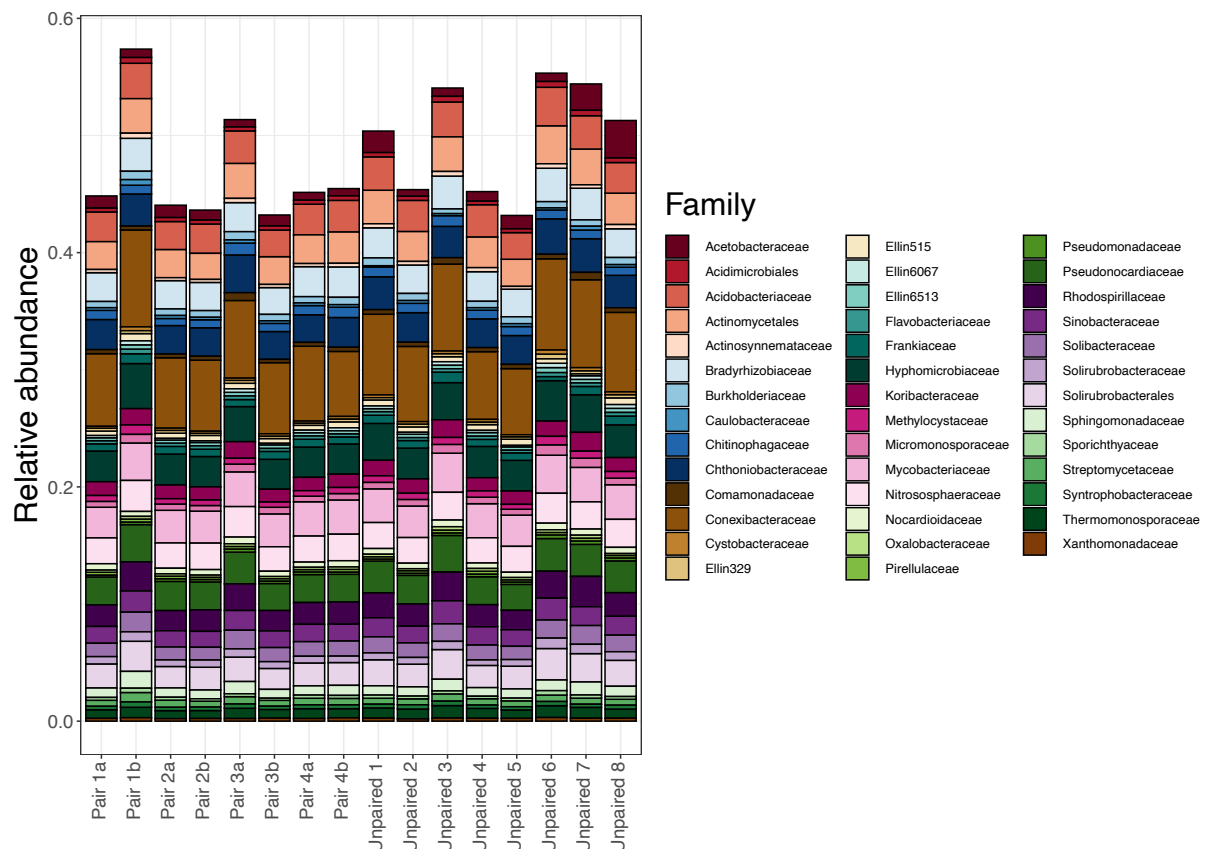


Figure 8 An exploratory bar chart visualising the relative abundance of the core bacterial microbiome present in participants' kombucha samples. Taxa are depicted at the Family level. The core is defined as Families with a read abundance $<0.002\%$.

Figure 9 illustrates the relative abundance of bacterial taxa at the phylum level. This demonstrates a strongly conserved phylogenetic structure of bacteria shared between samples. Furthermore, the structure is persistent despite the difference in kombucha SCOBY origin and geographic spread. In figure 9 the bar size indicates Actinobacteria and Proteobacteria are most dominant phyla, and Acidobacteria, Bacteroidetes, Verrucomicrobia, and Plantomycetes are moderately abundant. Proteobacteria have been reported as a dominant phylum in previous studies, with all other phylum reported in the literature present at some amount in these bacterial communities

(Chakravorty et al. 2016; Coton et al. 2017; De Filippis et al. 2018; Marsh et al. 2014; Reva et al. 2015). The wide range of phyla present however exceeds any previous findings on kombucha, with Acidobacteria and Verrucomicrobia having been reported in only one other study on Turkish kombucha (Arıkan et al. 2020; Chakravorty et al. 2016; Coton et al. 2017; De Filippis et al. 2018; Marsh et al. 2014; Reva et al. 2015).

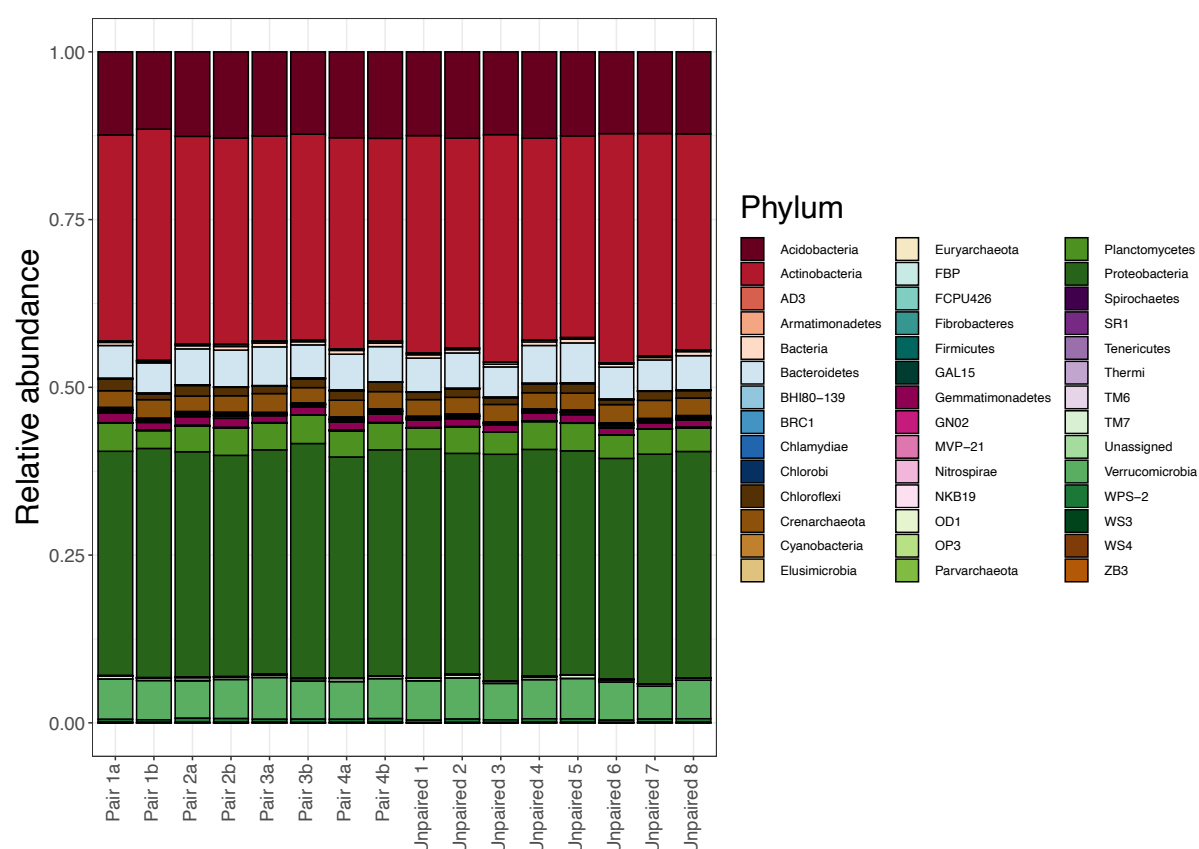


Figure 9 An exploratory bar chart visualising the relative abundance of the phylum level bacterial taxa present in participants' kombucha samples.

Figure 10 shows the relative abundance of bacterial genera. The core microbiome was defined as taxa with relative abundance greater than 0.1% and present in more than 50% of samples, using the microbiome package in R (Lahti & Shetty 2019). Similar to the phylum level bar chart there is uniformity between samples regardless of pairing or geographic distribution indicating a strongly conserved core. This uniformity further supports the Jaccard distance findings of 10% to 36% dissimilarity carried out on the core bacterial data. In figure 10 not all ESVs could be assigned a taxonomic identity at genus level with confidence, in these cases the next lowest taxonomic rank where

assignment could be made are displayed. Only 40-50% of each sample is being represented in this figure due to the removal of rare taxa, this suggests much of the diversity between samples is likely to be represented in the missing taxa. There is a noteworthy lack of shared genera with previous studies, with the exception of *Sphingomonadaceae* members of which have been associated with biofilm formation in drinking water, and identified in kombucha, water, and milk kefir (De Vries et al. 2019; Li et al. 2019; Marsh et al. 2014). Instead, the results seen in figure 10 indicate a complex conserved core that differs from the reported literature thus far (Arıkan et al. 2020; Chakravorty et al. 2016; Coton et al. 2017; De Filippis et al. 2018; Marsh et al. 2014; Reva et al. 2015; Villarreal-Soto et al. 2018).

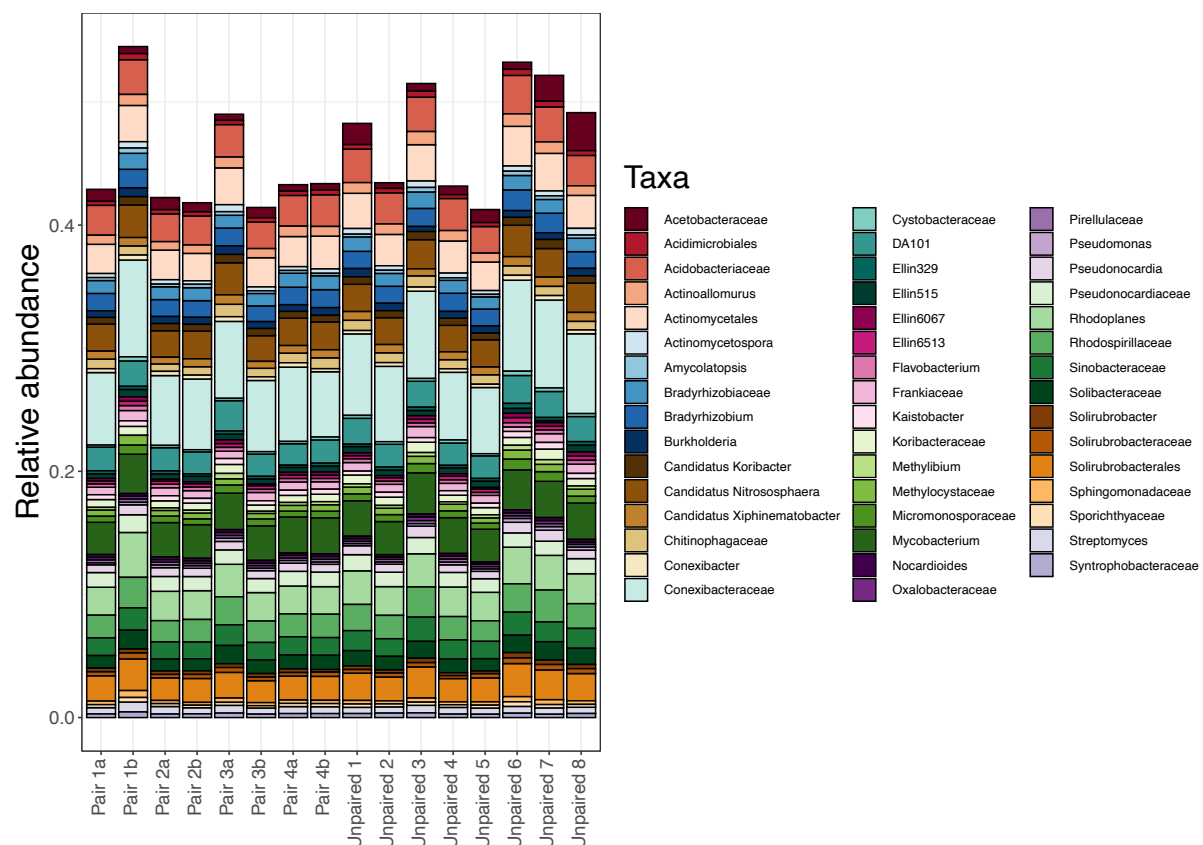


Figure 10 An exploratory bar chart visualising the relative abundance of core bacterial genera present in participants' kombucha samples. Core taxa were defined as taxa >0.1% relative abundance and in >50% samples. Where taxa could not be identified to genus level, the next highest taxonomic rank was used

Kombucha bacterial communities are unique despite strong core

Non-metric multidimensional scaling (NMDS) ordination was created to visualise community phylogenetic similarity and can be seen in figure 11. The distances between points in a UniFrac NMDS ordination are indicative of how similar a samples community is to the other communities being compared. The unweighted ordination is a measure of ESV presence and absence, whereas weighted takes taxonomic abundance data into account. Therefore, the clustering within the ellipsis seen in figure 11a points to how similar the samples are with a handful of outliers, this clustering is most likely due to the presence of a strong core bacterial community. When abundance is taken into account in the weighted UniFrac figure 11b there is some minor clustering seen in the red ellipsis between a number of unpaired and paired samples, indicating there is similarity in bacterial population between these samples. Other than one paired and one unpaired sample seen in the blue ellipsis in figure 11b, the distances in this figure demonstrate that each of these kombucha SCOBYs are distinct from the others despite a strong core community.

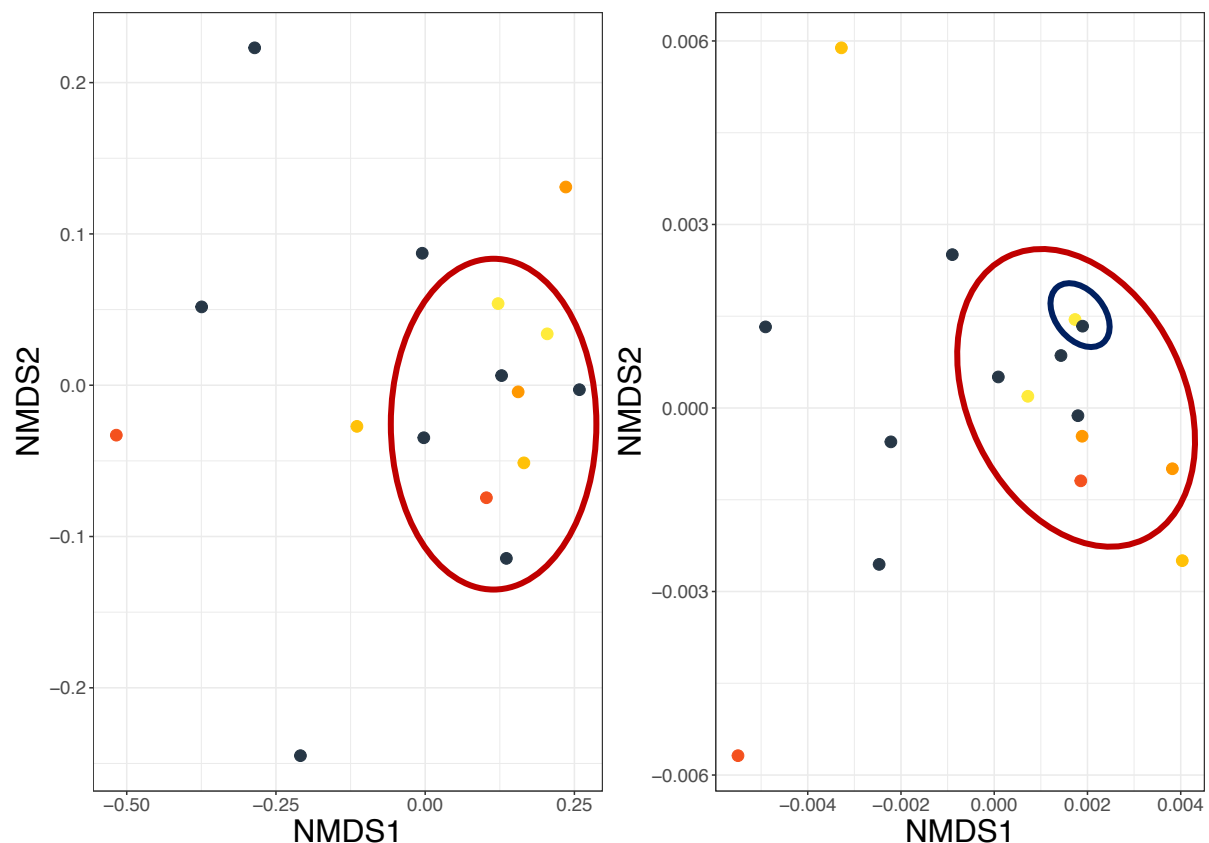


Figure 11 Unifrac unweighted (a - left) and weighted (b - right) NMDS ordinations were produced using the TSS bacterial ESV tables. The black dots indicate the unpaired samples and the paired samples are all matching colours. The 2D stress was < 0.2.

When the ESVs present in less than 50% of the samples were isolated, 5929 remained of 7101, this is a substantial number of rare ESVs. Bar charts visualising the relative abundance in the rare bacterial genera were created and can be seen in figure 12. Crossover of taxa between samples can be seen in this figure and is reflected in the Jaccard distances of 77% to 100% dissimilarity, this finding would also account for the spread of samples in the unweighted UniFrac ordination in the figure 11a. A number of the crossover genera are notable, including *Acetobacter*, *Gluconacetobacter*, and *Gluconobacter* as they are well known members of the acetic acid bacterial (AAB) group (De Filippis et al. 2018; Gomes et al. 2018; Marsh et al. 2014). These AAB are present and in many cases dominant in other reported kombucha communities (Arıkan et al. 2020; Chakravorty et al. 2016; Coton et al. 2017; De Filippis et al. 2018; Marsh et al. 2014; Reva et al. 2015; Villarreal-Soto et al. 2018). Despite this crossover, the data in figure 12 and the Jaccard dissimilarity distances clearly show that each kombucha SCOBY has a unique collection of rare taxa. There could be a number of explanations for the individuality of these kombucha communities. One being there is some effect human interaction and production practices are having on the community. The other could be the effect of population dynamics like a founder effect impacting the bacterial composition when a kombucha SCOBY is transferred between participants. This is a trend not seen in any other findings on the kombucha SCOBY in the literature and could be due to the aforementioned difference in DNA extraction technique or other difference in approach (Arıkan et al. 2020; Chakravorty et al. 2016; Coton et al. 2017; De Filippis et al. 2018; Marsh et al. 2014; Reva et al. 2015; Villarreal-Soto et al. 2018).

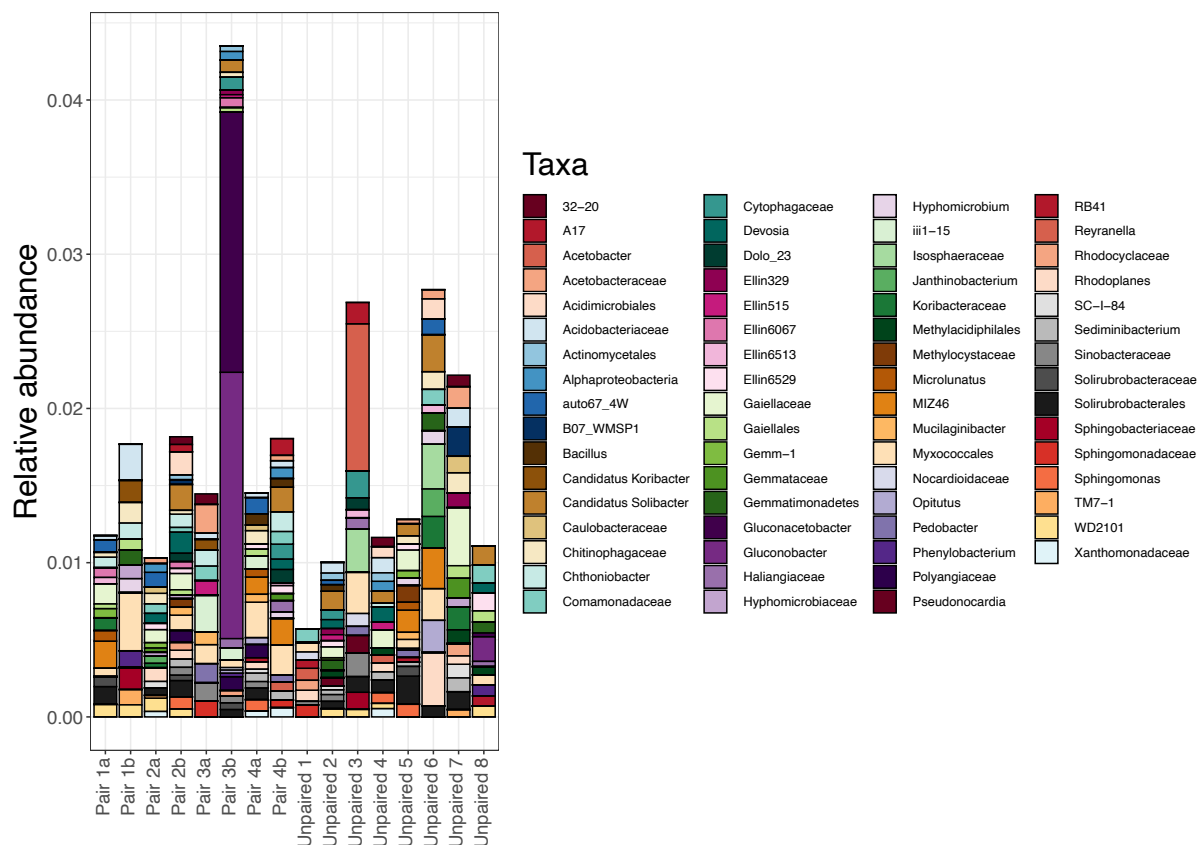


Figure 12 An exploratory bar chart visualising the relative abundance of rare bacterial genera present in participants' kombucha samples. Rare genera presented here constitute the top 20% most abundant ESVs which were present in less than four samples. Where genus could not be identified the next highest taxonomic rank was used.

Functional pathway analysis of bacterial community with PICRUSt ⁷

The values from an analysis of the bacterial data investigating functional diversity were used to create a principal co-ordinate analysis (PCoA) of a Bray-Curtis distance matrix and can be seen in figure 13 below. The distances between points in this PCoA ordination are indicative of how similar the functions of a particular community to the others being compared. In figure 13 samples are quite dispersed with some minor clustering, this is indicative of samples having a range of functional profiles with 89.5% of the variation in the data represented by Axis 1. Interestingly Axis 2, which represents 8.9% of the variation in the data, clearly separates samples into two distinct clusters, however these clusters could not be related to any explanatory parameters collected as part of this study.

⁷ See functional pathway in the glossary

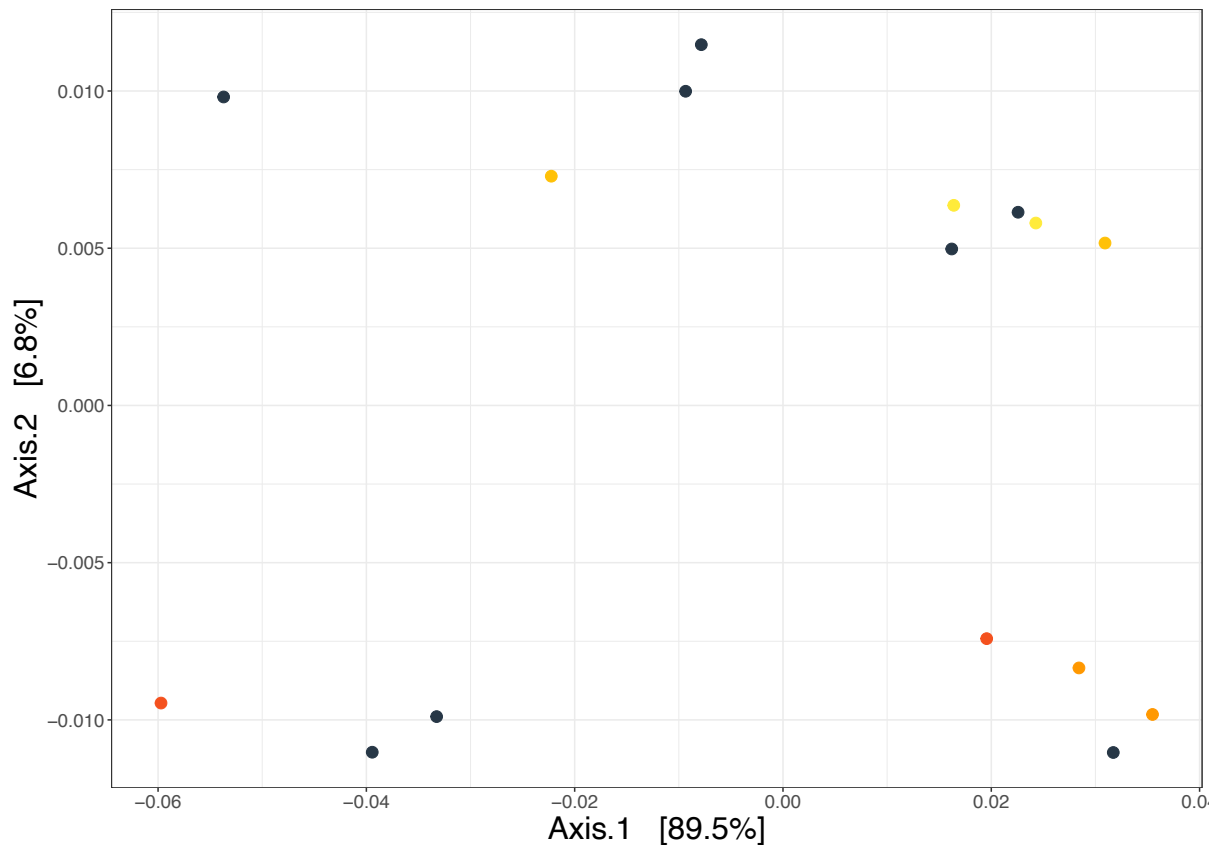


Figure 13 A principal coordinate analysis (PCoA) ordination of the PICRUST functional diversity data, created using Bray-Curtis distance metric. The black dots indicate the un-paired samples, and the paired samples are all matching colours.

Overall, 407 out of 427 functional pathways appeared in more than 50% of samples and 387 pathways were present in 100%, meaning there were few pathways unique to any one bacterial community. Of the large number of shared pathways there were many expected given the fermentative nature of kombucha. For example, the mixed acid, homolactic and heterolactic fermentation pathways were present across all samples. All of these pathways take sugars such as glucose, fructose, and sucrose and convert them into cellular energy, with by-products including acetate, ethanol, lactate and carbon dioxide (Caspi et al. 2020). Furthermore, appearing in all samples were fermentation pathways for short-chain fatty acids (SCFA) like acetate and butanoate. Studies have shown that these SCFAs play an important beneficial role in the health of human gut microbiota, and their presence in kombucha could play a part in kombucha's health giving reputation (Neis, Dejong & Rensen 2015; Silva, Bernardi & Frozza 2020).

Analysis using the MetaCyc database additionally found a range of pathways appearing in more than 50% of samples (Caspi et al. 2020). These included starch biosynthesis arising from the presence of cyanobacteria, the antibiotic Fosfomycin biosynthesis, and Beta-lactam antibiotic resistance, which are all commonly found in environmental microbial communities (Graham et al. 2016; Zeeman, Kossmann & Smith 2010). In this analysis it was also noted that the participant Hamish's kombucha sample had numerous unique pathways attributed to it unlike any other sample. For example, a pathway for the degradation of the toxic compound toluene was present, commonly found in gasoline contaminated water (Caspi et al. 2020). This could be due to a range of environmental and production practices particular to this kombucha sample. Hamish lives on a semi-rural property that practices permaculture, rainwater is used in the production of the kombucha, and the endemic microbial community present in the kitchen would in this case be impacted by the soil and produce brought in from the garden.

Simple but variable fungal community present in kombucha microbiota

Compared to the bacterial community, the fungal community present in participants' kombucha samples were simpler and more varied. Ascomycota and Basidiomycota are the only two phyla present across all samples, which is consistent with two previous studies looking at both liquid and biofilm samples (Marsh et al. 2014; Teoh, Heard & Cox 2004). One study was on Australian kombucha but looked exclusively at fungi; the other reported finding only Basidiomycota in their biofilms whereas this research used liquid samples only (Marsh et al. 2014; Teoh, Heard & Cox 2004).

Figure 14 is a UniFrac NMDS similar to the bacterial ordinations but for the fungal data. The unweighted data in figure 14a shows some clustering between samples, indicated by the ellipses. When weighted for taxonomic abundance however two strong clusters can be seen in the ellipses in figure 14b, so much so the dots are overlapping one another. There are also four samples dispersed widely across the figure 14b, suggesting the fungal communities in these samples are wildly different from the clustered ones.

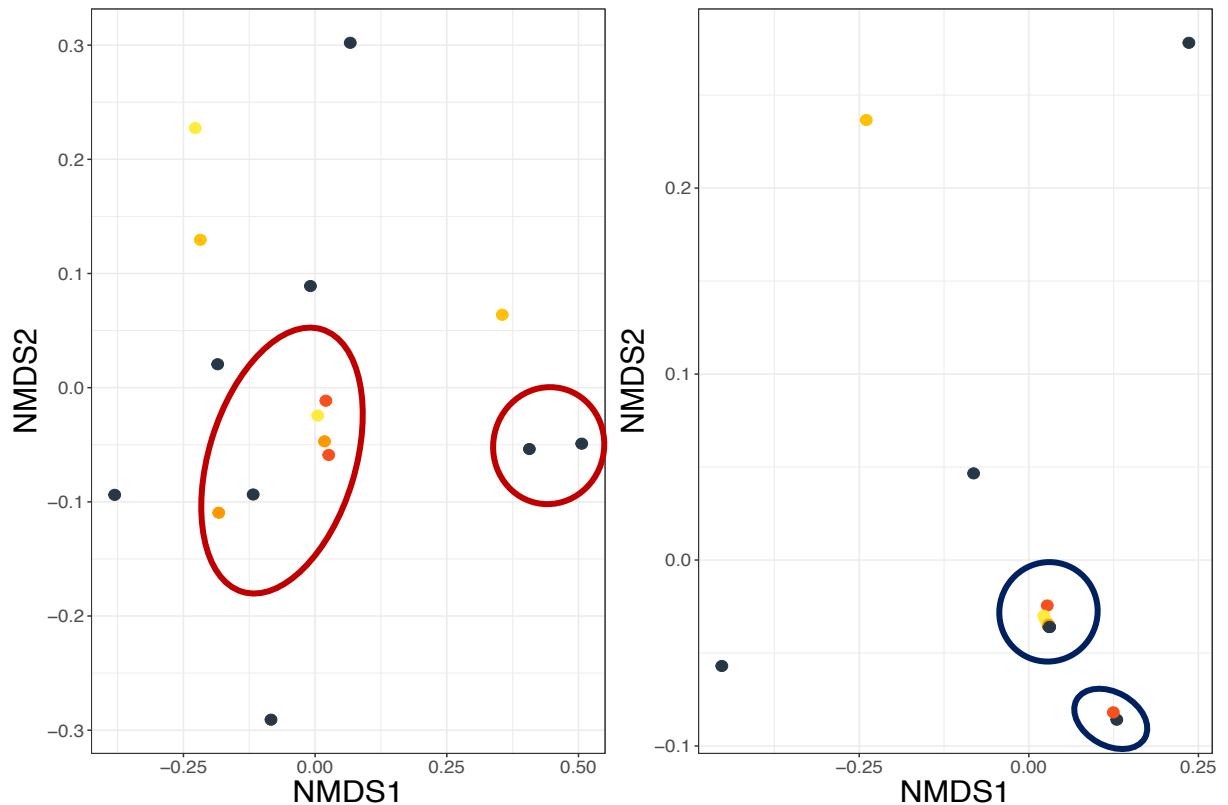


Figure 14 UniFrac unweighted (a - left) and weighted (b - right) NMDS ordinations were produced using the TSS and log-x transformed fungal ESV tables. The black dots indicate un-paired samples, and the paired samples are all in matching colours. The 2D stress was < 0.2.

Figure 15 is a bar chart showing the fungal relative abundance at a species level. This figure supports the data presented in figure 14b, as the clusters and outliers are reflected in the strong visual similarities between some samples and distinct differences in others seen in figure 15. There are at least nine fungal communities that are dominated by the genus *Zygosaccharomyces*, and four notably different community structures. This figure also reveals how small and relatively simple the fungal portion of the kombucha microbial community is in comparison to the bacterial, particularly those communities that are dominated by *Lachancea fermentati* or *Zygosaccharomyces lentus*. It also can be observed in this bar chart how varied each of the fungal communities are between different samples. There is no conserved core community presence as can be observed in the bacterial data. Many of the species listed in figure 15 have been found in a range of kombucha samples from around the world (Chakravorty et al. 2016; Coton et al. 2017; De Filippis et al. 2018; Marsh et al.

2014; Reva et al. 2015; Teoh, Heard & Cox 2004). With *Zygosaccharomyces* dominated fungal communities being common (Marsh et al. 2014). *L. fermentati* has also been reported as dominating the fungal community but only at day 7 of fermentation whereas the Pair 1a sample was collected at day 14 of fermentation (Chakravorty et al. 2016). Other species of note found in a small number of samples are *Brettanomyces/Dekkera bruxellensis* and *Botrytis caroliniana*, the former is both a spoilage microbe in wine and used extensively in Belgian beer, the latter is a grey mould disease often found on fruit (Li et al. 2012; Schifferdecker et al. 2014). There are a number of species present which have not been found in kombucha communities to date, *Malassezia restricta* for example can be seen in notable abundance in Pair 3b. *M. restricta* and *Malassezia sympodialis* are both lipophilic yeasts known to colonise the human skin surface (Sugita et al. 2004).

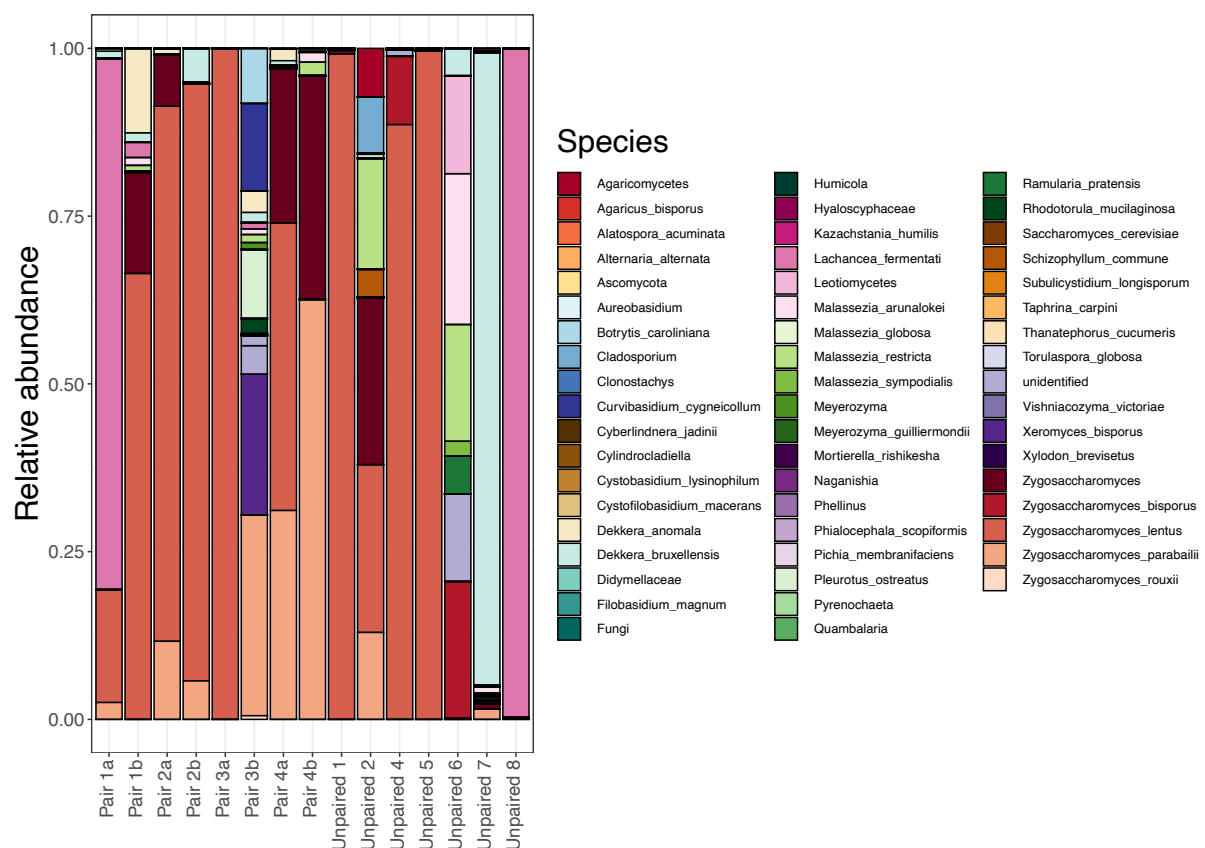


Figure 15 An exploratory bar chart visualising the relative abundance of the fungal species present in participants kombucha samples. Any singular names in the figure are indicative of higher taxonomic rank due to inability to be identified at a species level.

This chapter has presented and discussed the molecular microbiological results. It has demonstrated that, as hypothesised, a core bacterial microbiota is present across all kombucha samples. In fact, this core has a stronger presence than anticipated, demonstrated in both the relative abundance bar charts and functional analysis, which indicated that there were few pathways exclusive to one sample or another. Despite this, the findings also showed that there were unique rare taxa that set each sample apart from the others. The fungal results displayed notable similarity between some samples and diversity between others, with no core taxa present. These results present a complex image of the kombucha microbiota, including in terms of shared and diverse traits, with some expected and unexpected findings. The following concluding chapter aims to place these findings within the context of the proceeding ethnographic results, including by considering the particular relationships that can be seen to exist between the two sets of findings.

Chapter 7: Interdisciplinary findings, future directions, and conclusions

The previous three chapters examined both the ethnographic and molecular microbiological findings of this study; this chapter turns to what was found when these two sets of findings concerning kombucha and its human producers were combined. Firstly, this chapter will briefly revisit the ethnographic findings and molecular microbiological results in order to provide a basis from which the combined results can be understood. Following this, the results of the network analysis synthesising both the contextual detail gathered from the ethnographic data and the next-generation sequencing data will be discussed in relation to the overall aims of this research project. Finally, the limitations of the study along with possible directions for future research will be outlined, followed by a summary of overall conclusions.

In chapter four the preliminary and practical aspects of the ethnographic findings were discussed, drawing on both the survey and interview data. It was identified amongst the broader themes present in the data that women made up 82% of survey respondents, coming from a broad age range. It was also observed that the uptake of kombucha production amongst participants has increased, along with a growth in popularity of commercially produced kombucha products. In production, approximation and a blasé approach to quantities were consistent themes, particularly amongst experienced producers. Decisions about the types of tea and sugar that are added as part of the process of making kombucha depended on a range of variables, with the SCOBY's 'lineage' often taken into account, in addition to flavour and personal preference on the part of the brewer. Secondary ferments, what was added therein, or if a secondary ferment was conducted at all were likewise dependant on the producer's desires based on similar considerations of taste. It was common amongst those who had extant social networks with interests in fermentation not to engage with online communities, and only those who were alone in their interest sought out online groups to share knowledge, gain advice, or obtain a SCOBY. Overall, participants were exceedingly willing to share kombucha for free, particularly with their friends, unless they had a business based on selling kombucha products or teaching fermentation practices. When reflecting on the 'gifting' aspects of kombucha sociality, participants routinely provided instructions when sharing SCOBYs, suggesting

knowledge of kombucha production is not widespread. Thus, when people start on their home-made kombucha journeys they do so with degree of uncertainty and hesitancy. The hesitancy it may be surmised is at least partly due to the affective reactions the physical appearance and “feeling” SCOBYs provoke in people, in at least first and early encounters, particularly feelings abject disgust and revulsion, as explored in chapter 5. Finally, health and well-being comprised the most significant motivation behind the uptake of kombucha production, with general fermentation interest and enjoyment of the flavour being other central themes in the data.

Chapter five utilised participants’ phenomenologically embodied experiences of first encounters with the kombucha SCOBY to provide a basis from which to understand people’s historic and cultural milieu. This chapter was structured using the three intertwined analytic categories described in the introduction; the phenomenologically experiencing *individual* body, the *social* body, and the body *politic*, doing so in conjunction with the theme of microbial entanglement (Flachs & Orkin 2019; Scheper-Hughes & Lock 1987). Throughout the chapter, participants’ experiences are described and analysed using symbolic anthropological perspectives, including the broadly structural approach to the analysis of cultural categories offered by Mary Douglas (1966) in her seminal work *Purity and Danger*. Here it is argued that important ideological and categorical shifts can be observed, when once abject jars of SCOBY are embraced in a domestic space, moving from being viewed negatively as objects of repulsion to being treated more positively and familiarly as objects of personal affection. This chapter argued that, in the process of embracing their SCOBY within domestic space, participants begin by reflecting before subsequently adjusting, even rejecting, pre-existing and more widespread social perceptions of microbial life. Similarly, in engaging with discourses associated with post-Pasteurianism and eco-dietetics and taking up the understandings of microbial life associated with these, participants formed their own hybridised system of relating to microbial communities, the globalised food system, western biomedicine and the environment.

The microbiological results in chapter six demonstrated that across all participants’ samples, the kombucha that they produced shared a strong and complex conserved bacterial core community. Despite the strength of this core or shared set of cores, it was nevertheless found that each kombucha comprised a unique population of rare

taxa that differentiated that particular SCOBY from that of any of the other samples. This was even the case for those paired samples which would have started off as the same community. These findings differ from the reported literature published thus far which suggest a much simpler microbial community present in kombucha SCOBY (Arikan et al. 2020; Chakravorty et al. 2016; Coton et al. 2017; De Filippis et al. 2018; Marsh et al. 2013; Reva et al. 2015; Villarreal-Soto et al. 2018). These results could, in my research, be either or both a result of the methods used and the social and environmental context of the samples. In the investigation into the bacterial data functional diversity, it was found that, overall, there were a large number of shared pathways between communities, including a range of fermentation pathways that take sugars like glucose, fructose, and sucrose and convert them into cellular energy, creating a range of by-products. In this analysis it was also noted that Hamish's kombucha had numerous unique pathways that could be attributed to idiosyncratic environmental factors and production practices present in his case. In contrast to the bacterial data set, the fungal community results demonstrated that there was no conserved community core, and a notable similarity between some samples and diversity between others. All fungal samples had a narrower range of taxa, similar to previous findings with a few exceptions (Chakravorty et al. 2016; Coton et al. 2017; De Filippis et al. 2018; Marsh et al. 2013; Reva et al. 2015; Teoh, Heard & Cox 2004).

Interdisciplinary findings

The understanding of ideologically grounded kombucha production summarised in this chapter thus far contextualises the microbial community data resulting from the participant kombucha samples. In addition to the ethnographic data and analysis concerning participants changing relationships to their kombucha and indeed the kombucha producing community, where possible more specific information regarding methods and brewing environments was collected during the interview process to facilitate co-ordination and the capture of data across a range of biosocially entangled scales. Examples of this kind of information included, how much tea and sugar were used in production, how long a batch of kombucha was fermented for, and whether participants routinely used utensils or hands to interact with their SCOBY. This co-ordinated information was collated and used in combination with the microbial community data to perform network analyses to visualise the relationship between the kombucha SCOBYs and the contextual data.

The overall results of the network analysis indicated that no direct correlation between the ethnographic contextual and microbiological data could be found. All the variables that had been gathered were examined against both the bacterial and fungal community data. An example can be seen in figure 16, in which the whole bacterial community and paired participant information were visualised using Jaccard dissimilarity distances. Discernible patterns or significant line weight differences in this figure would indicate a correlative relationship, however there are none. When considered with the previous molecular microbiological findings it appears that if a kombucha SCOBY moves from one person to another it maintains a conserved core of bacteria. However, despite this strong core there were rare bacterial taxa present in each kombucha sample unique to the new social and biological environment. There is no relationship that could be found between the core or rare taxa and the methods used to continue the kombucha production. The fungal data demonstrates distinct differences between participants' kombucha samples however, and it does not appear related to any of the network variables either.

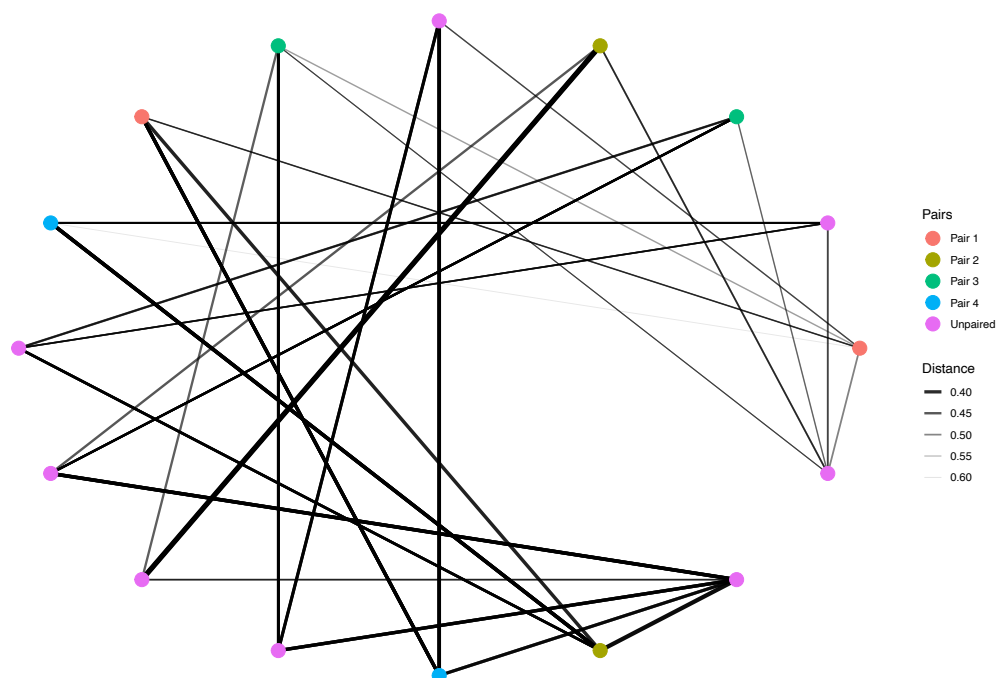


Figure 16 Network analysis visualisation of the whole sample bacterial community and paired variable, with line and line weight representing Jaccard dissimilarity distance values.

Although no direct correlation using the network analysis could be found, this does not denote a failure in the incorporation of ethnographic along with microbiological methods and data. In presenting the findings of this research, aspects of the relationships forming across biosocially entangled scales present in this research have been discussed in this and preceding chapters. Through the consciously co-ordinated data collection in this project, it has been established that the relational and interdependent microbial entanglement of kombucha and its producers is complex. This entangled relationship can be clearly seen through the intricate ways both participants and kombucha moved back and forth through the individual body, the social body and body politic, implicating everything from the phenomenologically experiencing human body to the SCOBY as a symbol of health and well-being, signifying the potentialities of human-microbial collaboration. When these outcomes and complexities are considered together it suggests a significant need for further research. Future research could explore these intertwined relationships in more depth, providing the opportunity to employ other methods of analysis beyond the scope of this exploration into the biosocial phenomenon of kombucha and its human producers.

In more practical terms, future research in this area could start by using a different method of interdisciplinary analysis. Network analysis in this setting was limited to comparing one variable at a time. As an alternative it would be advantageous to be able to explore more complex combinations of multiple variables. Other microbiological experiments could include the investigation of the kombucha microbial community in a closed system over time, doing so to understand the impact of founder effect, changes in community composition, and other factors that might arise such as the impact environmental microorganisms on the SCOBY. Such an experiment was originally conceived as part of this project, however due to technical issues and the impacts of COVID-19 it was unfortunately abandoned. Further ethnographic and experiential work with participants could also be conducted to explore other themes that arose in interviews, and that were not addressed in this thesis. These include engagement by participants with a range of alternative food communities, relationships with and production of different fermented foods, and what place workshops and other educational offerings have in the kombucha and fermentation communities. Other ideas for further research include active community engagement and collaboration with the project in a citizen science capacity, with SCOBYs being

swapped and samples taken by participants over time for example. Additionally, engaging with participants more fully throughout the process of analysing and examining the scientific lab results, as exemplified in the case of Hamish. For example, it would have been of interest to observe Hamish and other participants' responses to the data produced from his SCOBY which contained bacteria capable of degrading toxic petrochemical by-products, suggesting the presence of such contaminants despite he and his SCOBY residing in a semi-rural setting. Thus, considering the fractal perspective offered by Roberts and Sanz (2018), and the analytic approach to understanding the embodied, social and symbolic relation of people to SCOBYs. There may well have been an affective response of abjection and "matter out of place" directed towards the kombucha at a microbial and chemical level, as well as that of individual objects sitting in jars on kitchen benches.

Conclusion

This thesis and its research have taken the unusual and it is hoped ground-breaking step of combining the more often widely separated disciplinary technologies and approaches of anthropology and microbiology. Before this thesis concludes, I would like to take this opportunity to reflect briefly on the process and experience of undertaking this particular interdisciplinary research approach. While a fully rounded reflection on the experience of undertaking this project is beyond the scope of these concluding remarks, some acknowledgement may and perhaps ought to be made of the challenges of interdisciplinary research within university institutions, particularly as these will be important for conducting this kind of research into the future.

There were many difficult experiences related to this project that fell outside of the usual stresses and challenges of research, stemming in part from the question of how to approach this research epistemically, theoretically, and methodologically. More significantly in many ways was the evident fact that, although there is an accepted need for this kind of research, the necessity is seldom recognised in an official capacity and as such not supported or often undertaken. This lack of recognition and support leads to additional work on the part of researchers wishing to undertake such research, including in this case student researchers. This includes, for example, the experience of navigating bureaucratic struggles for official acknowledgement in more than one department, discipline area, or school (for example), each of which comes with its own

idiosyncratic set of norms and expectations of practice. These and the other challenges offered by interdisciplinary research were not insurmountable in my experience, however working across such disparate disciplinary areas while in many respects rewarding did involve added layers of administrative hurdles and other burdens. It would be my hope that, though embracing and supporting the importance of this kind of research in the future, such hurdles and burdens would be mitigated, leading to the encouragement of further research and its successful completion.

In consideration of these challenges faced, including the added labour of navigating and employing two very different disciplinary expectations and conventions of language from microbiology and anthropology, not to mention scientific and technical terminology and jargon, a conscious choice was made to not smooth these over unduly. Indeed, to do so would potentially have diluted the scholarly efficacy and authority of both disciplinary approaches. As a result, this thesis has explored the relationship between the kombucha SCOBY and its human producers in a domestic setting, doing so through a radically interdisciplinary lens. It has developed and implemented an innovative interdisciplinary methodological framework and mixed method approach, combining quantitative molecular techniques from microbiology and qualitative ethnographic techniques from anthropology to explore the shared biosocial space of kombucha and its producers, yielding results that were both expected and unexpected. As made clear in the ethnographic findings, there is an elaborate, microbially entangled relationship between kombucha and its producers that is observable analytically across a range of scales. This includes being intertwined between the *individual* body, the *social* body and the body *politic*, as observed in the way research participants' categorisation of their SCOBYs adjusted to reflect broader contexts and the shifting social significance of microorganisms, while the microbiopolitics of kombucha reflects discursive concerns about health, well-being, food production, and the environment.

The microbiological results revealed rich bacterial communities and a relatively simpler but highly variable fungal communities in the kombucha SCOBYs. In all samples there was a strong core bacterial community, as well as unique rare taxa across the different environments. Together this suggests further research is required for understanding the complexities of these microbial communities in relation to the

social and environmental conditions of their production. Within the limits of the network analysis undertaken there were no direct links between the ethnographic contextual and environmental variables and the kombucha microbial communities. Despite this, these findings suggest that more valuable research could be achieved in this interdisciplinary space. In successfully conducting this research, collecting and analysing both kinds of data, and demonstrating the extent to which domestic production of kombucha in Australia is a more complex and changing biosocial phenomenon than it may first appear, it is hoped that this project has contributed constructive groundwork towards future collaborations between microbiology and anthropology.

Appendices

Appendix One: Further ethics, recruitment, and ethnographic details

Due to this research involving human participants in the ethnographic portion and the need to obtain samples of food they produce, approval from a university ethics committee was required. This was sought from the low risk Arts, Commerce, and Social Sciences Human Ethics Sub-committee. Ethics was obtained and its approval number is: HEC19138.

A survey created using Qualtrics was the primary means of recruitment as it was hoped to create a 'snowball' effect with sharing and posting. It was distributed primarily on Facebook through general feed sharing and posted on the "Melbourne & Surrounds Fermentation Starter Culture Swap" and "Preserving & Fermenting Australia" groups. The survey was also distributed throughout the Physiology, Anatomy, and Microbiology, and Ecology, Environment, and Evolution departmental emailing lists.

In order to obtain paired participant SCOBY samples for analysis a range of participants were asked if anyone they had shared kombucha with might be interested in participation; with the caveat of only if they felt comfortable reaching out to them. 5 of 16 participants were recruited this way enabling paired samples to be analysed.

All participant data collected, coded, and used in this thesis is de-identified and/or pseudonyms are used.

Appendix Two: Survey and interview schedules

Survey schedule

Section 1 – Demographics

Please select your gender:

- Female
- Male
- Non-binary
- Prefer not to say

Please choose the appropriate age range:

- 18 – 24
- 25 – 29
- 30 – 34
- 35 – 39
- 40 – 44
- 45 – 49
- 50 – 54
- 55 – 59
- 60 – 64
- 65 – 69
- 70 – 74
- 75 – 79
- 80+

Please select the area you live in:

- Melbourne Metropolitan Area
- Western Victoria
- Northern Victoria
- Eastern Victoria

Section 2 – Kombucha – General questions

Do you make kombucha?

- Yes
- No

If no, do you make any variants of kombucha like Jun (green tea and honey)?

- Yes
- No*

*If no they will exit the survey with no other questions asked. Only up to about 2 - 3 minutes of time spent at this point.

If yes, where did you receive your starter/SCOBY from:

- Friend or acquaintance
- Family
- Exchanged or gifted from someone in an interest group
- Bought it from a business that sells it
- Other*

*If other there will be a text box for what other method of exchange was used

Approximately how long have you been making kombucha for:

- < than 6 months
- 1 year
- 2-4 years
- 5 + years
- 10+ years
- 20+ years
- > than 30 years

Why do you make and/or consume kombucha? (can choose as many options as are applicable)

- I like the flavour
- It is a novelty
- I have a general interest in fermented foods/beverage
- For the health benefits
- It is a part of my cultural heritage/tradition
- For sale in small business/artisanal production

Where/how did you learn to make kombucha? (can choose as many options as are applicable)

- The internet (website, youtube etc.)
- From the person who gave you your starter/SCOBY
- Attended a class on fermented foods
- Instructions that came with your starter/SCOBY (if purchased)
- Other*

*If other, there will be a text box to describe where/how they learnt

Do you give/share/sell your kombucha starters/SCOBYs?

- Yes*
- No**

*If yes, a text box will appear. Asking: Who do you give/share/sell your starter/SCOBY with, and do you teach or instruct them in production practices?

**If no, a text box will appear. Asking: Why?

Section 3 – Further information

Please describe, in as much detail as you would like to give, why you chose to start making and/or consuming kombucha:

- Open text box

Please describe, in as much detail as you can, the process/method you use to make your kombucha:

- Open text box

Please describe, in as much detail as you can, what you understand of the microbes that live in the kombucha you produce:

- Open text box

If you have anything else you would like to mention about the kombucha you make or want to provide further context to any of your previous answers, please do so below:

- Open text box

Section 4 – Contact details

Thank you for participating in this survey. The responses recorded here will remain anonymous to ensure you will not be identified in any resulting publications. All data collected in this study will be stored in a secure location, however you may request a summary of the published results by contacting Jacquelyn Evans, Dr Ashley Franks, or Dr John Taylor on the contact details provided below.

As a part of this study we would also like to take further interviews and gather samples of kombucha for microbiological analysis. This is being done in order to gain further insight into the impact sharing and production practices are potentially having on the microbial communities present in the kombucha.

If you live in Melbourne, or rurally within a few hours' drive of Melbourne and would be happy to be contacted for further information and/or interview and/or provide a kombucha sample, please leave your details below and we will contact you shortly. Otherwise, please contact Jacquelyn Evans on the details provided below for any questions or expressions of interest.

First name:

Email address:

And/or contact number:

We would like to remind you that participation in the interview stage of this study is also voluntary. Your responses in the interview will remain anonymous if you wish them to be, ensuring you will not be identified in any publication utilising this data. Just as the survey, the interview data will be securely kept, and results of the study can be provided on request.

Finally, if you have any further questions about this project please contact Jacquelyn Evans at 17992187@students.latrobe.edu.au. Any questions Jacquelyn cannot answer, or complaints about your treatment in this study, please contact Dr Ashley Franks, Associate Professor, Department of Microbiology, by email at a.franks@latrobe.edu.au. Or John Taylor, Senior Lecturer, Department of Social Inquiry, by email at john.taylor@latrobe.edu.au.

Semi-structured interview schedule

Please note: Not all the questions listed in this document may be asked or will be in an altered manner depending on responses given to previous questions. These questions may also be altered due to the information provided by the participant in their survey.

- Can you please remind me how long you have been making kombucha for and where or who you got your SCOBY/starter from?
 - o If it is family or friends etc where they got their starter from, ask at the end if their friends/family/etc are not already involved would they be interested in being a part of the study
- Can you give me more detail about what inspired you to start making kombucha, and why you continue to produce it?
- Can you please describe for me the relationship feel you might have with your kombucha as a living thing?
 - o Further prompts or questions: Have you named your kombucha? Can you please give me more detail on how you understand the microbes living in your kombucha?
- We asked in the survey where you learnt to make kombucha, could you please explain in more depth where/how you learnt to make it?
 - o Note any distinct features of method, and ask for details on the reasoning for things being done this way
- Can you please remind me what your method of production is? And do you make any variants on the black tea and sugar kombucha?
 - o Depending on their answer, prompt about kind of vessel, cleaning/sterilising practices, where does the kombucha sit while fermenting etc.
- Have you noticed any changes to taste, appearance or other features of your kombucha since you received it? Why do you think it has changed?
 - o If they have been making a long time might not remember, but might also have other observations of change

- Can you please tell me more about who you have shared your kombucha with?
 - o Further prompts and questions depending on answers: Do you teach them how to make kombucha? Or direct them to particular information sources? Are you part of an interest group that shares kombucha and what are normal the sharing practices in this group?
- Is there anything further you would like to tell me, or think we've missed anything you think is important about kombucha?

Appendix Three: Qiagen DNeasy® Powersoil kit protocol

Quick-Start Protocol

June 2016

DNeasy® PowerSoil® Kit

The DNeasy PowerSoil Kit can be stored at room temperature (15–25°C) until the expiry date printed on the box label.

Further information

- Safety Data Sheets: www.qiagen.com/safety
- Technical assistance: support.qiagen.com

Notes before starting

- Perform all centrifugation steps at room temperature (15–25°C).
- If Solution C1 has precipitated, heat at 60°C until precipitate dissolves.
- 2 ml collection tubes are provided.

1. Add 0.25 g of soil sample to the PowerBead Tube provided. Gently vortex to mix.
2. Add 60 µl of Solution C1 and invert several times or vortex briefly.

Note: Solution C1 may be added to the PowerBead tube before adding soil sample

3. Secure PowerBead Tubes horizontally using a Vortex Adapter tube holder (cat. no. 13000-V1-24).

4. Vortex at maximum speed for 10 min.

Note: If using the 24-place Vortex Adapter for more than 12 preps, increase the vortex time by 5–10 min.

5. Centrifuge tubes at 10,000 x g for 30 s.
6. Transfer the supernatant to a clean 2 ml collection tube.

Note: Expect between 400–500 µl of supernatant. Supernatant may still contain some soil particles.

7. Add 250 µl of Solution C2 and vortex for 5 s. Incubate at 2–8°C for 5 min.

— Sample to Insight —



Note: You can skip the 5 min incubation. However, if you have already validated the DNeasy PowerSoil extractions with this incubation we recommend you retain the step.

8. Centrifuge the tubes for 1 min at 10,000 x g.
9. Avoiding the pellet, transfer up to 600 µl of supernatant to a clean 2 ml collection tube.
10. Add 200 µl of Solution C3 and vortex briefly. Incubate at 2–8°C for 5 min.

Note: You can skip the 5 min incubation. However, if you have already validated the PowerSoil extractions with this incubation we recommend you retain the step.

11. Centrifuge the tubes for 1 min at 10,000 x g.
12. Avoiding the pellet, transfer up to 750 µl of supernatant to a clean 2 ml collection tube.
13. Shake to mix Solution C4 and add 1200 µl to the supernatant. Vortex for 5 s.
14. Load 675 µl onto an MB Spin Column and centrifuge at 10,000 x g for 1 min. Discard flow through.
15. Repeat step 14 twice, until all of the sample has been processed.
16. Add 500 µl of Solution C5. Centrifuge for 30 s at 10,000 x g.
17. Discard the flow through. Centrifuge again for 1 min at 10,000 x g.
18. Carefully place the MB Spin Column into a clean 2 ml collection tube. Avoid splashing any Solution C5 onto the column.
19. Add 100 µl of Solution C6 to the center of the white filter membrane. Alternatively, you can use sterile DNA-Free PCR Grade Water for this step (cat. no. 17000–10).
20. Centrifuge at room temperature for 30 s at 10,000 x g. Discard the MB Spin Column.

The DNA is now ready for downstream applications.

Note: Solution C6 is 10 mM Tris-HCl, pH 8.5. We recommend storing DNA frozen (–20° to –80°C) as Solution C6 does not contain EDTA. To concentrate DNA see the Hints & Troubleshooting Guide.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. Trademarks: QIAGEN®, Sample to Insight®, DNeasy®, PowerSoil® (QIAGEN Group). 1103425 06/2016 HB-2179-001 © 2016 QIAGEN, all rights reserved.

Ordering www.qiagen.com/contact | Technical Support support.qiagen.com | Website www.qiagen.com

Appendix Four: Detailed description of bioinformatic Methods

All steps in the bioinformatic pipeline were conducted in the program QIIME2 (Bolyen et al. 2019). The forward and reverse reads were merged, and the primers trimmed using *cutadapt* for both the bacterial and fungal sequences. Denoising and subsequent trimming of low-quality sequences was completed utilising the program DADA2 (Callahan et al. 2016). Bacterial sequences were taxonomically assigned using a Native Bayes classifier trained on the V4 16S rRNA region from the GreenGenes reference database version 13.8 (<https://greengenes.secondgenome.com>). The fungal sequences were taxonomically assigned using the full ITS region from the UNITE version 8 dynamic classified representative sequence set (<https://unite.ut.ee/repository.php>). The QIIME2 website (<http://view.qiime2.org>) was then used to create an ESV table for the bacterial and fungal data from the imported taxonomy-assigned sequences which specifies the number of reads (sequences) associated with unique ESVs within each sample. PICRUSt2 was used to assign pathway abundances based on the MetaCyc database to the bacterial data set.

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