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Inoculation, Growth and Bactericidal Effects of Three Kombucha Cultures

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Abstract: Kombucha, a domesticated consortium of several microorganisms grown on sugared tea, has been valued as a nutritive health aid for over a millennium. In this study, three cultures of kombucha were obtained from diverse sources. Different inoculation methods were compared, and the wet and dry weights of the nascent pellicles were measured when cultured on several carbon sources. In addition, the anti-bacterial properties of the fermented kombucha teas were tested against *Escherichia coli* and *Staphylococcus epidermis*. Inoculation with macerated pellicles gave the fastest kombucha growth. The best carbon sources for growth of the nascent kombucha pellicles were sucrose, glucose, and fructose. On maltose, galactose, and lactose, not only did the kombucha pellicles grow poorly but 25% were also contaminated by common airborne molds. Good growth of the kombucha cultures was correlated with low pH of the fermented tea. Antibacterial effects of concentrated fermented teas and vinegar were similar to those of 1 mmol ampicillin against *Escherichia coli* or 0.01 mmol penicillin against *Staphylococcus epidermis*. When the pH of concentrated kombucha teas was neutralized, their bactericidal effects were no better than unfermented controls.

Keywords: kombucha; microbial consortium; fermented tea; SCOBY; functional beverage

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1. Introduction

Kombucha is a popular functional beverage with many purported health-promoting properties. It is made by inoculating sugared tea with a microbial consortium of bacteria and yeasts growing together in a polysaccharide matrix [1]. In the lay literature, the kombucha inoculum is often called a "SCOBY" (Symbiotic Culture of Bacteria and Yeasts) or a "mother culture". During the one-to-two-week fermentation, the SCOBY is visible as a gelatinous film or pellicle floating on the surface of the sweetened fluid. This nascent pellicle is often called a "baby" culture and superficially resembles a jelly-like fungus. Many of the common names for kombucha are derived from the appearance of the culture, e.g., "tea fungus", "Manchurian mushroom," "Russian jelly", and "gout jelly" [2,3]. Sometimes the pellicle is applied externally as a poultice for wounds or as a cosmetic treatment for aging skin. Several South American groups have patented a "Bioskin" for the treatment of burns and other skin lesions [4]. Most commonly, however, home brewers merely use the nascent pellicle as an inoculum for preparing their own kombucha beverage.

The taste of the fermented tea has been compared to wine, champagne, cider, and vinegar. Kombucha is categorized as a functional beverage, i.e., a drink that promises health benefits beyond any inherent nutritional value. Purported benefits of kombucha

tea include anti-inflammatory [5] and antioxidant properties [6], leading to reduction in cardiovascular disease such as hypercholesterolemia and hypertension [7] reduction in cancer progression [1]; as well as improved gastrointestinal functions and invigoration of liver function [8,9]. Antimicrobial properties are also widely reported. In published studies, the antibacterial properties of the fermented teas have been attributed to organic acids, particularly acetic acid (low pH), as well as large proteins and catechins [1,10] Unfermented teas also have demonstrated antibacterial properties [11,12].

Several laboratories have analyzed the chemical constituents of kombucha teas, and the main metabolites reported were acetic acid, ethanol, gluconic acid, glucuronic acid, and lactic acid, as well as citric, malic, malonic, tartaric, oxalic, succinic, and pyruvic acids. In addition, sugars such as sucrose, glucose, and fructose were present [1,2] reported usnic acid and hypothesized that the antibacterial properties of the fermented tea were due to this metabolite.

The microbial constituents of kombucha vary between culture sources and media on which cultures are grown. *Acetobacter xylinum* and yeasts in the genus *Saccharomyces* are the most reported isolates identified from traditional culture methods. Other bacteria isolated from different kombucha consortia include *Acetobacter aceti*, *Acetobacter pasteurianus*, and *Pediococcus* spp. *Gluconobacter oxydans* [9,13] (Note: *Acetobacter* is also called *Gluconacebacter* and more recently *Komagataeibacter* [14]). The yeasts found in kombucha also vary and include *Brettanomyces* sp., *Candida* sp., *Kloeckera/Hanseniaspora* sp., *Kluyveromyces* sp., *Pichia* sp., *Saccharomyces* sp., *Saccharomycoides* sp., *Schizosaccharomyces* sp., *Torulopsis* sp., [8,15,16], and *Pichia* sp. [9,17]. Kombucha cultures grown on different teas showed similar bacterial components but different yeast communities [18]. Interestingly, samples fermented at 30 °C (instead of cooler temperatures like 20 °C) have shown higher diversity of microbes including sub-dominant bacterial populations (e.g., *Acinetobacter*, *Propionibacterium*) and lactic acid bacteria (*Lactobacillus*, *Streptococcus*) [19] The microbial load of kombucha teas, as well as the biomass of the resultant cultures, vary according to the type of tea used, with black teas having larger baby cultures than green and herbal teas [20].

In this study, the "SCOBY" (the gelatinous mass consisting of bacteria and yeasts embedded in a polysaccharide matrix) will be called the "pellicle" or the "kombucha culture" and the tea on which it is grown will be referred to as the "fermented tea" or the "kombucha tea". We obtained three different kombucha cultures and compared their growth and pH on different sugared teas under controlled laboratory conditions. In addition, we studied different inoculation techniques. Finally, we tested the bactericidal effects of filtered, unconcentrated, and concentrated kombucha teas against *Escherichia coli* and *Staphylococcus epidermis*.

2. Materials and Methods

2.1. Kombucha Cultures

Three independently obtained kombucha cultures were selected for their varied origins. Since home brewers obtain their SCOBYs from local kombucha aficionados or buy them commercially, we obtained two cultures from people we knew who kept their own kombucha cultures and purchased the third culture from a commercial supplier. Rather than stock numbers, the cultures were given names as is the custom among those who drink kombucha teas. "Fritz" was a gift from Mr. Fritz Owens of New Orleans, LA, "Toby" was a gift from Dr. Toby Feibelman of Frankfurt, Germany, and "Olinka" was purchased from Paper Ships, a holistic health store in San Anselmo, CA. Stock cultures of kombucha pellicles were stored in sealable plastic bags in plastic containers in a refrigerator at 5 °C.

The standard tea medium (STM) for maintaining stock cultures was prepared by bringing 500 mL dH_20 to a boil in a Fernbach flask, adding four Lipton brand tea bags (containing 2.5 g of black tea leaves), and steeping for 5 min. The tea bags were removed, 62 g of sucrose was added, the volume was brought up to 1000 mL with dH_20, and the tea–sucrose mixture was autoclaved. After cooling, a 7 +/− 1 g (wet weight) section of a previously grown kombucha pellicle (i.e., the SCOBY or "mother culture") was cut with

a sterile dissecting knife and placed onto the STM. The cultures were incubated without agitation, at 28 °C, away from direct light for ten days, at which time nascent pellicles had covered the surface of the fluid. Sections of these nascent pellicles were removed and became "mother" cultures in subsequent experiments comparing inoculation techniques and different carbon sources.

2.1.1. Comparison of Inoculation Techniques

In an experiment comparing inoculation by macerated or whole pellicles, 100 mL of the STM in 250 mL Erlenmeyer flasks was inoculated with a "Toby" mother pellicle of 4, 6, and 8 g, either as an intact section of pellicle or as a macerate. Macerated pellicles were prepared by blending the "mother" pellicle and 100 mL of STM in a sterile Waring blender on its highest setting for 1 min; the STM plus macerate was then returned to the flask [21]. All cultures were incubated at 28 °C away from direct light and without agitation for 15 days. Trials were run in triplicate.

2.1.2. Carbon Sources, pH, and Wet and Dry Weights

In experiments comparing carbon sources, 65 g/L of monosaccharides (fructose, galactose, glucose, and mannose) or 62 g/L of disaccharides (lactose and maltose) were substituted for sucrose in STM. One hundred ml aliquots of the various media were delivered into 250 mL Erlenmeyer flasks and autoclaved. A piece of a "mother" pellicle (7 +/− 0.1 g) grown on STM was cut with a sterile knife and placed into the cooled media. Tea without sugar and deionized water were included as controls. All cultures were incubated at 28 °C away from direct light and without agitation for 15 days. Fructose was run in triplicate; other trials were run in sextuplicate. Cultures visibly contaminated with molds were not included in pH or weight measurements.

Measurements of pH were made with a Corning pH meter 140, equilibrated with commercial buffers of pH 4, 7, and 10. The pH was recorded before inoculation, immediately after inoculation, and after 1, 3, 5, 7, 9, 11, 13, and 15 days of incubation. After the nascent kombucha pellicle had grown to form a layer, the electrode was inserted into the fermented tea medium underneath the pellicle and allowed to equilibrate.

Wet weights of the nascent pellicles were taken after 8 and 15 days of incubation. On Day 8, the nascent pellicle was separated from the original inoculum with sterile forceps and blotted on sterile paper towels, folding the paper towels over the pellicle four times, and then gently pressing. The wet pellicle was then weighed on a Mettler balance and returned to its fermented tea medium for another week. At 15 days, the wet nascent pellicle was again separated, blotted, and weighed. Then, these pellicles were warm air dried on a vegetable desiccator (Sigg Dorrex AGSIG Fraeunfel Type 1780) for 3–5 h, and dry weights were recorded. Averages were taken for both wet and dry weights, and a *t*-test was performed to determine significant differences.

2.1.3. Antibacterial Effects

The three kombucha strains were cultured on STM for 10 days, and the pellicles were discarded. The fermented tea was first filtered through Whatman filter paper no. 50 (7 cm diameter) using a vacuum filter apparatus and then re-filtered through a 22 μm Millipore filter. Ten ml aliquots of each tea were concentrated to 1 mL under partial vacuum in a rotary vacuuming device. Controls of sugared tea, unsugared tea, and a sucrose solution were also concentrated tenfold. In some experiments, the concentrates were neutralized using 1N NaOH.

Cultures of *Staphylococcus epidermis* (isolate used in Tulane University microbiology teaching lab) and *Escherichia coli* (strain DH 5 α, BRL Life Sciences) were obtained from Dr. David Mullin, Department of Cell and Molecular Biology, Tulane University. These strains represent one common Gram-positive bacterium (*S. epidermis*) and one common Gram-negative (*E. coli*) bacterium. Cultures were maintained on Luria broth (Difco) overnight at 37 °C and 150 rpm. Overnight cultures of *E. coli* and *S. epidermis* were then

combined with 45 mL autoclaved Luria agar (cooled to approximately 40 °C), mixed gently, and poured into sterile, disposable Petri plates.

Penicillin G (Fisher Scientific, Pittsburgh, USA) and ampicillin (Sigma-Aldrich, St. Louis, MO, USA)) were used as positive controls for *S.s epidermis* and *E. coli*, respectively. The antibiotic stock cultures were diluted were diluted in a series of tenfold dilutions from 0.1 mol/mL to 1 µmol/mL in sterile deionized water. Usnic acid (Sigma) was also tested at concentrations ranging from 1 mol/mL to 1 µmol/mo, made by dissolving the usnic acid in 22 µm filtered acetone. Sterile H_2O, vinegar (distilled grain, 5% acidity, Heinz, Pittsburgh, PA, USA), and filtered acetone were included as controls.

A hole was punched in the center of the solidified agar with a sterile 6 mm plug, creating a well. The wells were then filled with 200 µl of the concentrated teas, controls, and various dilutions of ampicillin, penicillin, and usnic acid. Plates were incubated overnight at 37 °C in the dark. Diameters of zones of bacterial growth were measured in two directions at right angles to one another for each plate. The two measurements were averaged, and 6 mm was subtracted (the diameter of the plug).

3. Results

Inoculation of Whole and Macerated Pellicles

The wet and dry weights of nascent pellicles obtained after using 4, 6, or 8 g of macerated or unmacerated "mother" cultures is given in Table 1. The weight of the original inoculum was not as important as the method for inoculation. At 8 days, the weights of pellicles obtained from macerated inocula were about double (12.4–18.4 g) those of pellicles from traditional unmacerated inocula (5.0–7.6 g). However, after 15 days of incubation, there were few significant differences between the wet weights of nascent cultures obtained using 4, 6, or 8 g inocula whether macerated or whole. There were no significant differences for the dry weights, which, after 15 days of cultivation, ranged from 800–970 mg for all cultures.

Table 1. Wet and dry weights of nascent pellicles after inoculation with 4, 6, or 8 g (wet weight) of macerated or whole Toby mother cultures.

Inoculum	Type	Weight (g)		
Weight		**8 days (wet)**	**15 days (wet)**	**15 days (dry)**
4 g	Whole	7.6 ± 2.1^{c}	20.3 ± 1.7^{abc}	0.95 ± 0.17^{f}
	Macerated	12.8 ± 3.5^{d}	22.0 ± 6.5^{ab}	0.97 ± 2.1^{f}
6 g	Whole	5.0 ± 1.2^{ef}	18.7 ± 3.2^{bc}	0.80 ± 0.14^{f}
	Macerated	17.3 ± 3.3^{c}	19.4 ± 2.8^{abc}	0.86 ± 0.14^{f}
8 g	Whole	6.7 ± 1.8^{c}	23.6 ± 4.9^{abc}	0.96 ± 0.21^{f}
	Macerated	18.4 ± 2.9^{c}	20.6 ± 1.6^{a}	0.93 ± 0.07^{f}

Trials followed by the same letter are not significantly different from one another.

The wet and dry weights of the nascent pellicles for the three kombucha cultures grown on different carbon source are given in Table 2. Despite the high standard deviations, definite differences could be detected between carbon sources. Sucrose yielded significantly larger pellicles than all other sugars. Glucose and fructose also supported good growth, with both giving significantly larger pellicles than galactose, lactose, maltose, and mannose. Mold contamination was the highest for cultures with the poorest growth. The "Toby" strain showed the least amount of contamination (10%) as compared to 17% for both "Fritz" and "Olinka". Surprisingly, the wet weights of the mannose, galactose, maltose, and lactose cultures were not significantly different from controls grown on an infusion of tea lacking sugar. Even on pure water, in which the only carbon source was that carried forward with the inoculum, a clear gelatinous mass covered the surface of the medium. The wet weights of these "pseudo-pellicles" ranged from 2.0–5.4 g; however, the corresponding dry weights were always less than 0.64 g.

Compared to "Olinka" and "Toby", "Fritz" culture yielded the overall highest average wet weights on all sugars, but this difference was not significant. Since kombucha consortia do not have 'canonical' microbial components, it is to be expected that consortia from different sources would vary. In summary, there were significant differences between the wet weights of pellicles grown on different media, but there were no significant differences in these weights between the three kombucha cultures on any given media.

Table 2. Wet and dry weights (g) of nascent pellicles of three kombucha strains grown on tea amended with seven different sugars after 8 or 15 days.

Carbon Source	Kombucha Consortium	Number of Contaminated Cultures [a]	Weight (g) [b]		
			8 days (wet)	15 days (wet)	15 days (dry)
Water control	Fritz	0	2.8 ± 1.8	3.9 ± 2.6	0.04 ± 0.01
	Toby	0	2.2 ± 1.0	5.4 ± 4.1	0.01 ± 0.01
	Olinka	0	2.0 ± 1.4	4.5 ± 2.7	0.02 ± 0.01
Tea control [ab]	Fritz	1	4.0 ± 0.8	5.7 ± 1.6	0.08 ± 0.02
	Toby	2	3.3 ± 0.9	6.9 ± 2.4	0.06 ± 0.03
	Olinka	2	2.7 ± 1.3	5.3 ± 2.0	0.07 ± 0.03
Mannose [ab]	Fritz	1	2.6 ± 0.4	6.8 ± 2.9	0.3 ± 0.07
	Toby	1	2.8 ± 1.3	7.3 ± 3.1	0.3 ± 0.08
	Olinka	0	2.9 ± 1.4	8.0 ± 1.2	0.25 ± 0.14
Galactose [b]	Fritz	1	5.1 ± 1.7	±2.9	0.59 ± 0.11
	Toby	0	4.0 ± 0.9	7.0 ± 1.8	0.19 ± 0.14
	Olinka	3	6.3 ± 3.6	8.3 ± 2.9	0.47 ± 0.18
Glucose [c]	Fritz	1	7.1 ± 2.1	19.9 ± 4.6	0.64 ± 0.33
	Toby	0	3.6 ± 1.2	13.4 ± 6.2	0.63 ± 0.30
	Olinka	0	5.8 ± 4.9	8.5 ± 7.6	0.38 ± 0.27
Fructose [c]	Fritz	0	5.0 ± 1.3	13.4 ± 1.7	0.53 ± 0.35
	Toby	0	6.8 ± 0.8	9.2 ± 1.0	0.40 ± 0.10
	Olinka	0	8.4 ± 3.8	15.2 ± 5.3	0.37 ± 0.06
Maltose [b]	Fritz	2	7.1 ± 0.9	9.0 ± 3.8	0.55 ± 0.19
	Toby	2	4.1 ± 0.57	7.1 ± 4.0	0.45 ± 0.21
	Olinka	4	5.9 ± 4.1	6.2 ± 4.2	0.26 ± 0.08
Lactose [b]	Fritz	2	5.5 ± 1.6	6.0 ± 4.9	0.40 ± 0.17
	Toby	1	6.3 ± 1.8	8.9 ± 1.0	0.64 ± 0.15
	Olinka	1	4.6 ± 2.0	6.4 ± 2.1	0.34 ± 0.17
Sucrose [d]	Fritz	1	13.0 ± 4.0	23.1 ± 8.4	0.84 ± 0.33
	Toby	0	6.7 ± 1.0	15.5 ± 6.0	0.73 ± 0.08
	Olinka	0	9.5 ± 6.2	15.9 ± 8.9	0.53 ± 0.24

[1] Kombucha pellicles with macroscopically visible mold growth on the surface were scored as contaminated.
[2] Media followed by the same letter are not significantly different from one another. Except for fructose, all strains were grown in sextuplicate. Strains grown on fructose were in triplicate.

The pH of the cultures on all the media, for the three different kombucha consortia, was measured every other day over the course of the 15-day incubation (data not shown). Teas without added sugar had an initial pH of 4.5. The pH of the teas with added sugar ranged from 3.2 for galactose, glucose, and fructose to 5.1 for sucrose. Almost immediately upon inoculation, the pH dropped for all teas to 3.2 (+/− 0.1). Over the course of the next 11 days, there was little change in pH for cultures on tea alone, galactose, lactose, and maltose, while cultures on fructose, glucose, mannose, and sucrose continued to become acidic. Around the eleventh day, cultures on tea alone, galactose, lactose, and maltose showed a slight increase in pH (3.3 to 4.0) while other cultures continued to decline to values between approximate 1.7 and 2. In general, those cultures with the best growth and lowest rates of contamination had fermented teas with the lowest pH's.

The inhibition of bacterial growth was tested for unconcentrated and concentrated teas grown on STM for 10 days. For the *S. epidermis* strain tested, none of the unconcentrated teas showed any inhibition of bacterial growth. For *E. coli* strain DH 5 α, unconcentrated teas

showed similar inhibition to controls of tea + sucrose or of tea only. The bactericidal effects of concentrated "Fritz", "Toby", and "Olinka" teas are shown in Table 3. For concentrated teas, the antibacterial effect was greatest for the "Toby" strain against *E. coli* strain DH 5 α. In general, both fermented and unfermented teas showed greater activity against *E. coli* strain DH 5 α than *S. epidermis*. When the pH of the kombucha teas was neutralized, they continued to exhibit bactericidal effects, especially against *E. coli* strain DH 5 α; however, this effect was no greater than that exhibited by unfermented tea + sucrose controls. Usnic acid was more active against *S. epidermis* than *E. coli* strain DH 5 α (see Table 3).

Table 3. Inhibition of *Staphylococcus epidermis* and *Escherichia coli* DH 5 α by concentrated kombucha tea ferments and controls.

Substance Tested	Concentration	pH Neutralized	*Staphylococcus epidermis*	*Escherichia coli* DH 5 α
"Fritz"				
(10 day)	10x	No	++	+++
	10x	Yes	+	++
"Toby"				
(10 day)	10x	No	++	++++
	10x	Yes	+	++
"Olinka"				
(10 day)	10x	No	++	++
	10x	Yes	-	++
Tea	10x	No	-	++
	10x	Yes	+	+
Sucrose	10x	no	-	-
	10x	yes	+	-
Tea + sucrose	10x	no	+	++
(=STM)	10x	yes	+	++
Usnic acid	10^{-1}	-	++	++
	10^{-2}	-	+++	+
	10^{-3}–10^{-4}	-	++	+
Acetone	1	-	+	-
Vinegar	1	-	++	++
Water	1	-	-	-

++++ >20 mm inhibition (=Penicillin 0.1–0.01 mol/mL against *S. epidermis* or Ampicillin 0.1–0.01 mL/m against *E. coli*); +++ = ≥20 mm–>15 mm (=Penicillin 0.1 mmol/mL against *S. epidermis* or Ampicillin 0.1 mlmol/mL against *E. coli*); ++ = ≥15 mm–>10 mm (=Penicillin 0.01 mmol/mL against *S. epidermis* or Ampicillin 0.01 mmol/mL against *E. coli*); + = ≥10 mm–>0 mm (=Penicillin 0.001 mmol/mL against *S. epidermis* or Ampicillin 0.001 mmol/mL against *E. coli*); - = no zone of inhibition.

4. Discussion

Macerated cultures showed significantly larger pellicles after one week on STM than did cultures that were inoculated with whole pieces, suggesting that macerated inocula provide a way of speeding up the kombucha fermentation. With increasing demand for commercial preparations in the functional beverage market, growers will have economic incentives for making the process more efficient. The traditional recipe almost always calls for an infusion of black tea made with sucrose (table sugar or saccharose). Many studies, like ours, have shown that this traditional method is sound and that sucrose is a better carbon source than other common sugars [1,17]. In our experiments, fructose and glucose also supported good growth, acidification, and little contamination. Other works have reported that kombucha cultures also grow well on sugar beet molasses where they are reported to produce more biomass as measured by a wet weight [22]. When kombucha has been cultivated on milk, where the main component sugar is the disaccharide lactose (glucose + galactose), kinetic studies showed good growth on glucose but not on galactose [23]. Our data show similar poorer growth on galactose.

Many home preparers of kombucha teas vary the recipe to change the flavor. Different research groups have worked on alternative raw materials such as fruit or vegetable juices,

herbal infusions, milk, and food industry by-products instead of tea, resulting in an end product with varied properties [24]. Even though the herbal teas contained the same amount of sugar, fermentation on black tea produced more lactic acid and gluconic acid. In their work on "Biofilm" production by *Acetobacter xylinum*, [25] compared infusions of black tea, coffee, cacao, cola nut, guarana, and "mate" and found the highest wet and dry weights were obtained for black tea.

In addition to changing the liquid component, kombucha drinkers have experimented with using alternative sweeteners in place of white sugar. The manual by Frank [2] instructs diet-conscious users that "sugar is not added to kombucha in order to make the beverage taste sweeter, but to form a good nutrient solution for the culture". Frank [2] also warns that artificial sweeteners such as saccharin and cyclamate will starve the culture. These admonitions made more sense to us after we had observed the size and wet weight of the "pseudo-pellicles" formed in our experiments on both water and unsweetened tea. Despite the absence of a sugar, these controls yielded a gel-like layer that covered the surface of the liquid. These "pseudo-pellicles" are almost entirely composed of water, with a wet weight to dry weight ratio of about 165:1, reflecting the enormous absorptive capacity of bacterial cellulose. Scientifically naïve individuals could easily mistake a "pseudo-pellicle" for genuine growth of the culture.

Healthy kombucha consortia have a wet-to-dry ratio of approximately 30:1, yield strongly acidified teas, and are less prone to contamination. Since many common molds produce dangerous mycotoxins [26,27]), mold contamination can pose a genuine health risk. In our experiments, media were autoclaved, transfers were performed under a clean hood, and every attempt was made to maintain good sterile technique. Under these conditions, only 2 of the 45 cultures grown on sucrose, glucose, or fructose were contaminated (4%), while 23 of the 90 cultures grown on other media were contaminated (25%). Given that most kombucha teas are prepared in home kitchens without the benefit of sterility, the danger of mold contamination is real. This risk would increase when the culture is grown on too little sucrose or on an inappropriate carbon-source substitute. It is important to educate users not only about hygienic methods of culture propagation, but also about importance of properly "feeding" their kombucha consortia with traditional recipes that use "real" sugar (preferably sucrose) and "real" tea (=*Camellia sinensis)*, not an herbal substitute.

Kombucha teas are believed to provide varied advantages for human health [28]. Nevertheless, several metareviews of the published literature on kombucha have determined that its purported health benefits are not supported by well-designed, controlled clinical trials [9,17,29]. For example, the tea is widely claimed to have antibiotic effects [9]). Our experiments indicate that there is no evidence of bactericidal effects beyond the inhibition produced by vinegar (acetic acid) and unfermented tea controls. Both acetic acid and the phenols in tea are known to have antibacterial properties. Our data supports early studies by Hesseltine [30] who was unable to demonstrate antibiotic activity against *Agrobacterium tumifaciens*, and by Steinkraus et al. [31], who also had negative results against *Escherichia coli*, *Helicobacter pylori*, and *Staphylocccus aureus*, as well as a more recent studies by Velicanski et al. [32] who exhibited the same antibacterial activity for kombucha and acetic acid solutions, or Silva et al. [33], who suggested that the antibacterial effects were more related to the compounds present in tea infusions than to those produced during the kombucha fermentation process.

As the popularity of kombucha has gown and its commercial production increased, it has generally been assumed that kombucha teas will not hurt consumers. However, there are increasing concerns about the safety of some home and commercial kombucha teas [34,35] and our data indicate that contamination with potentially mycotoxigenic molds is a realistic possibility. It is reassuring that guidelines for safe processing have been published [36] and international regulatory agencies in Europe, Japan, and the USA are developing standardized regulations for functional food products of all kinds [37].

5. Conclusions

Kombucha has become a staple functional beverage in the health food industry and is often used as part of alternative medicine regimens. As it is likely that the consumption of kombucha will continue to grow, it is important for private consumers as well as commercial distributers of kombucha cultures and teas to be provided with accurate information about the proper "care and feeding" of this unique consortial life form, as well as its actual medicinal properties. This study adds to the growing body of literature on kombucha and highlights that macerated inocula may accelerate kombucha fermentation, that sucrose is the preferred carbon source to minimize contamination, that the absorptive capacity of bacterial cellulose can lead to the formation of "pseudo-pellicles", and that kombucha does not appear to have unique antibacterial properties. Finally, it should be pointed out that these laboratory-based findings may be helpful to experimental microbial ecologists who implement the kombucha consortium as a model system for studying multispecies cooperation and aspects of biofilm formation [13].

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