Hands On Biofilm! Utilising a public audience in a citizen science project to assess yield variability when culturing kombucha pellicle

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Abstract

The pellicle biofilm generated during the Kombucha tea fermentation process has, when dried, textile-like properties that may have real-life applications. However, pellicle yield can vary depending on inoculation and incubation conditions, which affects research investigations on the properties of the pellicle.

To generate data on variability to help define optimum pellicle growth conditions, as part of a public engagement event about biofilm, a citizen science activity was hosted whereby visitors to a science festival were invited to select incubation conditions and inoculate different media with liquid or solid (pellicle). More than 220 samples were inoculated (in excess of 1200 visitors mainly in family groups). The most popular incubation conditions were coconut water or tea medium, 30C/room temperature and liquid inoculum. The most productive/reproducible in terms of yield and variability were tea medium, 30C and liquid inoculum, which reflect some of the conditions most used in the domestic setting for kombucha culture.

The event provided both useful research data and generated public interest in a research area of which many will have been unaware. Interest in the results of the activity, available several weeks after the activity, was sustained using email contact and FlickR for dissemination of images and data.

Introduction

Kombucha, also known as tea fungus, or tea mushroom, is a symbiotic culture of bacteria and yeasts and has been used for thousands of years in the fermentation of drinks. Kombucha tea can be prepared at home by placing the fungus (or starter culture / 'mother' pellicle) in a pre-brewed, sweetened tea broth for several days to produce a 'pleasantly fruity sour-like sparkling flavour' (Jayabalan *et al.*, 2014). Pre-prepared Kombucha drinks are also commercially available in a variety of liquid formulations (Malbaša *et al.*, 2011, Coelho, 2020) such as lemon, pomegranate, and beer, and are sold based on their purported health benefits (Abaci *et al.*, 2022).

However, after an extended brewing period in drink preparation (approx. 4 weeks) a new Kombucha pellicle develops on the surface of the brewing liquid (referred to as a 'daughter' pellicle). When this pellicle is removed and dried, it is deemed to be similar in animal leather in aesthetic, and therefore has been investigated in a research context for its potential properties as an alternative textile or biomaterial for clothing (Wood, 2019, Wood *et al.*, 2022).

The importance of sourcing novel biomaterials should not be underestimated in the context of the textile and fashion industries, where there is increasing pressure to reduce overconsumption and waste. Many clothing items are made from non-biodegradable materials that cannot easily be disposed of; the Ellen Macarthur Foundation (2017) states that the equivalent of one rubbish truck of waste clothing is burned every second. Equally, unsustainable practice in 'natural' textile production (e.g. cotton and wool) has devastating impacts on the environment and there are concerns regarding animal welfare in the production of leather products (Wood, 2019). The fashion industry is seeking new ways in which to produce textiles. The term biomaterials in terms of the fashion industry is often linked to those that are marketed as 'plant based'. Whilst these products have been labelled 'vegetable' or 'vegan' leathers, many of these products still rely on a synthetic component to render them fit for purpose and therefore do not fully address the potential impacts on the environment. Kombucha based materials have also been labelled as a leather alternatives (or alt-leathers) as they have a similar aesthetic to animal skin once dried. However, untreated, Kombucha based alt-leather is highly hydrophilic rendering it unsuitable for most apparel applications in its natural state (Wood et al., 2023).

Research in this field to date consists of Kombucha 'fabric' usually grown in artisan or domestic settings, except for a few limited lab -based studies (Domskiene *et al.*, 2019, Provin *et al.*, 2021). Relatively little is known about the bulk reproducibility of the pellicle, and this information is necessary for research projects, particularly in textile manufacture, when reproducibility of yield and nature of pellicle is important. Research has demonstrated that across the literature a variety of different external factors affect variability, such as: culture medium, incubation temperature, incubation duration, mode of inoculum, depth of culture medium and surface area variability (Wood *et al.*, 2022). In addition, repeated subculture could affect the pellicle microbiome and therefore the properties of the resultant pellicle (Wood *et al.*, 2022). Thus there is a need to identify conditions that best create the most reproducible/reliable yield to enable more in depth research on the potential of the end textile in variety of uses.

Previous studies, such as those conducted by Lee (2023), Fernandes et al. (2019), Garcia et al. (2019), and Chan et al. (2018) have alluded to the concept of 'growing your own clothes at home', exploring the potential of using bacterial cellulose pellicles in applications such as jackets and shoes. These studies have used a Kombucha SCOBY in green or black tea as a starter to produce larger pellicle sheets to create garment shapes. They have also explored techniques common in the fashion industry such as stitching and shaping, alongside the addition of trims such as zippers, buttons, and ribs. The concept has captured the interest of some mainstream media outlets (Shaw, 2012) , with 'recipes' for growing being circulated publicly on sites such as Pinterest (Ross, 2023) and Materiom

(Aberg, 2023). Given this interest, the development of bacterial cellulose textiles provides an ideal topic to design and deliver citizen science.

Citizen science is a term that is used in relation to large scale public participation in research projects (Dickinson *et al.*, 2012). It is a term that is distinct from 'amateur science' in that whilst the participant does not need specific scientific knowledge (Land-Zandstra *et al.*, 2021), the project is set up by professional researchers with a clear methodology and specific aims in place, often with a view to creating a publishable set of data and findings. It is not a new concept, with the earliest examples noted as Europe-wide bird surveys in the eighteenth century or the creation of the Astronomical Society of the Pacific in 1889 (Dickinson *et al.*, 2012). Historically, citizen science projects have been part of ecological or environmental studies, but with the increase of the reach of the internet, these types of projects have gained popularity in a diverse spectrum of disciplines. Hands-on activities delivered in accessible locations such as museums offer an opportunity to engage the public in citizen science.

The aim of this work was to investigate the variability of kombucha pellicle yield using visitors to a public engagement event during Manchester Science Festival to select the incubation conditions that give the most reproducible yield, and to assess participant engagement in a long-term experiment.

Methods

The overall event was designed to raise awareness of the nature and existence of biofilm (Verran *et al.*, 2020), and of the importance of 'good germs' in fermented food and drink (Verran *et al.*, 2018) and in the human microbiome, and in the development of sustainable biotextiles (specifically kombucha) (Wood, 2019).

Structure and layout of the event

The citizen science component of the event was structured so that visitors were introduced first to biofilms in general, and then specifically to kombucha. The team delivering this information was primarily composed of five undergraduate and one postgraduate students, with three academic supervisors (JW, JR, JV). Two five litre Kilner jars had been inoculated five weeks previously and stored at room temperature so that the pellicle biofilms were visible for observation. Aliquots of the broth from these jars were extracted for use as liquid inoculum in the experiment. For the solid inoculum, the pellicle was cut into approx. 0.5cm^2 portions and stored in a covered petri dish until required. A poster board (fig 1) was used to help explain the aim of the experiment, whereby

participants could select, with guidance, inoculation and incubation variables for the growth of their own kombucha biofilm.

Citizen science experiment – variables and inoculation

These variables were: inoculum - pellicle or liquid; culture medium – coconut water, beer, sweetened black tea or the medium most used in the literature, H&S medium (Schramm & Hestrin, 1954); incubation temperature (30C or 'room temperature', measured at 22 °C +/- 2 °C). Three hundred 25mL Universal bottles were prepared in advance of the event, each containing 10mL of a sterile prepared culture medium. Seventy-five bottles were prepared for each medium.

Once these variables had been selected, the participants noted them on a postcard that had been issued on arrival. Each inoculated vessel was given a reference number (participant reference), as well as information on the selected variables. Participants were also invited to share their email addresses so that they could be contacted at the end of the experiment before results were to be presented (8 weeks later). The project was assessed and approved by the Manchester Metropolitan University Ethics Committee (application number 41586) which included the retention of personal data for purposes of contacting visitors after the event. Risk assessment was carried out with input from the host (museum) to assess not only the risk of the activities, but also the practicalities of delivering in an active Museum site. Liability insurance was provided by Manchester Metropolitan University.

Participants either carried out the inoculation process themselves (observed) or watched the delivery team carry out the inoculation process. Lids were screwed on tightly (because of subsequent transport), and at the end of the day were taken to the laboratory for incubation at the designated temperature. After five weeks, it was decided to extend the incubation for an additional three weeks because growth had been relatively slow. Lids were checked to ensure that they were sufficiently loose for aerobic conditions.

Citizen science experiment – Incubation and recording results

Thus, after eight weeks, pellicles were harvested. Photographs were taken of all incubation vessels; and approximate pellicle thickness was measured in mm using a ruler held alongside the bottle. Then, pellicles were decanted from the vessels, placed onto filter paper and weighed (g) both wet (immediately after removal) and dry (after 48 hours drying to constant weight, and were photographed again. Frequency charts were compiled for each incubation condition (to determine the most popular combinations), and then the conditions that yielded the highest dry quantity of pellicle were identified.

Images and data were posted on FlickR (https://www.flickr.com/photos/196712535@N06/) so that participants could see the results of the study. Emails were sent after the advertised date of data release (to see how many participants remembered the date), and the data were advertised on Twitter one week after that (to see what the general interest for the study might be).

Results and discussion

Overall participation

There were over 1200 visitors to the event overall, primarily in family groups. Subjective observations from the delivery team noted obvious excitement and engagement in the citizen science activity (fig 2). Family groups were keen to hear about the experiment and took care thinking about their incubation conditions. There were 221 bottles inoculated during the day from a total of 300 prepared. The most frequently selected medium was coconut (fig 3); the most popular inoculum was liquid; the most popular incubation temperature was 30°C.

Producing kombucha-derived pellicles

In terms of successfully obtaining pellicle (Fig 4), coconut and tea were most productive (Coconut = 28 and Tea = 27 pellicles grown). Tea was the medium most used to cultivate kombucha (Coelho, 2020); coconut water would likely provide a highly nutritious and complex medium that would facilitate growth. Pellicle was not apparent in all inoculated bottles (for example, in the tea medium, the solid inoculum yielded no biofilm). There was also evidence of contamination (cloudy culture medium) in some of the inoculated bottles: usually pellicle was absent in these samples.

When pellicles were dried, those grown in tea at both 30 °C and room temperature, using a liquid inoculum, produced the thickest and heaviest pellicles (fig 5). A liquid inoculum would enable cell dispersal (in comparison to a pellicle fragment inoculum), and potentially enhance growth. Interestingly, these data aligned well with the observed 'best'/most reliable incubation conditions as observed previously in our laboratories (tea broth, inoculated with liquid and incubated at 30 °C/room temperature (Wood *et al.*, 2023)) and might be useful for people who wish to grow kombucha at home, most often using a tea medium as per kombucha suppliers' recommendations (HappyKombucha.co.uk). As a drink (and therefore brewed for a limited amount of time, approximately 4 days) Kombucha is commercially available in a variety of liquid media, such as lemonade and passionfruit (e.g. HollandandBarrett.com), agreeing with our findings (and others) that a variety of media can support microbial activity and therefore subsequent pellicle growth. Kombucha beer is also commercially available (gunbrewery.co.uk). For commercially available beverages, the brewing process takes days rather than weeks, thus a pellicle is not observed in these

products. In the domestic setting no evidence could be found of use of any culture medium other than tea.

Reproducibility of pellicle prodution

In terms of reproducibility of yield and control of experimental variables, all of the culture media are complex (undefined) - but the process of making and inoculating the (most commonly used and most reliable) tea medium can at least be defined.

The information gained from this study helps us to speculate on the potential variability of pellicle production in different laboratories, using different volumes/surface area of medium iIt would have been useful to inoculate all unused bottles to provide more data, but this was not done).

In brief, with more than 200 'independent' inoculations, the highest yield was generated using the following conditions: liquid inoculum, 30 °C or room temperature incubation, tea medium. Little information is known regarding the effects of vessel volume and liquid surface area on yield.

Citizen science experiment

In terms of a citizen science experiment, it is not easy to retain participant interest in the experiment when the incubation period is so long, with no intermediate contact with participants. In a separate study, at a school science club, the experiment extended across a half term, thereby interfering with holidays and examination periods. It was not possible to carry out the pellicle harvesting with the students (vacation), although they were able to inoculate and examine the dried pellicles, as well produce posters on their work (Wood *et al.*, 2022). Thus the FlickR site was deemed to be an opportune means of continuing engagement with participants in this study. There was considerable work involved in observing, photographing, and weighing the pellicles, as well as posting images and information on the website (Fig 6). However, when results were released on FlickR, there were very few hits on the site (27). An email reminder (Supp. Fig 1) to 114 participants who provided contact information increased visits to the site significantly, with 4647 views (109 viewers), and with some images receiving more than 70 'hits' over the six-week window of study. Later, a Twitter post was made to flag the event overall, and during a subsequent three-week window, views rose to 5340 (122 individuals).

The interim contact clearly re-invigorated participant interest. The lengthy culture period required for biofilm generation does not sit easily with the provision of feedback to participants and evaluation of their engagement – unlike the culture of bacteria where data and images can be obtained in a few days, enabling rapid feedback (Verran *et al.*, 2020).The use of FlickR provided a

useful record of results, and a means of re-engaging audiences: a valuable approach to disseminating experimental results in a citizen science biofilm project.

Conclusion

Data on kombucha pellicle variability caused by varying experimental conditions were obtained, assisting our research in this area. Public engagement with the project was excellent at the point of interaction, but additional strategies are important to sustain interest at distance over the long incubation period required.

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