Brewers' Research and Education Fund

Fellowship 2017 Research proposal

BeerDecoded: Exploration of beer metagenomes



■ Principal investigator (PI): Jonathan Sobel Ph.D.

■ Host institution: Hackuarium

■ Full title: Exploration of beer metagenomes

Proposal short name: BeerDecodedProposal duration: 12 months

BeerDecoded is a citizen science project of the association Hackuarium, a non-profit that promotes public understanding of science by making laboratory tools accessible to all. The goal of BeerDecoded is to extend scientific knowledge about beer while having a conversation with the general public on issues related to food, genomics and the role of technology in society. Over the past two years we have run a crowdfunding campaign to finance the first stage of the project (EUR 10,000), we have participated in several events in Switzerland and abroad and have now obtained the metagenomic profiles for 39 beers. However, we currently do not have access to information about the recipe of each of these beers, limiting the potential for understanding the role of the microorganisms in shaping the taste of each brew. BrewDogs, a craft brewery from Scotland recently released all their recipes. We would like to use both this resource and also our analysis capability, including a portable DNA sequencer (minION) to build a proof of concept of beer metagenome analysis workable schools, citizen science laboratories, craft breweries or industrial plants. These analyses will allow anyone to detect microbial contaminants and potentially identify microorganisms that affect the flavour of the beers. Finally, our goal is to set up a public repository where brewers and beer scientists could share their analyses and browse our knowledgebase.

Introduction

Beers have always been a playground for scientists. For instance, one of the most widely used statistical tests, namely the Student T-test, was developed by William Sealy Gosset, an employee of the Guinness brewery. Even Joule demonstrated the first law of thermodynamics using the family's brewing equipment. Beer is a simple beverage based on only a few ingredients [1]. Still, it is unfair to say that beer gets its flavour from just its recipe. Malted barley provides sugar and body, and its roasting impacts flavour. Hop flowers bring bitterness as well as fruit notes in some craft beers. Dissolved minerals in the water affect the flavours that come through from grain and hops. Yeasts provide alcohol, carbon dioxide and hundreds of aroma molecules. There is more: brewing is a process, and the brewer's "ingredients" do not work alone during the brewing process. Hence the importance of the recipe as well as the potential presence of contaminant microorganisms [2].

Unveiling the natural fermentation

Some breweries optimised their processes to maximize the presence of environmental "wild" yeast species into the fermentation process. In fact, beer is a complex ecosystem, where several strains of yeast and also other fungi and bacteria may impact the final taste of the beverage [3]. It is fair to say that this process optimisation is still more art than science, and we would like to make it more reproducible using molecular biology. How can we discover unappreciated microorganisms present in the final product? How can we identify which microbes contribute to the unique flavour of each brew? How can we ultimately modify the brewing process to control the role of these microbes?

DNA is the building block of life. Every living being contains unique DNA fingerprints that discriminate one organism from another. A recent comparative genomic study of 157 brewing yeast strains by the Verstrepen lab published in the scientific journal Cell, looked at the yeast's DNA (as raw material) and demonstrated that industrial yeasts originated from only a few domesticated ancestors [4]. Our approach is to isolate and study the DNA traces that are present in finished beers. Our long term goal is to be able to directly relate these DNA traces with the brewing recipes and with the taste profiles of each brew. In our lab we demonstrated that it is possible to extract DNA directly from bottled beer and to identify the minute presence of 10-30 different wild yeast species in commercial beers. So, yes, it is possible to identify the microbes in beer based on DNA. For example, from our preliminary data we can identify which wild yeasts are inside two Belgian beers (Figure 1).

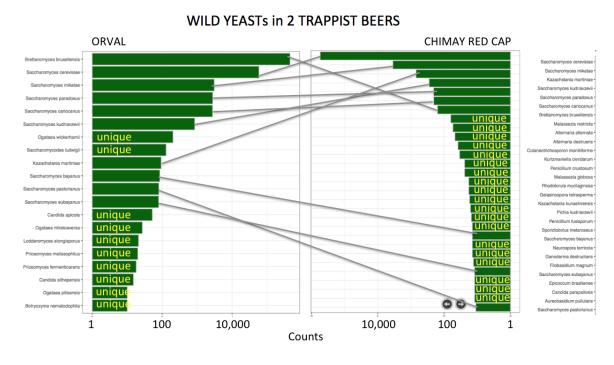


Figure 1: Relative abundance of DNA from yeast species in two belgian beers

And so what? Our work is unveiling a new layer of knowledge around the wild yeast present in

commercial beer. We assume that this knowledge could be useful to help brewers brew better beer. But how to make sense of this information? Asking sommeliers, or mining digital taste profiles (i.e. from Untappd etc..) one could establish a relationship between the presence of Candida apicola and the orange zest flavor. But how could a brewer build on that knowledge? Simply pitching Candida apicola into the fermentation process would probably not work because brewing is more than a recipe: it is a complex process. So, if a brewer wanted to increase the presence of wild Candida apicola, it could be important to identify processes and ingredients that are responsible for its presence in the bottled beer. More importantly, a brewer may want to modify a process to minimise the presence of beer spoilage microbes. Unfortunately, breweries often keep their processes, recipes and know-how strictly secret, making difficult to infer causal associations between a desired/unwanted wild-yeast and a process. This is where the UK market is proving different and why the specific outcomes of our research could benefit the UK sector. Recently a UK brewery (Brewdog) published all the recipes and processes of their 250+ beers as an act to give back to the community of homebrewers. Today in the UK, there is the key to connect wild yeast with brewing processes and the BREF grant could make it happen.

Milestone 1. Mapping wild fermenters in public brewing recipes

Brewdog's open recipes put us in the unique position to i) measure wild microbes inside Brewdog beers, ii) try to directly associate the microbial presence to the process (i.e. by correlation with ingredients/ brewing steps unique in different beers), iii) suggest process modifications to increase the presence of desired wild yeast in the fermentation. As many of these ingredients and processes are specifically available in the UK, we expect the UK sector to be the more direct beneficiary of the knowledge that we will generate with the BREF grant. We expect UK brewers to test our predictions and we foresee support from them. We (or other parties) could engage in an iterative process with the brewers, where new beers are tested and processes modified in a sustainable virtual cycle of scientific brewing.

Milestone 2. Making wild fermenters mapping accessible to brewers

To generate our preliminary results, we used instrumentation that is generally available in academic labs. Researchers queue to access these instruments; and it thus took us months to gather the sequencing data, because of the queuing list. Our current operations are not scalable, in fact. Given a typical brewery's production timescales, it is unlikely that UK brewers could use metagenomic parameters to optimise their brewing processes with this current practice. Can we analyse the beer DNA directly in a brewery? BREF will support us to adapt our protocol to brewery timescales.

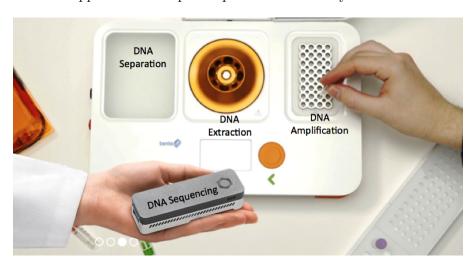


Figure 2: Experimental setup

Recently, a UK company released a portable DNA extraction/amplification lab (Bento.bio) and another UK company (Oxford Nanopore [5; 6]) released a portable DNA sequencer. We want to adapt our analysis process to these portable instruments and operate them directly in a brewery (Figure 2).

With the BREF support, we could use this portable instrumentation to create a rapid, "in-day" protocol that could be easily replicated by any brewery in the UK market with minimal (<3000GBP) investment. The same protocol could be used by schools for educational activities around wild fermentation.

Preliminary results

In 2015, we crowd-funded EUR 10,000 for a small pilot experiment. We sampled about 120 beers, from which we extracted DNA. We used a targeted metagenomic approach using DNA sequences from the ITS locus of the ribosomal DNA cluster, which provide species-specific identification information. DNA extracts from 39 beers, were sequenced using an Illumina MiSeq sequencer from the genomic facility at the University of Lausanne, Switzerland, providing high quality data on the many organisms contained in each beer. This pilot experiment demonstrated that we could accurately detect various species of yeasts and other fungi using ITS amplicons in beers (Figure 3A). This analysis revealed that some beers, such as **Wienerwald**, or the **Chimay Red Cap**, have more than 30 different fungal species. In addition, using a hierarchical clustering approach (Figure 3B), we could built a proximity tree between the different beers in our data-set. This analysis revealed, for instance, that two stouts (**Hackuarium Fakufaku** and **La Nebuleuse Malt Capone**) brewed in the same city have a similar wild yeast content.

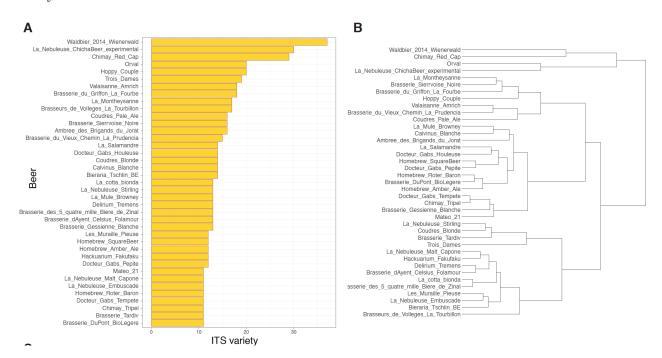


Figure 3: Analysis of 39 beer metagenomes using ITS amplicons. A) ITS variation in our 39 beers. B) Hierarchical clustering the 39 beer metagenomes.

Proposed work

- 1. Selection of **Brewdog** beers with highest deterministic potential
- 2. Selective DNA sequencing
- 3. Development of a workable protocol in a non-standard research infrastructure, typically a brewery with limited laboratory facilities
- 4. Release of an open-access repository for beer metagenome sequencing analysis

Selection of Brewdog beers with testable processes

To associate processes/ingredients with metagenomic content, we need to select those beers in which only few variables change in the brewing process. We will calculate process variance among Brewdog beer's brewing processes and select a path of minimal variance to identify 10-15 beers that are more meaningful for the subsequent DNA analysis.

Selective DNA sequencing

Using the minION portable instrument from Oxford Nanopore [5; 6], a British company developing state-of-the-art sequencing technology, we will explore another highly conserved loci between different species of bacteria, namely 16s RNA. With this approach, we will identify the complete bacterial community living in each beer ecosystem. In addition we will explore chloroplast specific primers to detect hop and grain DNA, and study the diversity in these important ingredients. We will also associate the presence of specific microbes to specific brewing processes and ingredients using machine learning algorithms. These steps will permit us to approach UK breweries and propose a joint scientific brewing project to test our predictions.

Development of a workable protocol in a non-standard lab structure

In Hackuarium (our citizen science lab), we are concerned about outreach of science and more precisely about understanding of molecular biology. We want to export science outside of traditional labs (i.e. [7]). We are developing low-cost approaches, including protocols, and use of lab equipment such as the Open Nanodrop (a machine that allow QC and DNA quantification). We will use our expertise to propose a workable protocol for Beer DNA analysis in any brewery, with a low-cost setup.

Production of an open-access repository for beer sequencing analysis

Our goal is to share our findings in an open access and open source manner. We aim at building a public repository where beer scientists, brewers and citizen scientists can browse our results, as well as share their own data.

Budget

We ask for 10000£ to cover a fraction of the following costs:

- Laboratory consumables (DNA extraction kits, PCR reagents, ...)
- Server and access cloud computing (i.e. Amazon Aws)
- Equipment (MinION sequencer & Bento Lab,flow cells,...)
- Intern fellowship (Lab work, Bioinformatic analyses)

Team

Gianpaolo Rando, Ph.D. Project lead Jonathan Sobel, Ph.D. Bioinformatics, Project lead Vanessa Lorenzo, Interaction design Luc Henry, DPhil. Science outreach Rachel Aronoff, Ph.D. Microbiology

Hackuarium

A community of passionate individuals working in a non-institutional, open and fully equipped laboratory part of UniverCité, a vibrant ecosystem including also a Coworking space, a Makerspace, and a Hackerspace. Founded in August 2014 and growing steadily since, Hackuarium is now the home of 40+ people working part-time on a variety of projects ranging from Antarctic exploration to bioelectronic sensors for water pollutants and, of course beer DNA.

Information and Contacts

- http://www.hackuarium.ch/en/
- http://www.genome.beer
- Twitter:@beerdecoded

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