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The Idea
Engineering of self-cloning brewer's yeast for novel terpene profiles in beer.

Terpene flavor compounds are an important target for food improvement in a number of areas. One such area is the brewing of beer, particularly newer "craft" beers that feature large quantities of newer American varieties of hops bred for their aroma and flavor characteristics rather than their bittering properties. Beer fermentation by brewer's yeast (Saccharomyces cerevisiae) results in changes to the profile of terpene compounds extracted from hops in a number of ways including degradation of flavor-active compounds, transformation between different classes, and hydrolysis of glycosidically bound precursors to make them flavor-active. All of these processes present targets for modification of yeast to change the profile of flavor compounds in finished beer. Genetically modifying yeast for a food product is problematic due to EU regulations on such products but by using so-called "self-cloning" techniques to transfer sequences from one region of the yeast genome to another (i.e. cisgenesis), we can make useful modifications without creating an organism that is considered a "GMO" by current regulation. Our project will be composed of a laboratory component involving molecular biology for the creation of new yeast strains and gas chromatography combined with mass spectrometry (GC-MS) to analyze terpene profiles, a modeling and data analysis component in which we try to understand how terpene profiles influence flavor perception, and a legal component in which we attempt to create a legal pathway for bringing self-cloning yeast to the home brewing community.
Who we are
Paul Grant (pg384@cam.ac.uk) is a postdoctoral research associate in the department of plant sciences. He is a molecular biologist by training, currently working on a synthetic biology project involving engineering of cell-cell communication in microbes for the creation of multicellular patterning.

Sebastian Ahnert (sea31@cam.ac.uk) is a Royal Society University Research Fellow in the Theory of Condensed Matter (TCM) group of the Cavendish Laboratory at the University of Cambridge, and a Fellow of King's College, Cambridge.

Orr Yarkoni (oy210@cam.ac.uk) is a postdoctoral research associate in the pathology department. He is currently working on an arsenic biosensor that is in the process of obtaining approval for use in the field for testing well water for levels of arsenic.
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1
Implementation
We will use the methods described in Hirosawa et al. (2004) to replace the promoters of target genes with high-expressing constitutive promoters using self-cloning techniques that leave behind only yeast-derived sequences. We will perform this procedure on three different strains of yeast commonly used in both home and commercial brewing. Our primary target will be exo-β-1,3-glucanase (Exg1) that functions as a glucoside hydrolase for the release of glycosidically bound terpenes, but we will also investigate other targets that may have an influence on the terpene profile of fermented beer. After confirmation of successfully modified strains through sequencing and RT-qPCR, we will compare the modified yeast strains to their parental strains in lab-scale fermentations of malt extract boiled with different varieties of hops. We will use GC-MS to create a profile of the various hop-derived terpenes found in the different fermented solutions. We will also recruit volunteer participants in sensory panels to smell the solutions (as a safe alternative to tasting) and report differences in the detectable aroma profiles. We will use double blind triangle test methodologies to determine if aroma profiles between engineered and parental strains are significantly different. By combining genetic changes, profiling at the level of compounds, and profiling at the level of human-detectable aromas, we will be developing a

methodology for engineering flavors at the genetic level. Sebastian Ahnert has significant experience in data analysis involving flavor compounds (Ahn et al., 2011) and will use this data to develop new methods for understanding the link between genetic changes and aroma profiles. We will also investigate the possibilities for legally using our new yeast strains for producing beer for human consumption. Organisms produced by self-cloning are currently exempt from the majority of regulations that cover GMOs under the contained use regulations. However, they are (somewhat paradoxically) explicitly subject to the rules covering deliberate release. We will use the experience Orr Yarkoni has gained with bringing a GM product to market to explore the possibilities for developing a path to non-commercial (homebrew) use of modified yeast produced by self-cloning. References: Hirosawa I, Aritomi K, Hoshida H, Kashiwagi S, Nishizawa Y, Akada R. Construction of a self-cloning sake yeast that overexpresses alcohol acetyltransferase gene by a two-step gene replacement protocol. Appl Microbiol Biotechnol. 2004 Jul;65(1):68-73. Ahn YY, Ahnert SE, Bagrow JP, Barabási AL. Flavor network and the principles of food pairing. Sci Rep. 2011;1:196.

Benefits and outcomes

This project will provide a new opportunity for collaboration between the departments of pathology, physics, and plant sciences in order to develop techniques that are broadly applicable to the engineering of flavor through changes at the genetic level of organisms involved in the industrial processing of food. We will create new strains of yeast that will be suitable for use by the DIYbio community (as they will not require class I GM facilities). This provides a great resource for outreach and education, showing how self-cloning techniques to produce genetic changes can result in interesting differences in the aroma profile of a beer. Hopefully, this can serve to reduce barriers to the acceptance of making modifications to organisms involved in food production. Perhaps by targeting such a small change and focusing on sensory differences that are a matter of taste, we can reduce the stakes involved in genetic modification—we will not be making any claims as to the health benefits or the industrial superiority of our yeast strains, simply that they make the beer smell different. The homebrew community is similar to the DIYbio community in its interest in open exchange of information and techniques. It would be an exciting opportunity if we can develop a legal pathway by which the self-cloning techniques already in use by DIYbio enthusiasts can be brought to homebrewers in the form of new brewing strains. We recognize that the legal hurdles may result in us being unable to release our yeast strains for human consumption but we will at least put that legal framework to the test and at the same time develop techniques that will be useful for future work in engineering flavor profiles and create yeast strains that will be useful outreach and educational tools for demonstrating the impact of genetic differences on aromas that people can readily perceive.

Sponsor for the research and cost centre

Jim Haseloff. Department of plant sciences. jh295@cam.ac.uk.

Sponsor support confirmed?

1

Budget

Molecular biology reagents: £1000 Including primers, enzymes, plates, media, sequencing. Brewing ingredients: £500 Including yeast strains, hops, malt extract, labware for brewing. GC-MS analysis: £1500 Core facilities in the department of chemistry charge £22.50 per sample. Travel and outreach: £1000 We wish to present our findings at academic, DIYbio, and homebrew events.