



Making "hoppy" beer without hops

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ABSTRACT

Tasty "hoppy" beer is increasingly preferred by consumers. However, the hop plant is a demanding crop and it varies considerably in essential oil content. A new approach reports how to confer the "hoppy" flavour by genetically engineering



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Afterwork? It is time go for a beer and to relax! Beer is one of the most liked and consumed alcoholic beverages around the world. Over the past two decades, consumers have shown an increasing preference for beers that contain a "hoppy" flavour.

But how is beer produced? During the brewing process, yeast (*Saccharomyces cerevisiae*) "eats" grains and converts sugars into ethanol (a type of alcohol) and other by-products which bring characteristic tasty flavours to the beer. This biological process is called <u>alcoholic fermentation</u>, a way by which yeast produces its own energy to survive and – in exchange – delivers alcohol molecules to its surrounding "environment". This is the reason why yeast is essential to industrially produce alcoholic beverages including beer. Flowers

of the <u>hop plant</u> are typically added during fermentation to impart a hoppy flavour. However, hops are both a water and energy intensive crop and vary considerably in essential oil content, making it challenging to achieve a consistent hoppy taste in beer. The goal of this study was to propose a new approach to produce hoppy beers without using the hop plant.

The primary hoppy flavor determinants that have been reported are two biomolecules called <u>linalool</u> and <u>geraniol</u>. The authors of the study hypothesized that a yeast capable of producing these molecules, would be able to brew hoppy beers without using hop plants. To this end, the authors tried to identify plant-derived enzymes (proteins that facilitate chemical reactions converting a certain molecule





into product molecule(s)) that produce linalool and geraniol (linalool and geraniol synthases respectively), which could be genetically engineered in yeast. As these enzymes have not yet been found in hops, the authors genetically engineered an optimized lab yeast with different plant-derived linalool or geraniol synthases, and measured the amount of synthesized linalool and geraniol. Linalool synthase from water mint and geraniol synthase from basil were characterized as most the efficient enzymes.

However, brewer's yeast is normally used to produce beer commercially instead of the optimized lab yeast that has been experimented on. Therefore, the authors next engineered brewer's yeast capable of producing linalool and geraniol during beer fermentation. The brewer's yeast can naturally make only a small amount of the molecule that is needed for linalool and geranitol production. The authors decided to boost the production of this molecule by genetically engineering brewer's yeast. For this purpose, they selected relevant genes and engineered them into the yeast, as well as linalool synthase from water mint and geraniol synthase from basil. As expected, the engineered brewer's yeasts produced linalool and geraniol when grown under fermentation conditions similar to an industrial brewing process.

The amount of linalool and geraniol produced by the engineered brewer's yeasts, however, varied considerably among yeast strains. Importantly, in most of the yeasts that were tested, the production level was lower than commercial beers. This is because every genetically engineered yeast contains a different set of promoter DNA sequences. The promoter DNA sequence governs how often a gene is expressed and thus how much of its protein is generated. "Strong" promoters lead to high level of gene expression and protein production, whereas "weak" promoters give less. The authors characterized certain engineered yeasts, that contain a good combination of "strong" promoters which lead to a high linalool and geraniol production close to commercial beers.

Furthermore, the authors tested whether the engineered brewer's yeasts were capable of producing a constant amount of linalool and geraniol. Repetitive fermentations allowed them to confirm that the engineered yeasts produce the flavor-determining molecules more consistently than traditional brewing. This feature of the engineered yeasts can potentially resolve the "inconsistent" hop flavor problem of commercial beers.

Finally, the authors tested if the engineered yeasts would confer a hoppy flavor as perceived through human taste. Most importantly, according to human tasters, beers produced with the engineered yeast had a stronger hop flavor, compared to beer fermented with traditional brewer's yeast and dry hopping.

These results show that the genetically engineered yeasts are capable of giving a consistent and intense hop flavor in beer. This, in turn, can potentially achieve a cheaper, more efficient, and nature-friendly commercial beer production in the near future.