BioBeer: Biosynthesis of Resveratrol During Fermentation

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**Introduction**

Resveratrol, a phytoalexin produced by plants as a defense against pathogens, is found in a number of foods, including red wine, berries, and peanuts. The presence of resveratrol in red wine has been proposed to underlie the “French Paradox,” i.e., the observation that French citizens exhibit a relatively low rate of cardiovascular disease compared with the rest of the world given their diet of high-saturated fats. Studies over the past decade have provided evidence that resveratrol exhibits diverse health benefits, ranging from coronary disease prevention to decreased cholesterol levels and resistance to infection with oxidative stress. The health benefits associated with the consumption of resveratrol-enriched foods has led to a market for dietary supplements of this phytoalexin. Unfortunately, resveratrol supplements are thought to exhibit reduced bioactivity compared with resveratrol found in red wine. Wine is thought to maintain resveratrol in a bioactive form because the anthocyanins and polyphenols in red wine protect resveratrol from degradation and storage prevents light and air oxidation of this compound.

Beer is more popular in the United States than wine, but it does not contain high levels of resveratrol. To provide the health benefits of resveratrol to a wider populace in a lower cost than resveratrol supplements, we have engineered yeast to produce resveratrol. The goal is to engineer a brewing yeast strain containing an integrated genetic circuit. The yeast should be able to produce substantial levels of resveratrol during fermentation, which catalyzes the last two steps of resveratrol biosynthesis.

**Strategy**

To produce brewer’s yeast, hops, and malted barley are placed in a fermenter, where the yeast convert sugar into ethanol and carbon dioxide. Our yeast will be engineered to synthesize resveratrol in addition to these products. To accomplish this goal, a genetic circuit will be introduced into yeast that produces the enzymes that catalyze the conversion of tyrosine to resveratrol. These enzymes are tyrosine ammonia lyase (TAL), 4-coumarate CoA-ligase, and STS (stilbene synthase).

**Brewer’s yeast selection**

A strain used to brew Hefeeweizen at St. Arnold Brewery was chosen as a chassis for two reasons:

1. This strain is a top-fermenting ale yeast, so it is most likely an industrial Saccharomyces cerevisiae, which would make it very similar to lab strains. (Of course, we cannot use a lab strain as it would ferment beer.)
2. Hefeeweizen is an unfiltered beer, so the yeast is left in it when it is served. This means that any resveratrol stuck within the yeast will be consumed.

Engineering a brewing strain of yeast is challenging because these strains are expected to be polyploid and prototrophic. Thus, auxotrophic strains cannot be used to select for maintenance of our circuit as they can with laboratory yeast strains.

**HPLC Analysis**

We have been using high-pressure liquid chromatography (HPLC) with a reverse phase column to determine the presence of resveratrol in our samples. This method is sensitive to very low amounts of resveratrol and allows accurate quantification of the concentration of resveratrol present.

**Circuit Design**

**Case 1.** Express 4CL-STS during fermentation, which catalyzes the last two steps of resveratrol biosynthesis.

**Case 2.** Produces bleocin resistance when induced by galactose. It is used to select for brewer’s yeast containing the circuit.

**Case 3.** Constitutively produces TAL, which catalyzes the first step in resveratrol biosynthesis.

The construct will be able to integrate into the yeast genome at the PGAL1 locus because the first promoter and terminator sequences are from this locus. Since most industrial yeasts are polyploid, this should not significantly affect the fermentation abilities of brewing yeasts.

**Parts Completed**

Individual parts and project-specific constructs

8.2% alcohol

Volume consumed/year (gallons)

**Integration construct #1**

A circuit for 4-coumaric acid feeding

**Conclusion**

Brewer’s yeast represents an affordable solution for producing resveratrol-enriched beer. We have engineered yeast for resveratrol biosynthesis. This approach will be useful for delivering other types of air- and light-sensitive compounds (e.g., cannabinoids) to a wide population efficiently.

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