Designing Rose-scented *Saccharomyces cerevisiae*; a Customized Yeast to Enhance the Aroma of Baked Goods.

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With this synthetic biology project, we aim to transform *Saccharomyces cerevisiae*, or bread yeast, using the genes responsible for the rose aroma in order to give bread the rose scent. Chefs often use roses in dessert making due to its worldwide recognition as a symbol of love and romance. Different types of rose dishes, including rose crepe cake, rose mousse, and rose pound cake, are popular all around the world. The objective of this paper is to produce yeast that can inherently give bread an elegant rose odor during fermentation as a replacement to the conventional long process used by chefs. We discovered the following genes which account for the production of chemicals responsible for the rose odor: BBa_K1507006, a gene which has been successfully transformed into *Escherichia coli*, and BBa_K2281005 which has been successfully transformed into yeast. The latter group of researchers reported that the yeast proved to be appropriate for the production of the chemical which accounts for the rose scent. Future data will determine if the rose aroma successfully remains in the yeast and allows it to hold the scent during fermentation and produce rose-scented bread.

Keywords: *Citronellol, rose scent, Saccharomyces cerevisiae, bread*

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Watch a video introduction by the authors at http://bit.ly/2Z8XzBR

Background

Rose is not only famous as a symbol of love and romance but is often used in various dessert making. A few desserts such as Rose crepe cake, rose mousse, and rose pound cake are popular all around the world. However, currently rose-flavored compounds such as rose water, perfume, and oil are very difficult to use for beginner chefs since the flavor will vaporize during baking stage in the oven. Moreover, nowadays customers have a natural tendency against foods with too many additives, and rose flavored bread without additive would appear healthy and natural alternative to all the different additives. The objective of this paper is to produce yeast that can give bread an elegant rose odor during fermentation thus allowing everyone to easily make rose flavored baked goods. *Saccharomyces cerevisiae* is a strain of yeast most commonly used for bread making. We chose this specific strain because it is commonly used among professional chefs, home cooks, and everyone in between. This transformation of this yeast will make it widely accessible for all.

Systems Level

It is proposed to transform the rose aroma gene (Wang 2017) into yeast. Using BBa_K2281005, we will transform *S. cerevisiae,* a species of yeast specifically used for bread making. This transformation would test the ability to produce citronellol (a chemical which is found in rose oil and has the rose aroma) when the gene coding for its production is transformed into yeast. Figure 1. depicts the complete pathway of the production of citronellol in a transformed *Saccharomyces cerevisiae* cell.

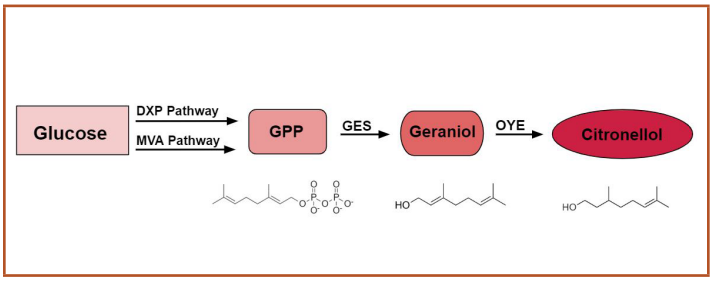

Figure 1. *The diagram depicts the pathway of glucose to citronellol, the product known for producing the classic rose scent. The process begins with glucose and undergoes three major steps to produce the end product citronellol. Through the Mevalonate (MVA) pathway and DXP reductoisomerase (part of the MEP pathway), which are two essential metabolic pathways, DMAPP and IPP are synthesized and transformed into GPP. The enzyme geraniol synthase (GES) is used to produce geraniol from GPP by removing the two phosphate groups. Finally, with the Old Yellow Enzyme (OYE), an allylic alcohol double bond from Geraniol was reduced, and the final product, citronellol is produced (Yuan et al. 2011).*

The LiAc transformation method will be used to transform the aroma gene into the yeast. Competent yeast are prepared and suspended in a LiAc solution with the plasmid DNA to be transformed. We plan to select the transformants using the URA3 complementation method as shown in Figure 2.

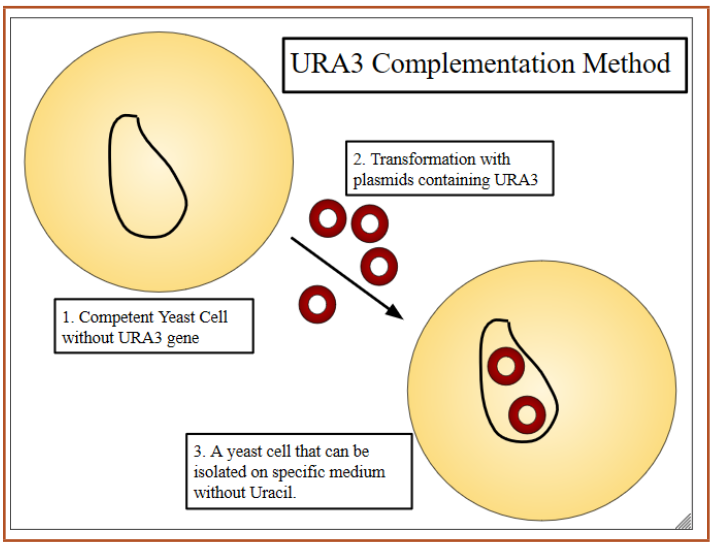

Figure 2. *The selection method for transformed S. cerevisiae relies on the URA3 method. The mutated cells used in the process lack a URA3 gene while the plasmid DNA has a copy of URA3 gene as well as the URA3 promoter. S. cerevisiae with a URA3 deletion is transformed with a plasmid containing a URA3 gene and promoter. Transformants are selected by growing the transformed yeast on a specific media without uracil. The genes of interest will be inserted into the plasmid and cloned into S. cerevisiae using this method.*

Device Level

The transformed *S. cerevisiae* will be used in bread-making because it gives the bread a pleasant rose odor by producing citronellol in a safe and environmental-friendly way. Using the selection process described in Figure 2, we would insert the plasmid which accounts for the genes that produce citronellol into the *S. cerevisiae* cells. This will allow the cells to produce citronellol.

As seen in Figure 1, the process starts with glucose and takes three major steps to produce the end product, Citronellol. Through the Mevalonate pathway (also known as the MVA pathway) and DXP reductoisomerase (part of the MEP pathway), which are two essential metabolic pathways, dimethylallyl pyrophosphate (DMAPP) and isopentenyl pyrophosphate (IPP) are synthesized and converted into geranyl pyrophosphate (GPP). The enzyme geraniol synthase (GES) is used to produce geraniol from GPP by removing the two phosphate groups. Finally, with the Old Yellow Enzyme (OYE), an allylic alcohol double bond from Geraniol reduces, resulting in the final product, citronellol (Yuan, Chen, Lu, Liu, & Zeng, 2011).

Parts Level

We will be using *S. cerevisiae* cells and need to either order competent cells or make them in a lab for transformations. Also, a transformation kit for *S. cerevisiae* would be useful in the process. One of such devices is available from Sigma Aldrich (Yeast Transformation Protocols). On the iGEM Parts Registry, we located the gene that will be used for our transformations and experiment. This is the BBa_K2281005 gene which will be inserted into the yeast in order to produce citronellol to create the pleasant rose scent.

Safety

Saccharomyces cerevisiae is widely used in the bakery industries, therefore is not harmful to people or the environment.

Citronellol, the main compound that produces the rose scent, is widely regarded as safe to use. Companies use it for the production of a variety of products, including perfumes and essential oils. Although slight irritability has appeared in certain patch tests, this only occurs when the citronellol is in high concentration and is applied directly to the skin for an extended period of time (HSDB 2015).

Discussions

Our project is the production of an eco-friendly and safe yeast that produces a rose odor when making bread. It will be useful to everyone from master chefs to amateur cooks all around the globe, as it will allow them to easily make rose scented pastries.

This project was accomplished through participation in the BioBuilderClub, an after-school program organized by BioBuilder Educational Foundation. BioBuilderClub engages high school teams around the world to combine engineering approaches and scientific know-how to design/build/test their own project ideas using synthetic biology.

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