

## Evolutionary Dynamics of a Secondary Metabolite Gene Cluster in Budding Yeasts

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### Project Goals

This project aims to improve our understanding of how microorganisms apportion carbon flux into biofuel-relevant metabolic pathways, including how we can rationally engineer flux to improve the production of specialty biofuels.

### Abstract

The focus of biomass conversion into biofuels has recently shifted away from the production of ethanol and into the production of specialty biofuels, such as isobutanol. For the yeast *Saccharomyces cerevisiae*, this presents several challenges, as native production of isobutanol is minimal compared to ethanol. One major challenge is that the native flux through the branched-chain amino acid (BCAA) pathway, from which isobutanol is produced, is quite low. We therefore sought to identify species of yeast with naturally higher flux through this pathway. To do this, we studied a small subset of yeast species that produce a pigment called pulcherrimin, which is identifiable by its characteristic red color when bound to iron. Pulcherrimin is a cyclic-dipeptide derivative produced from two leucine molecules, linking it directly to BCAA biosynthesis. We identified a four-gene cluster conserved among species that produced pulcherrimin, which we named the PULcherrimin gene cluster. Using targeted gene replacements in the pulcherrimin-producing yeast *Kluyveromyces lactis*, we found that all four genes play a role in either pulcherrimin biosynthesis or re-utilization of pulcherrimin-bound iron from culture medium. To our knowledge, this is the first demonstration of a functional secondary metabolite gene cluster in budding yeasts. In characterizing the species distribution of the *PUL* gene cluster, we found the presence of partial clusters in several more species, including *S. cerevisiae*. These partial clusters always consisted of the genes required for utilization of pulcherrimin, and not the biosynthesis genes. We performed targeted gene replacements in *S. cerevisiae* and confirmed the roles of these genes, neither of which had previously been assigned a known function. We found no evidence for acquisition of the *PUL* cluster through gene gains via horizontal gene transfer, and we therefore hypothesize that the *PUL* cluster was present in the ancestor of all budding yeasts, but was lost in most yeast lineages. We also predict a public goods dilemma that emerges from lineages that have lost the pulcherrimin biosynthesis genes, but maintain the genes involved in pulcherrimin utilization. Future work will focus on the molecular mechanisms by which pulcherrimin-producing lineages vary in their levels of production to better understand how they differently apportion their BCAA carbon flux.

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