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Principle Investigator: Michael Lentz

Academic Institution: University of North Florida, Jacksonville, FL, U.S.A.

Project Title: Biochemistry of phenolic acid decarboxylase enzyme from Brettanomyces yeast

Project Intro:

Craft brewers are experimenting with an ever-widening array of fermentative yeasts in an effort to create new and interesting beer flavors and aromas. Yeasts of the genus Brettanomyces are rapidly growing in popularity, both as a primary and co-fermenter. With these new strains comes potential risk, as these yeasts are less well characterized and unpredictable compared to their established Saccharomyces counterparts. A key aroma- and flavor-active feature of Brettanomyces is production of volatile phenolic compounds during fermentation through the metabolism of hydroxycinnamic acids (HCAs) extracted during the brewing process from grains and hops. These volatile compounds can be positive (spicy, smoky, "funky") or undesirable (plastic, burnt, medicinal). The types of phenolic compound produced are likely to be influenced by choice of raw ingredients, fermentation conditions, and yeast strain. This project seeks to eliminate yeast strain as an unknown variable by characterizing the biochemical pathway for volatile phenol production by Brettanomyces yeast. We will clone and sequence the gene for the key metabolic enzyme, phenolic acid decarboxylase (PAD) from a variety of species and strains. We will overexpress the cloned enzyme in recombinant bacteria, then characterize its biochemical and enzymatic properties. We hypothesize that differences in enzyme activity will correlate with genetic differences between strains. Our results will add a new level of understanding and predictability to "Brett beer" fermentation, by giving brewers additional information about fermentation performance of different Brettanomyces yeast strains.

Project Objectives:

Our overall goal is to understand the mechanisms of volatile phenol production by Brettanomyces yeast and their contribution to beer aroma and flavor. To address this goal, we will characterize the biochemical and enzymatic properties of phenolic acid decarboxylase (PAD), a key yeast enzyme in volatile phenol synthesis. The following objectives are proposed. 1. Clone, express, and purify PAD enzyme from new isolates. We have successfully put in place a system to clone and overexpress the Brettanomyces PAD enzyme in E. coli bacterial cells using standard PCR and T7 expression systems. We will acquire additional brewing isolates from both Brettanomyces bruxellensis and B. anomalus, the two common brewing species, as well as other species in the genus in order to obtain a wide cross-section of PAD enzyme variation. To maximize our ability to address the role of strain variation, we will also include wild isolates and wine spoilage strains. 2. Biochemical characterization of PAD enzyme. We will quantify Vmax (reaction rate) and Km (substrate binding affinity) of PAD enzyme extracts prepared from induced E. coli cells. We will determine these values using ferulic acid, p-coumaric acid, caffeic acid, and sinapic acid as substrates, the most common HCAs in beer raw ingredients. We will also determine pH and temperature sensitivity of the purified enzymes. By evaluating a sufficient number of strains, we expect to identify and describe a correlation between substrate specificity and brewing potential. These features will provide

important new insights into the molecular mechanism of beer aroma and flavor characteristics generated by Brettanomyces. 3. PAD gene sequence variation. Several complete genome sequences are available for Brettanomyces strains, and additional PAD gene sequences are also available. We have compared these sequences using online genetic analysis tools. Our comparison of these sequences reveals a high degree of conservation, but also variation. Protein sequence variation may account for some, or all of the functional variation described in objective #2. To determine any correlation between gene sequence and functional variation, we will sequence the PAD gene from our cloned isolates. Sequence alignments will be performed, and variation will be analyzed against differences in enzyme properties determined in objective #2. We hypothesize that amino acid substitutions in different strains will alter enzyme activity and/or substrate specificity, accounting for at least some of the variation in PAD activity we have observed in functional assays. We previously completed a more general HCA metabolism analysis on local environmental Brettanomyces isolates as well as several commercially available brewing strains. Results demonstrate significant variation among strains relative to PAD activity, particularly towards ferulic and p-coumaric acids. This data supports previous work by others and demonstrates considerable variation in this key organoleptic activity. This proposed research will characterize the phenolic acid decarboxylase (PAD) enzyme in a variety of commercial brewing and other stins of Brettanomyces yeast.