

# Oregon Wine Advisory Board Research Progress Report

1990 - 1991

---

## Development and Evaluation of a Protease-Excreting Wine Yeast to Prevent Protein Hazes in White Wines

**Principal Investigator:** Alan T. Bakalinsky, Assistant Professor, Food Science and Technology.

**Cooperator:** Barney T. Watson, Instructor, Food Science and Technology.

### Objectives:

1. Isolation or construction of an acid protease-excreting laboratory strain of *Saccharomyces cerevisiae*.
2. Evaluation of enzyme activity against model protein, protein-tannin, and grape protein substrates.
3. Selection or screening of isolates producing greater enzyme activity with improved specificity.
4. Construction of an acid protease-excreting wine strain of *Saccharomyces cerevisiae*.
5. Evaluation of fermentation behavior and extracellular protease activity of promising isolates during vinification.

### Background and Justification:

The formation of primarily proteinaceous hazes in bottled white wines or the potential for such an occurrence are major concerns in white wine production. The low molecular weight grape proteins believed responsible interact irreversibly with reactive grape-derived polyphenolic constituents to form insoluble complexes. The presence of hazes of any sort renders bottled wine defective and necessitates costly retreatment and rebottling. Since white wines constitute approximately 60% of the production in Oregon, the problem is of local significance. Protein hazes are limited to white wines because proteins originally present in red varieties are precipitated naturally during fermentation by the abundance of grape tannin.

The current preferred preventive method, bentonite fining, involves the removal of protein from wine prior to bottling by cation exchange. Its use has well-recognized disadvantages: 1) the treated wine is not always rendered free of potentially unstable and insoluble proteins; 2) the voluminous lees produced are costly to process by filtration resulting in an inevitable loss of wine; 3) the treatment itself can sometimes strip wine of important flavor constituents. The search for an acceptable and effective substitute to bentonite has generated interest in silica fining, other cation-exchange materials, the use of free and immobilized proteases and immobilized tannin. Reports of the successful application of acid proteases--the only class of proteases active at wine pH--have encouraged my interest in trying to develop a wine yeast strain capable of producing such an enzyme naturally during vinification.

Certain variant strains of the yeast *Saccharomyces cerevisiae* are known to excrete enzymes normally localized in the vacuole. The basis for this aberrant behavior has been determined in at least one case (PNAS 1986, 83:3248). I wish to exploit these findings by developing a strain capable of overproducing

and excreting an acid protease of high activity and broad specificity capable of hydrolyzing the unstable proteins responsible for protein hazes in white wines. Since genetic methods currently used with well-characterized laboratory yeast strains have been shown to be readily applicable to the wine strains, much of the procedural groundwork required by this project has already been laid. Successful development of such a strain, compatible in other respects with winemaking practices, should eliminate need for any additional post-fermentation stabilization treatments. Moreover, the hydrolysis products--amino acids and peptides--will enrich the grape juice in available nitrogen and may reduce the troublesome occurrence of costly, unfinished fermentations caused by its deficiency.

### **Procedures:**

An acid protease-excreting laboratory strain (which is not the same as a wine strain) producing an undetermined amount of extracellular protease activity is available now for evaluation under laboratory conditions. Enzyme activity and specificity will be analyzed in routine culture media and in model wines prior to the introduction or modification of the protease-excreting character into wine strains. I have developed wine strain derivatives into which I will initially introduce the trait from the laboratory strain. The derivative strains are easier to manipulate directly than their parents yet appear to retain the important winemaking characteristics for which wine yeasts are principally valued-- ethanol and SO<sub>2</sub> tolerance, a vigorous fermentative capacity, and propensity for not producing off-flavors or aromas. Activity of the excreted enzyme will be monitored under laboratory conditions to determine its ability to hydrolyze a variety of model proteins under fermentation conditions. If activity appears to be adequate, the enzyme will be tested against grape proteins and if possible, the specific proteins and protein-polyphenolic complexes that have been implicated in haze formation. Unfortunately, these constituents are difficult to isolate in quantity since they are present in wine at very low concentration, in spite of their potential to precipitate. If enzyme activity and/or specificity appears to be inadequate, we will attempt to select improved clones of the protease-excreting strain by growing it in media containing the specific proteins or protein-polyphenolic complexes as sole nitrogen sources. Depending on the results of these assays, the protease-excreting strain will be used in trial fermentations to assess its performance under more typical conditions of use. The wines so produced will be subjected to chemical and sensory analysis to evaluate possible off-flavors or aromas resulting from the increased peptide content since some peptides are known to possess a bitter flavor. The potential of the acid protease itself to interact with polyphenolic compounds and form a haze will be carefully examined.

### **Progress Report 1989-1990:**

1. Formulations for agar-based culture media containing casein as a model protein have been developed and tested for conveniently assaying acid protease activity among large numbers of yeast isolates. Modifications are being tested to make the media more selective.
2. An electrophoretic method for fractionating wine proteins has been tested and shown to be useful for potentially monitoring protease activity with respect to the different wine protein fractions. Current efforts are aimed towards increasing the method's sensitivity to allow detection of wine proteins present at very low concentration.
3. Wine strain derivatives into which the desirable protease-excreting character may be introduced prior to production strains have been evaluated in small-scale fermentations to confirm their suitability. Required chemical analyses (residual sugar, SO<sub>2</sub>, ethanol, acidity) are essentially complete. Sensory evaluation is scheduled for May, 1990.
4. A laboratory yeast strain possessing the potential to excrete an acid protease is now being evaluated for enzyme activity and specificity.