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Quantification and examination of zymolectin cell surface levels and other cell characteristics in brewing yeast using a flow cytometric assay

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Abstract

This study sought to address the lack of understanding of zymolectin regulation using two new approaches: 1.) an assay that measured zymolectin concentrations during lab scale fermentations with a flow cytometer and an avidin, Texas Red® fluorescent probe and 2.) a deconvolution technique to detect sub-populations within histograms prepared from flow cytometry data. During the lab scale fermentations, Langmuir analysis of the flow cytometer fluorescence measurements revealed that mean zymolectin levels first decreased then later increased during the fermentations. Histograms constructed from the fluorescence data suggested the flow cytometer was able to detect sub-populations of cells with more and less zymolectins. Furthermore, analyses of the forward scatter data sets using the deconvolution software identified three sub-populations of cells present during fermentations. Future work plans to extract more information from the large data sets with improved gating methods based on cell size and age.

Introduction

There is disagreement in the literature around whether zymolectin activity is **genetically induced** or **constitutive**.

Genetically induced

1.) Studies by Stratford and Carter (1993) showed that zymolectins are produced during the exponential growth phase and activated at flocculation onset.

2.) Rhymes and Smart (2000) noted an increase in levels of the Flo 1 protein in two yeast strains at 15 and 48 hours growth time while employing immunofluorescence staining with polyclonal anti-Flo 1p antibodies.

Constitutive

1.) Patelakis et al. (1998) found there was no significant change in zymolectin density over the course of a fermentation, suggesting the *FLO1* gene, which codes for proteins involved in zymolectin assembly, acts in a constitutive manner. We have confirmed these observations in later studies using two different industrial strains.

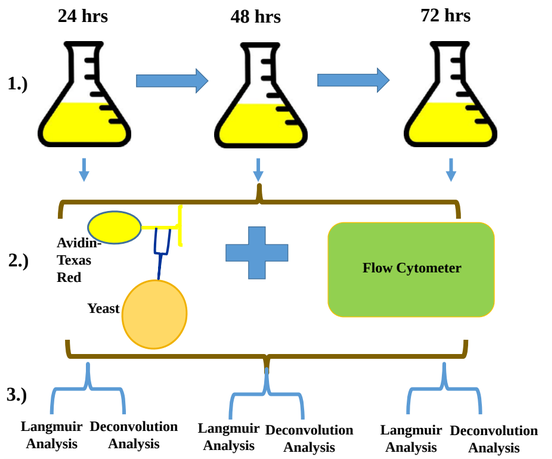
2.) Work by Higgins and colleagues at C.U.B. did not find any up-regulation of flocculation controlling *FLO* genes (personal communication, 2001) at 0, 1, 4, 7 , 10 and 24 hours into a fermentation.

Purpose of Research and Hypothesis

This study sought to more adequately and accurately count zymolectins on an individual cell level with a flow cytometer to re-examine the disagreement in the literature around zymolectin activity regulation.

It was believed that flow cytometer techniques would generate data which would facilitate the detection of sub-populations of cells with different levels of zymolectins, thus supporting the hypothesis that zymolectin activity is genetically induced.

Experimental Techniques



Materials and Methods

1.) 250 mL Erlenmeyer flasks with 100 ml YEPD broth and the SMA yeast were incubated at 100 rpm at 30˚ C on an orbital shaker to produce cultures 24, 48 and 72 hours old.

2.) An avidin, Texas Red® conjugate probe with an excitation wavelength at approximately 595 nm and an emission wavelength at about 615 nm was prepared at concentrations of 1.5, 1.4 1.2, 1.0, 0.8, 0.6, 0.4, 0.2 and 0.1 mg/mL. 1390 µl of cells at an approximate concentration of 1 x 10^6 cells/ml were incubated with 10 µl of each probe concentration.Samples were run through a Partec Cyflow® SL flow cytometer equipped with a 488-nm air cooled argon ion laser.

3.) Langmuir analysis was done as described by Patelakis et al. (1998) and deconvolution was applied with the Multipeak Fitting 2 function of Igor Pro 6.2. This software feature employs an automatic peak-finding algorithm that searches for peaks by finding maxima in the smoothed second derivative of the data.

Results and Discussion

Table 1. Zymolectins per cell as determined by Langmuir analysis.

Measures	24hr	48hr	72hr
Estimated SMA Cell Concentration (cells per ml)	1.12E+06	1.48E+06	1.00E+06
Total Bound Avidin as Free Avidin Approaches Infinity (µg)	1.20	1.06	17.73
Avidin Bound per Cell (pg)	1.08	0.72	17.73
Molecular weight of avidin, Texas Red® conjugate (pg/mol)	6.66E+16	6.66E+16	6.66E+16
Moles of avidin, Texas Red® per cell	1.62E-17	1.08E-17	2.65E-16
Avogadro's Constant (molecules per mole)	6.02E+23	6.02E+23	6.02E+23
Zymolectins per cell	9.73E+06	6.50E+06	1.59E+08

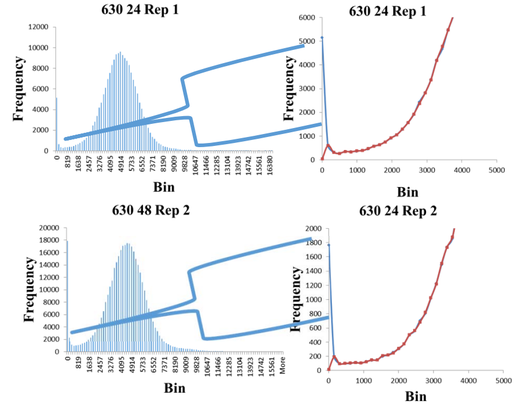


Figure 1. Histograms constructed from the fluorescence data that suggest the flow cytometer was able to detect sub-populations of cells with more and less zymolectins, as emphasized in the enlarged section on the right.

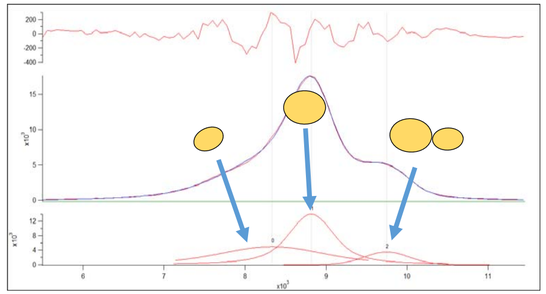


Figure 2. Deconvolution analysis of the forward scatter histograms data that identified three sub-populations of cells present during the fermentations, presumed to be daughter cells, mother cells and mother cells with buds.

During the lab scale fermentations, Langmuir analysis of the flow cytometer fluorescence measurements (Table 1) suggested that mean zymolectin levels first decreased then later increased. Histograms constructed from the fluorescence data suggested the flow cytometer was able to detect sub-populations of cells with more and less zymolectins (Figure 1). Deconvolution analysis of the forward scatter measurements using the Multipeak Fitting 2 function in Igor Pro 6.2 identified three sub-populations of cells present during the fermentations (Figure 2). These sub-populations were presumed to represent daughter cells, mother cells and mother cells with buds. Over the course of the fermentations, presumed sub-populations of daughter cells increased and presumed sub-populations of mother cells with buds decreased, implying cell size (measured by the flow cytometer) tended towards uniformity.

Conclusion and Future Work

These initial results suggest zymolectin levels were not constitutive during the lab scale fermentations and could be genetically induced. It is believed this new assay has more specificity and sensitivity then past methods that attempted to study zymolectin presence on the yeast cell. Future work plans to validate the assumptions made herein and extract more pertinent information from the large data sets with improved gating methods based on cell size and age.

References

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