Examination of Growth and Capsule Formation in Mutant Strains of the Oleaginous Yeast, *Cryptococcus neoformans* to be used in Biodiesel Production

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Abstract

The burning of fossil fuels is a main cause of global climate change due to the release trapped carbon in the form of CO_2 generated in combustion engines. There is a need to find more carbon neutral and renewable fuels that can be used to power combustion engines in airplanes, trains, and automobiles, since for the power of the combustion engine in unlikely to be replaced in the foreseeable future. Biodiesel has the potential to be a renewable source of cleaner burning energy that can be used to replace the finite resource of fossil fuels. Biodiesels can be made from biological matter with high concentrations of long carbon chains in the form of fatty acids found in fats and oils. Biodiesels have been successfully generated from the fatty acids in animal fats, and plant-based oils, but these sources are ethically, and logistically challenging sources that result in minimal savings on CO_2 release. Our lab has explored the used of oleaginous yeasts that can converting plant sugars to high concentrations of fatty acids bound in fats and phospholipids. One possible system is the encapsulated yeast *Cryptococcus neoformans* that has been thoroughly studied due to its ability to cause illness in immunocompromised individuals. Our preliminary results show that a strain of *C. neoformans* containing a mutation causing a defect in capsule formation that is avirulent, can produce high levels of lipids that can be chemically converted to multiple fatty acid methyl esters that make up biodiesel. In this study, we have been striving to optimize biodiesel production by examining capsule formation in multiple mutant strains and examining the effect media with different Carbon to Nitrogen ratios has on biomass and biodiesel production. We hope that our results will provide a new, renewable system to generate biodiesel on a large-scale.

Introduction

- The combustion of fossil fuels is largely to blame for the gradual warning of our planet: 81% of the 97.1 quadrillion BTUs consumed in the US annually (1).
- Biodiesel, which is produced by the esterification of a lipid with an alcohol is suggested to be an environmentally friendly substitute to fossil fuels (2,3).
- Naturally oleaginous yeasts are a renewable form of lipids that do not compete with food sources such as edible oils (4).
- *Rhodotorula glutinis* is an oleaginous yeast that is recognized for its lipid producing abilities (2,5).
- *Cryptococcus neoformans* is a pathogenic, capsular yeast very similar to *R. glutinis.* Its pathogenicity is directly related to its capsule which protects it from the body and allows it to cause meningoencephalitis and upper respiratory infections in immunocompromised individuals (6,7).
- A capsular yeast is avirulent (8).
- *C. neoformans* has many molecular tools already established that can potentially be used to increase its natural lipid producing ability (8).

Methods

Cell Growth → Centrifugation → Freezing of Cells → Lyophilization

Mechanical and Chemical Extraction → Lipid Quantification after Rotorary Evaporation of Solvents

Saponification → Esterification → Liquid Extraction → India Ink staining

YPD	Basal Media
Yeast Extract + Bactopeptone + Dextrose	1 g of yeast + 120g of dextrose + 0.024 g of NH4H2PO4 + 30g MgSO4- 7H20 + 60g K-H2PO4 + 13.24g CaCl2 + 1g CuSO4-5H2O + 0.02g ZnSO4-7H2O + 0.04g MnSO4-H2O +

Results

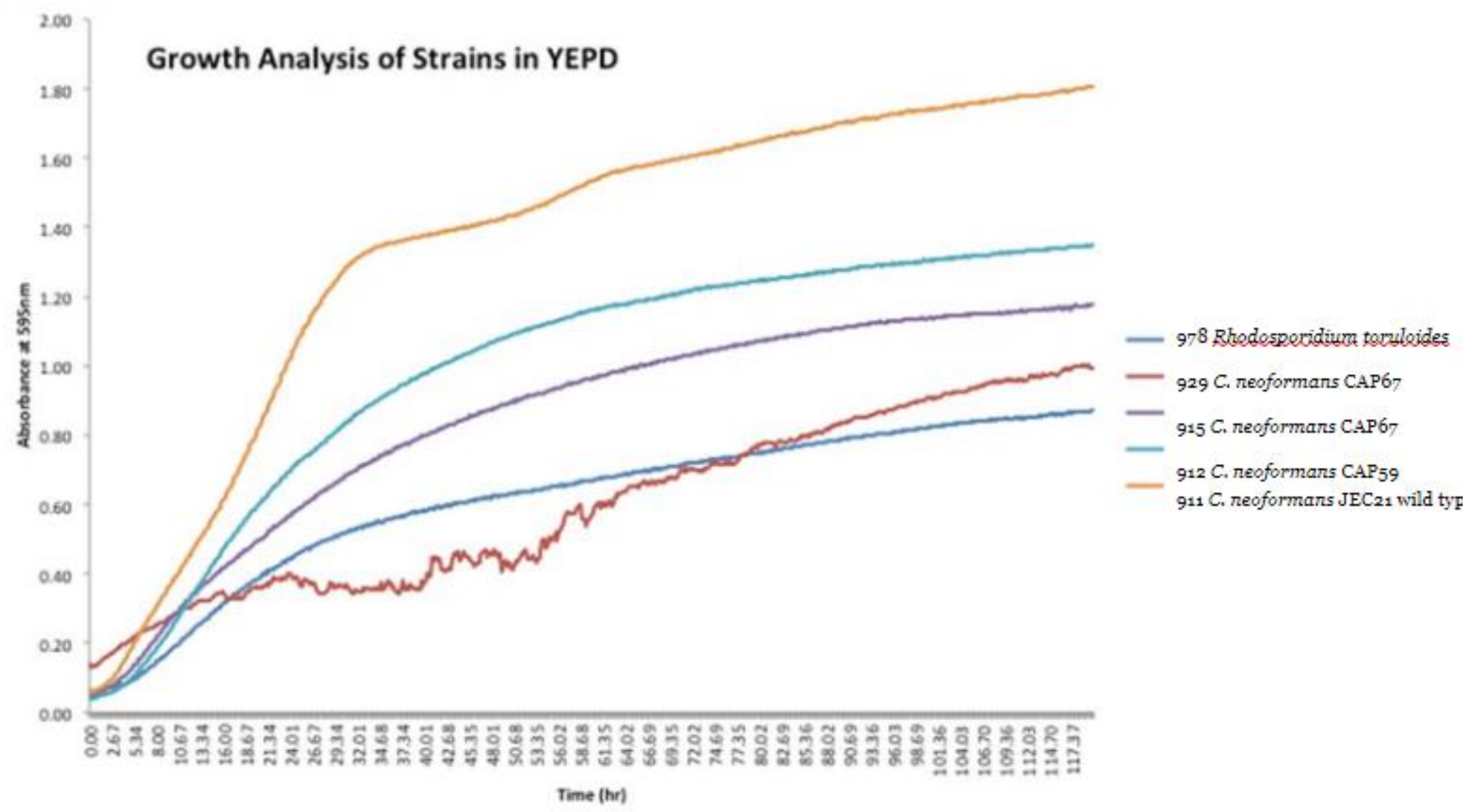
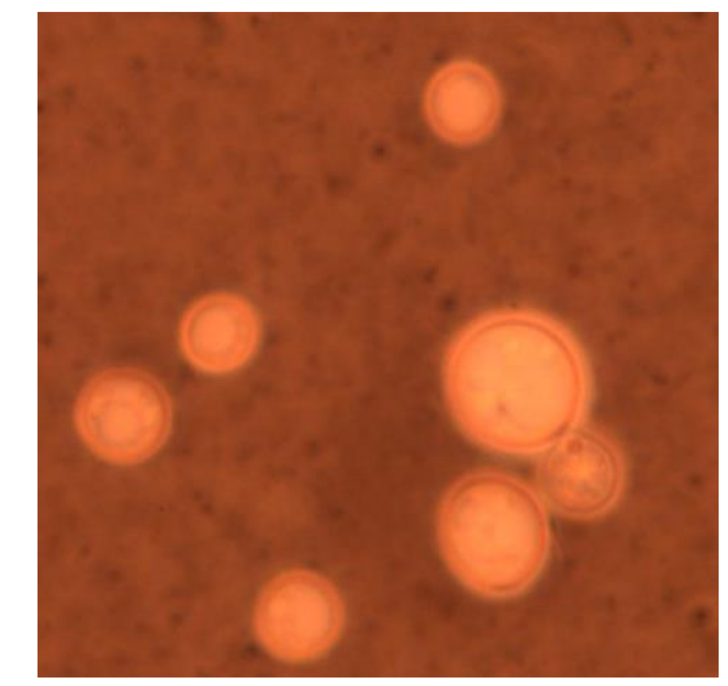


Figure 1. Growth curves of *C. neoformans* in YEPD medium.

Strain (Media)	Lipid Yield (wt. % by dry mass)
CAP59 *C. neoformans* (YPD)	16.0 (+/-) 3.5
CAP59 *C. neoformans* (basal media)	19.3 (+/-) 0.2
JEC21 Wild type *C. neoformans* (YPD)	5.22 (+/-) 2.29
JEC21 Wild type *C. neoformans* (Basal media)	8.11



913 *C. neoformans* Serotype D Wild Type

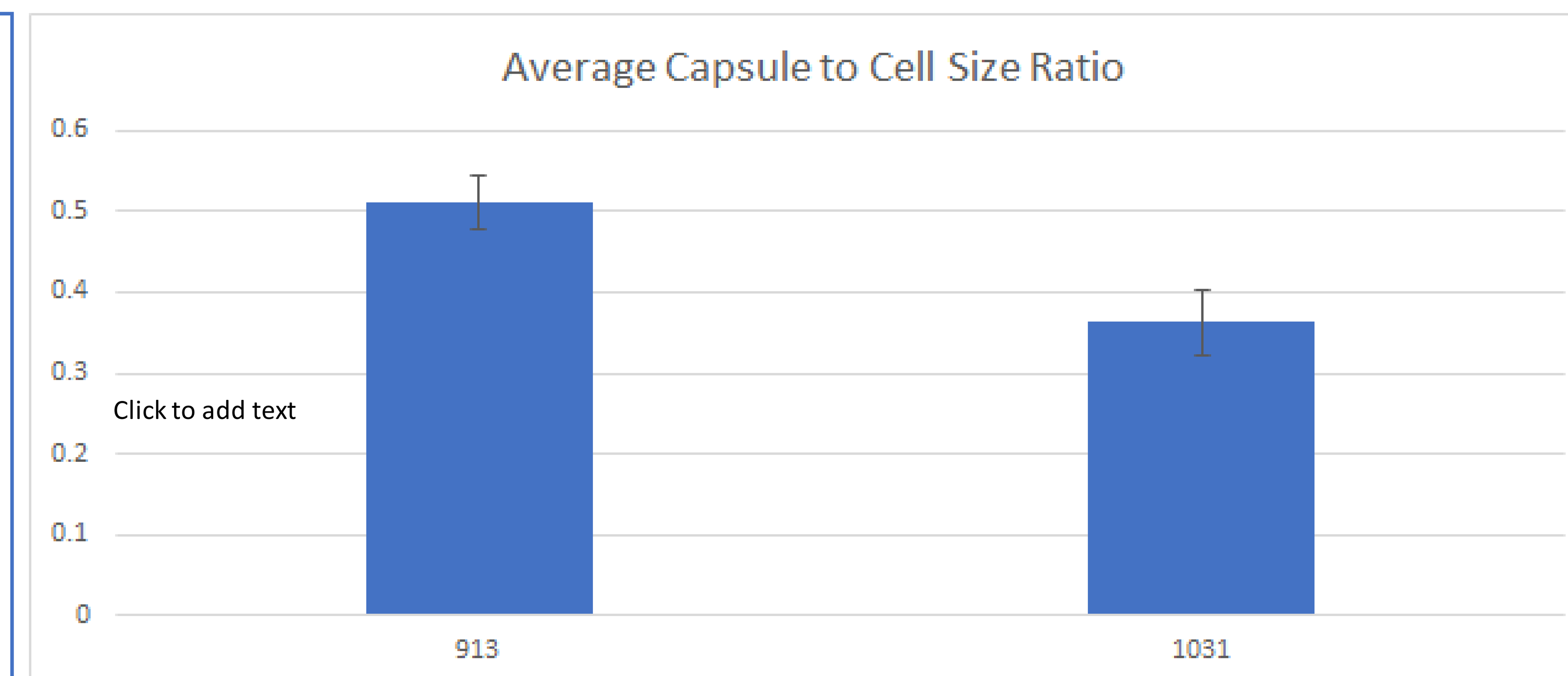
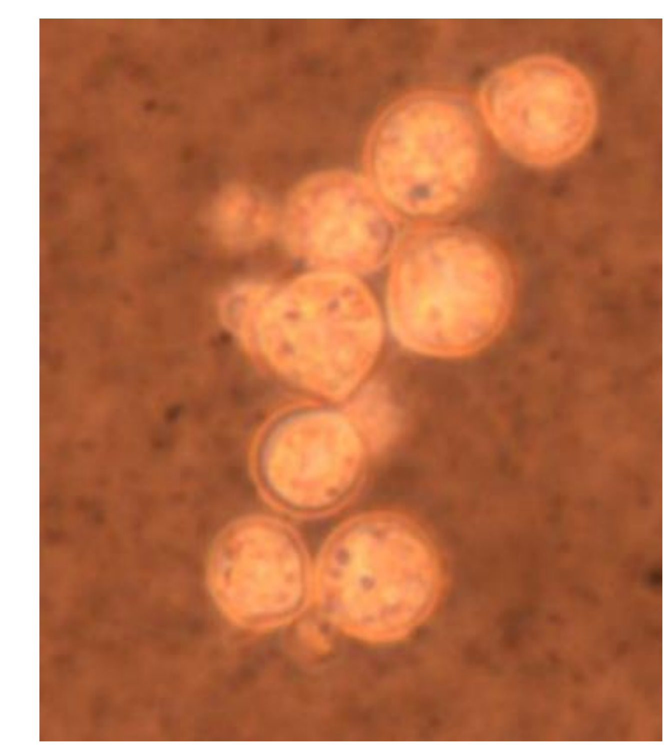


Figure 2. 913 *C. neoformans* serotype D Wild Type and 10301 *C. neoformans* Serotype A KN99a cap59Δ average capsule to cell size ratio grown in YEPD media

Strain	Average Capsule to Cell Size Ratio	SEM
913 *C. neoformans* Serotype D Wild Type	0.511217766	0.032678
1031 *C. neoformans* Serotype A KN99a cap59Δ	0.363088418	0.040524



1031 *C. neoformans* Serotype A KN99a cap59Δ

Future Work

- Test additional acapsular mutant strains
- Inducing capsule mutations in wildtype *Cryptococcus*
- Further optimization of mechanical and chemical extraction methods
- Additional growth curve analysis with all *Cryptococcus* and *Rhodoturula* strains
- Analyze additional *Cryptococcus* mutant and wildtype strains
- Apply India ink to strains and analyze capsules
- Test different recipes of mediums and how well strains grow

Conclusions

- For *Cryptococcus*, strains grown in Basal Media produce better lipid yields by percent than when grown in YPD
- CAP59 shows promising quantities of lipid production but does not form a pellet well after centrifugation
- Found that it is possible for *C. neoformans* to grow on media with the plant sugars lignin and cellulose
- *Cryptococcus* is also able to grow on media containing malt extract
- Mutant CAP59 cannot produce capsule as well as Wild Type
- Incubation with CO_2 will induce capsule growth in both Wild Type and Mutant strains

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Acknowledgements

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