# Characterizing fungal inhibitors in drought-stressed switchgrass

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**Project goals: The overarching goal of this project is to identify fungal inhibitors from drought-stressed switchgrass using extraction techniques and use this information to develop strategies to overcome biomass variability and ensure consistent biofuel generation.**

Development of economically viable and greener pathways to synthesize renewable energy has become an important research theme in recent years. Lignocellulosic biomass is a promising group of feedstocks that can be used for second-generation biofuel production. Recent research has showed that biomass characteristics are altered by environmental growth conditions, and directly influence the extent of biomass conversion to fuels. Specifically, it was reported that drought experienced during the growth of switchgrass led to complete inhibition of yeast growth during fermentation [1]. Our goal in this project was to characterize specific compounds that led to this inhibition and to determine whether the microbial-inhibitors are plant-generated compounds, by-products of the pretreatment process, or a combination of both. Switchgrass harvested in drought (2012) and non-drought (2010) years were pretreated using Ammonia Fiber Expansion (AFEX). Untreated and AFEX processed samples were then extracted using solvents (i.e. water, ethanol, and ethyl acetate) to selectively remove potential inhibitory compounds and determine whether pretreatment affects the inhibition. High solids loading enzymatic hydrolysis was performed on all samples followed by fermentation using yeast strain *Saccharomyces cerevisiae* (GLBRCY945). Cell growth ($OD_{600}$), sugar consumption, and ethanol production were used to evaluate fermentation performance. Extracts were analyzed using liquid chromatography-mass spectrometry (LC-MS) to identify potential inhibitory compounds. Tandem MS analysis was conducted to annotate the identities of unknown inhibitors. We found that water extraction of drought-year switchgrass before AFEX pretreatment was most effective in overcoming yeast inhibition. We also identified numerous saponins, a class of plant-generated triterpene glycosides, which were significantly more abundant in the water extracts from drought-year (inhibitory) switchgrass. These compounds are commonly known as natural laundry detergents and have been reported as toxic to eukaryotic cells by various researchers [2]. Our analysis showed that plant generated compounds inhibited the conversion and that including a water extract step might reduce the inhibition and increase the biofuel yield from biomass.

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## References

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[2] Heng W., et al (2014). *Industrial Crops and Products*, 61:422-429.