2021 ASBC Research Council Grantee

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Project Title*: Identification of* Fusarium graminearum *strains producing different hydrophobin structural variants, and evaluation of the expression of hydrophobin genes in the field and during malting*

Project Intro:

Hydrophobins are small-secreted proteins found in filamentous fungi that play roles in fungal growth and development. The genome of Fusarium graminearum contains five genes encoding for hydrophobins (FgHyd1-5). Fusarium can produce hydrophobins during malting which can survive throughout the brewing and end up in beer. Hydrophobins are one of the factors inducing the primary beer gushing which have been studied for decades. Currently, there is no effective method developed for gushing prediction in large numbers of samples. Although people try to detect hydrophobins quantitatively, it is assumed that the ability of hydrophobins to induce gushing also varies due to differences in their structure (i.e., resulting from variation in amino acid sequence). In this study, the hydrophobin production rate will be measured using F. graminearum strains showing different amino acid sequences in hydrophobin genes, and their rate of hydrophobin production during malting will be assessed. The different hydrophobin structural variants will be purified, and their gushing-inducing abilities will be measured. Transcript levels of Fusarium hydrophobins will be investigated via RNA extraction and RT-PCR, to study the differences in hydrophobin gene expression in Fusarium strains in barley and during malting. Quarantin et al. (2019) have characterized the role of Fusarium hydrophobins in fungal growth and plant infection. However, they only investigated hydrophobins in a single F. graminearum genotype, and the hydrophobin gene expression during malting needs to be clarified. This study will provide information on induction and regulation of hydrophobin production in F. graminearum, thus can improve the prediction of beer gushing.

Project Objectives:

1. Identify F. graminearum strains that possess differences in hydrophobin protein sequence a. From prior research, we know the DNA sequences of each of the five hydrophobin genes (hyd1-hyd5), for 7 different Fusarium graminearum strains. This will be helpful to identify the amino acid differences in the hydrophobin proteins produced by different Fusarium strains. After identifying the differences in hydrophobin gene sequences, primers will be designed for five genes encoding for hydrophobins and these primers will be used to assess the gene expression.

2. Evaluate the rate of hydrophobin production among the Fusarium strains in culture a. In vitro fungal culturing will be done in selected media. The hydrophobins will be extracted and purified from wet mycelium with 1% SDS in 100 mM Tris-HCl buffer (pH 9) followed by KCl precipitation to remove SDS and hydrophobic interaction chromatography. The hydrophobin content will be determined using Reverse phase High-Performance Liquid Chromatography in the department of Chemistry. Protein analysis to

detect hydrophobins will be done via SDS-PAGE and immunoblotting. The purified hydrophobins will be used for gushing experiments at the Canadian Malting Barley Technical Centre.

3. Assess the rate of hydrophobin production in Fusarium strains during the malting process a. Barley plants will be grown in growth chambers. For each Fusarium strain to be included, spores will be produced by cultivation in mung bean broth. Spore suspensions will be adjusted to a standard dose and used to spray-inoculate barley plants at anthesis. Harvested grain will be used to perform lab-based malting trials according to the procedure in my current research and malt extract will be collected from micro malted barley grain. Reverse phase High-Performance Liquid Chromatography in the department of Chemistry will be used to quantify the hydrophobin protein and to measure the rate of hydrophobin production at each stage during malting.

4. Investigate differences in hydrophobin gene expression of Fusarium strains in the field and during malting a. Expression analysis will be performed using RNA extraction from spikelets collected from barley spikes inoculated with F. graminearum strains. And RNA extraction will also be done at each step during the malting of barley infected with individual strains of F. graminearum. Complementary DNA will be prepared using a cDNA synthesis kit and the designed primers will be used for expression analysis. The expression level of hydrophobin genes will be analyzed by RT-PCR.