



2022 ASBC Research Council Grantee

Principle Investigator: Gregg Johnson

Academic Institution: Point Park University, Pittsburgh, PA, U.S.A.

Project Title: Identifying the Root Cause of Hop Creep

Project Intro:

Hop creep is a phenomenon in which dry hopped beer undergoes an unpredictable secondary fermentation. The unpredictable nature can generate product that needs to be disposed of due to unsafe pressure in containers or a difference between measured and labeled alcohol content, which may violate labeling standards. Hop creep is likely caused by enzymes such as alpha and beta amylase in the hops cones converting unfermentable sugars left over after the primary fermentation into fermentable sugars. The fermentable sugars that result are then fermented by yeast remaining in the fermenter or final packaging. The initial source of the enzymes is unclear. They may be natively produced by the hops plants or introduced via microbes traveling along with the hops. Kirkpatrick and Shellhammer (2018) measured the enzyme power of the hops but did not differentiate between native enzymes or microbial enzymes. Teraoka et al. (2021) produced results that suggest the source of the enzymes are likely microbial, but more evidence is needed to support this hypothesis. We hypothesize, based on the work of Teraoka et al. (2021), that the enzymes' origins are microbial. To test this, we will culture microbes present on the hops plants and test for hop creep from the microbes directly using 16sRNA sequencing to identify the species. To test the alternate hypothesis regarding native enzymes, we will use molecular biological techniques to establish the presence or absence of native enzymes in the hops with rtPCR and/or protein analysis.

Project Objectives:

We propose 4 sets of experiments to address the following question: Does the enzyme activity that converts nonfermentable sugars into fermentable sugars after dry-hopping originate in the hops material natively or with microbes growing on the hops material? We hypothesize that the activity is from a microbial source based on the results of Teraoka et al. (2021)

- To sample bacteria living on hops plants and culture them, demonstrate whether they have the capacity to cause hop creep, and identify the species of bacteria with DNA sequencing. Identifying a microbial species capable of causing a second fermentation that is growing on hops is evidence of the microbial source hypothesis and may offer insight to the brewing community to control hop creep.
- To use rtPCR and/or western blotting to establish if native hops alpha and beta amylase are present in hop cones. Their presence would support the native hops hypothesis and we predict they will not be present in significant levels.
- To establish whether azides inhibit plant sourced amylase enzymes, an unanswered question from Teraoka et al. (2021). We predict azides will not inhibit amylase, which supports the microbial source hypothesis.

- To show whether sanitized hop cones do or do not have the capacity to cause hop creep; if it loses the capacity to create fermentable sugars this supports the microbial source hypothesis. Kirkpatrick, K. and Shellhammer, T. (2018). Evidence of dextrin hydrolyzing enzymes in cascade hops (*humulus lupulus*). *Journal of Agricultural and Food Chemistry*, 66(34), 9121-9126. Teraoka, R., Kawauchi, M., and Bamforth, C. (2021). Do starch-degrading enzymes in hop samples originate in microorganisms? *MBAA TQ*, 58(3), 143-147.