A Search for Diastatic Enzymes Endogenous to *Humulus lupulus* and Produced by Microbes Associated with Pellet Hops Driving "Hop Creep" of Dry Hopped Beer

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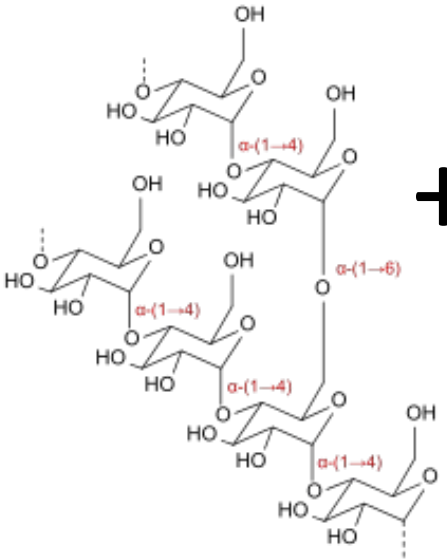
“Hop creep” is the diastatic power of hops

Dextrin with α-(1→4) and α-(1→6) bonds in finished beer

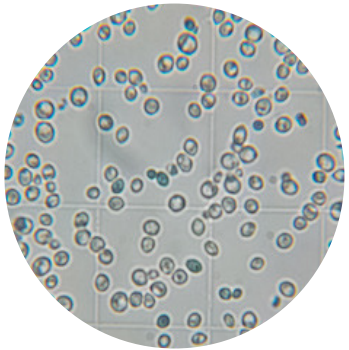


+

Pellet hops

+

Yeast



Fermentation products

CO_2

EtOH

Diacetyl

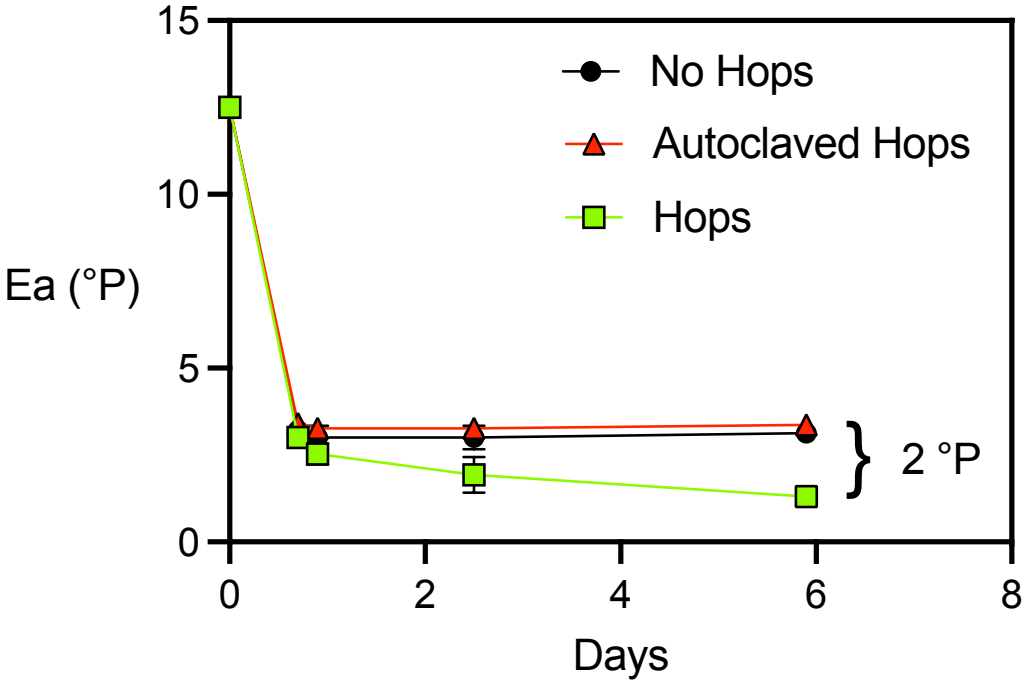
Etc.

en.wikipedia.org/wiki/Dextrin

www.yakimachief.com

braukaiser.com

Pellet hops produce 2°P over attenuation



Forced attenuation
12°P wort
Chico ale yeast

Exogenous

Fungi on hops

David Gent, USDA Agricultural Research Service, Bugwood.org

Endogenous

Hop cone with lupulin glands

Baldurmen , CC BY-SA 4.0, commons.wikimedia.org

Some questions to address

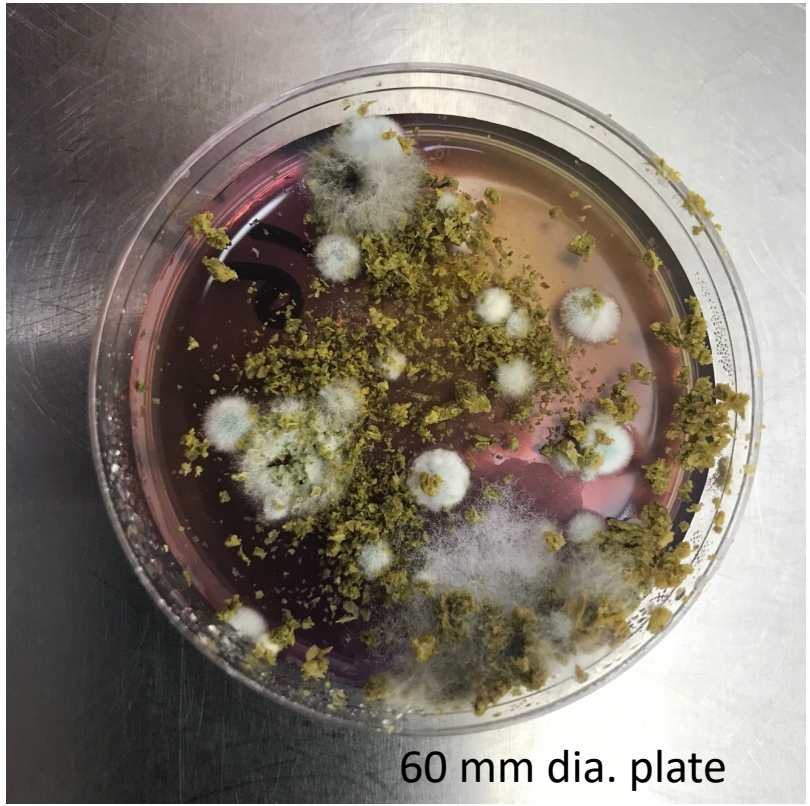
- What are the sources of amylases driving hop creep?
  - Do microbes play a role?
    - Amylase-producing microbes on pellet hops
    - Bacteria
    - Fungi
  - Does *Humulus lupulus* (hops) produce amylases?
- Understanding the source presents options for control

Spoiler Alert

Approach to explore impact of microbes

- Isolate microbes from hops in pure culture
- What types of microbes are found on pellet hops?
    - Bacteria? Fungi?
    - Do any produce amylases?
    - Does adding the microbes to beer produce hop creep?

Agar plate inoculated with 0.1 g of Cascade pellet hops



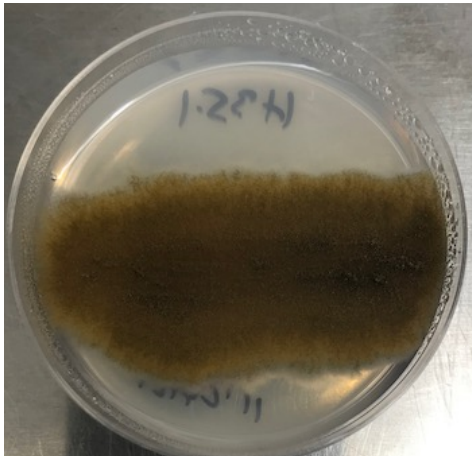
~18 fungal colonies
after seven days at 30°C

Fungi isolated from pellet hops

Penicillium sp.

63%

Alternaria sp.



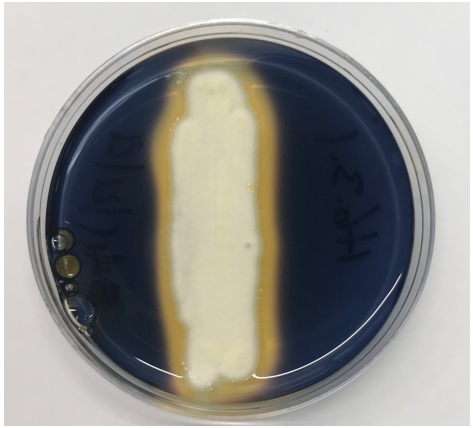
37%

Percent of fungus isolates

Azacca
Cascade
Centennial
Citra
Galaxy
Mosaic

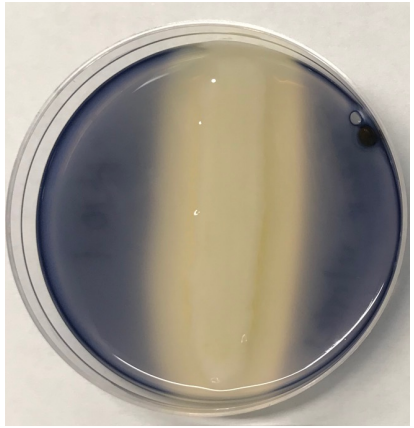
Starch degrading fungi and bacteria

Fungus



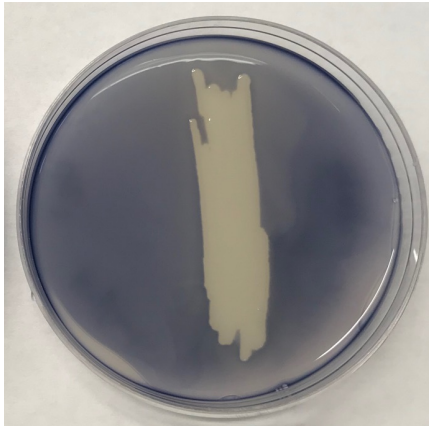
8 of 40 fungi

Bacterium

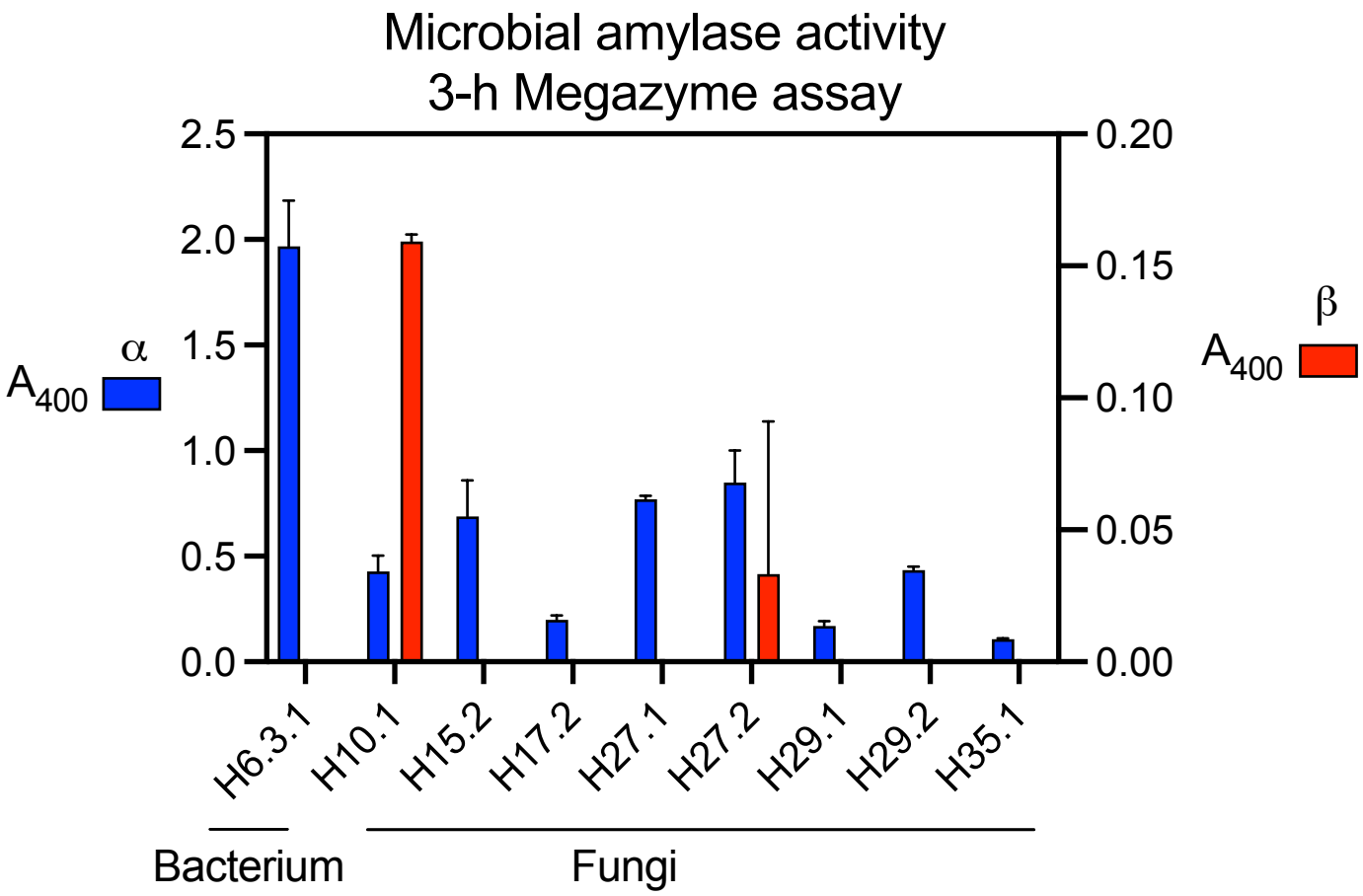


1 of 28 bacteria

Control



Clearing of iodine staining indicates starch degradation


Microbial amylase activity
3-h Megazyme assay
α
A400
β
A400
2.5
2.0
1.5
1.0
0.5
0.0
0.20
0.15
0.10
0.05
0.00
H6.3.1
H10.1
H15.2
H17.2
H27.1
H27.2
H29.1
H29.2
H35.1
Bacterium
Fungi

Do bacteria and fungi drive hop creep?

- Add microbes to wort and beer
  - Look for over attenuation and continued fermentation
    - Extract consumption
    - Alcohol production
- Controls with hops or no addition

Do bacteria and fungi drive hop creep?

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  - Look for over attenuation and continued fermentation
    - Extract consumption
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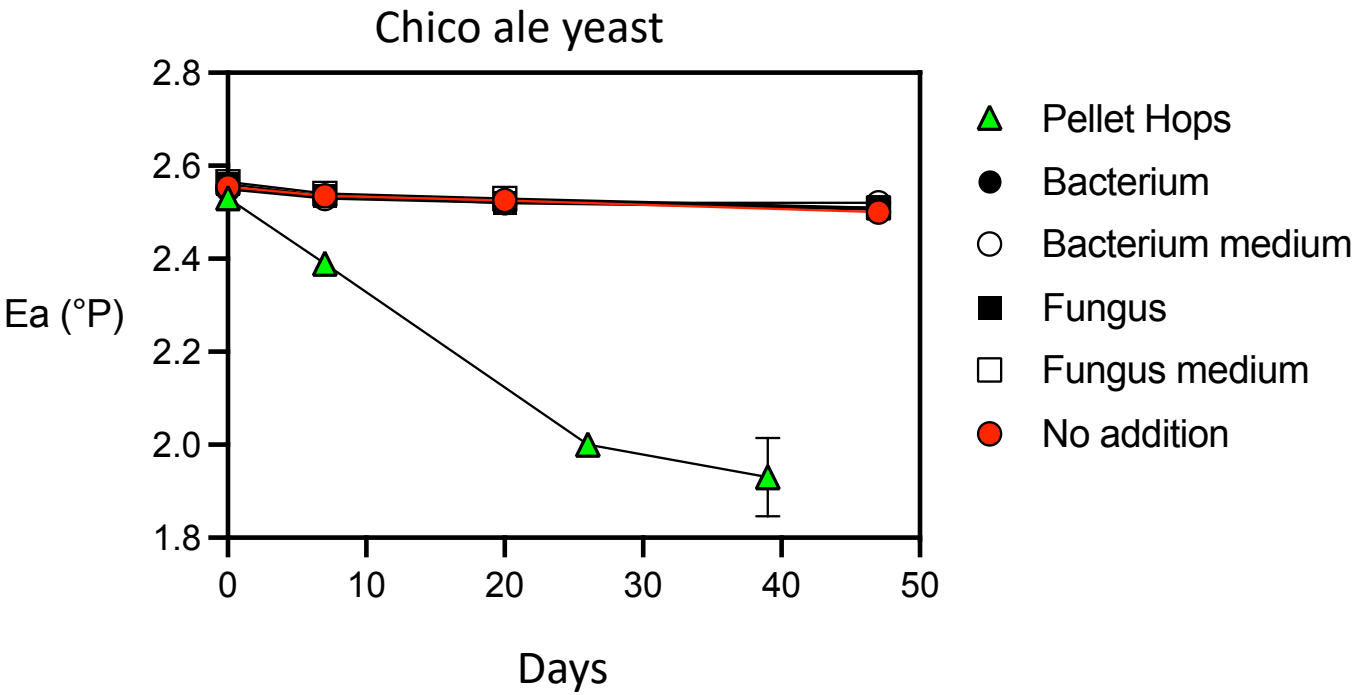
- Controls with hops or no addition

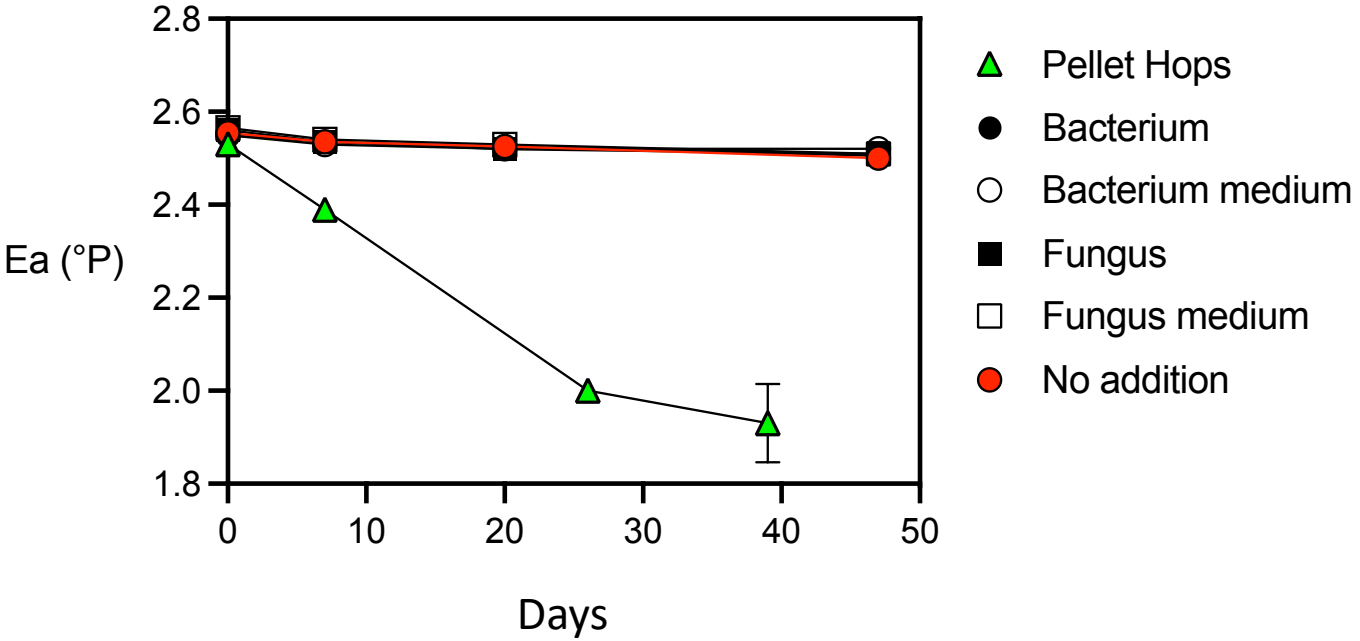
No evidence that these microbes drive hop creep

Continued fermentation of Pale Ale

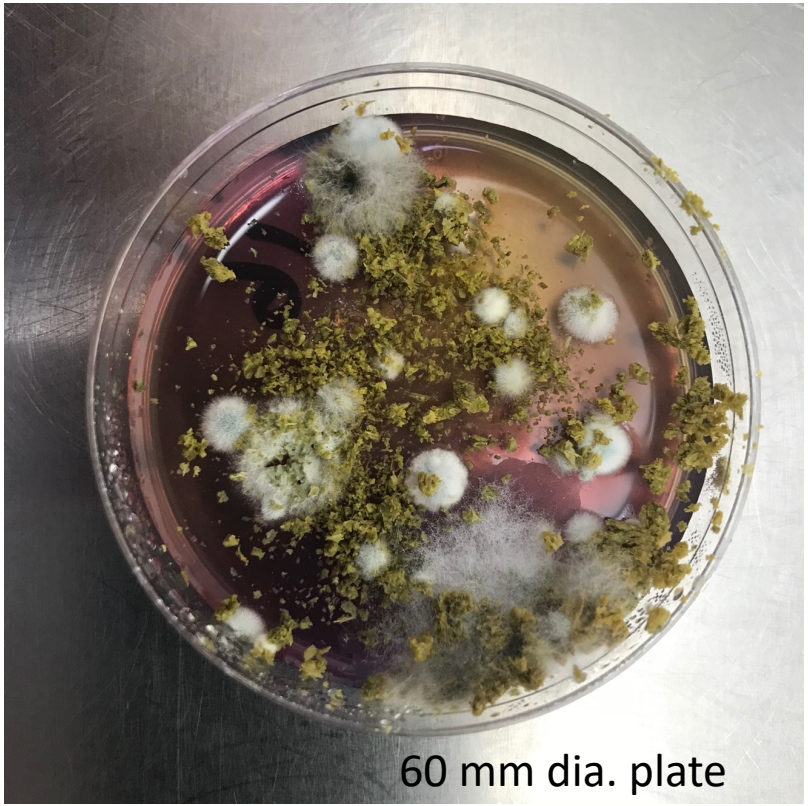


Continued fermentation of Pale Ale

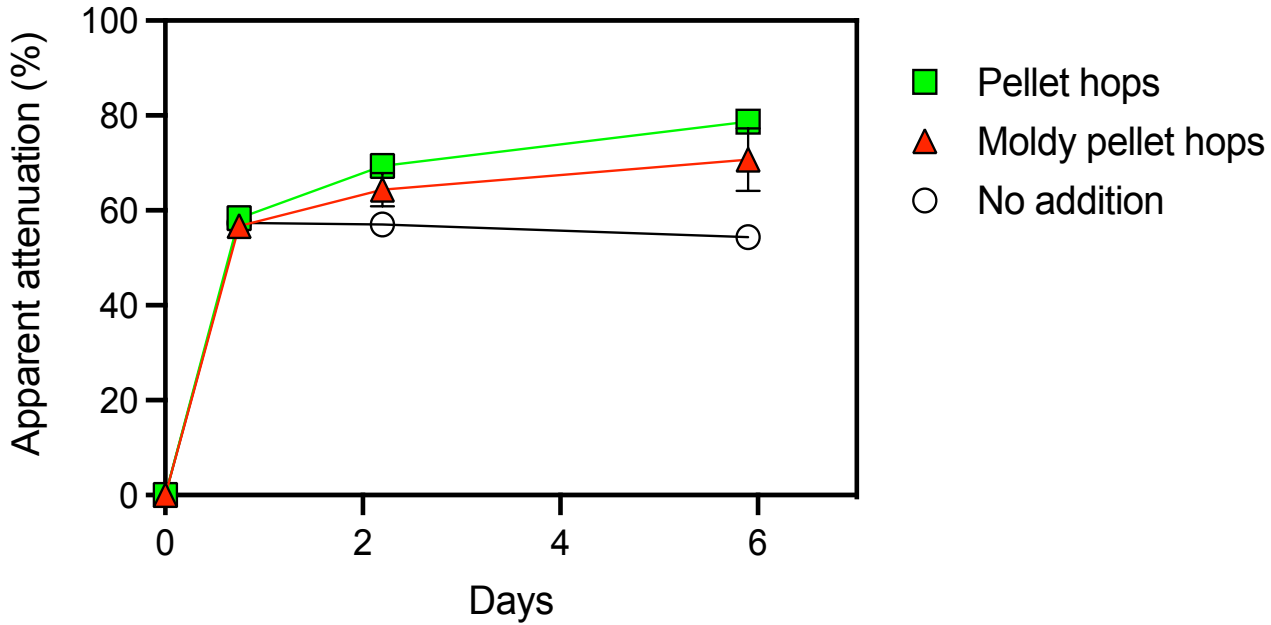
Spent bacterial and fungal media contained amylases!



Moldy pellet hops



Moldy pellet hops no more diastatic than fresh pellet hops



Search for endogenous hop amylases

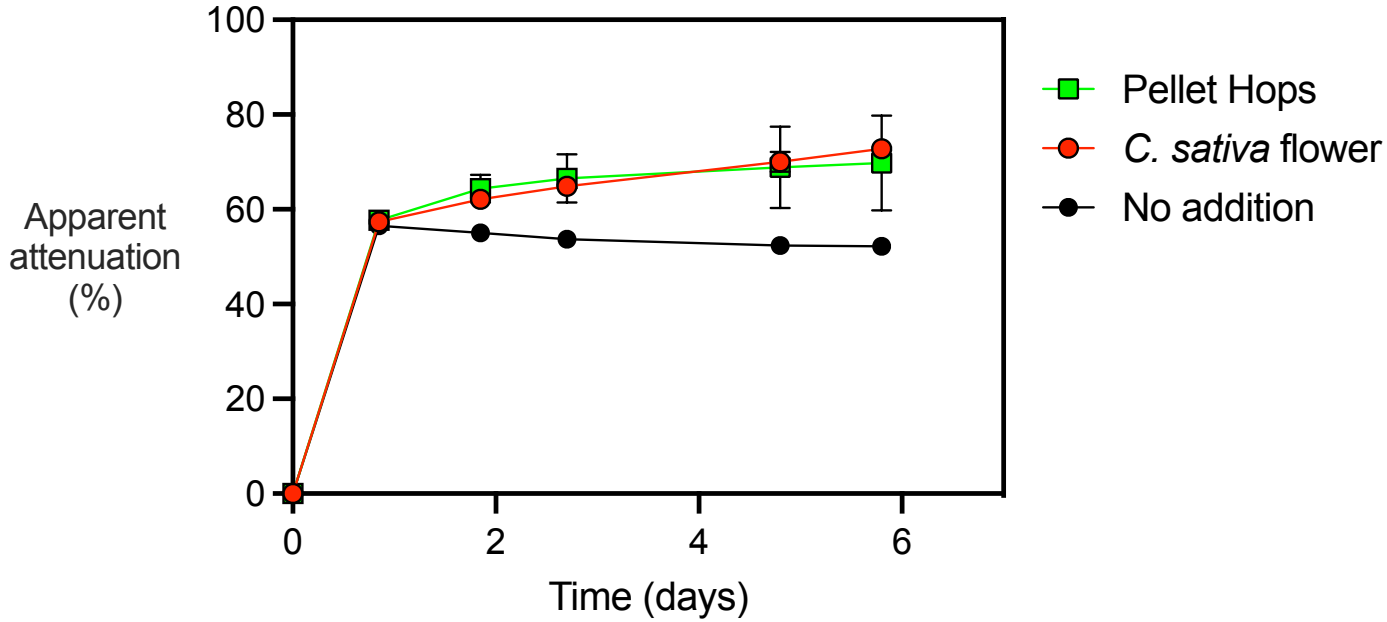
Search for endogenous hop amylases

- Success seemed unlikely
  - Starch metabolism not featured in hop physiology
  - No published literature on hop amylases
- Questions before proceeding
  - Is hop creep specific to hops?
  - Non-specific effect of any plant material?

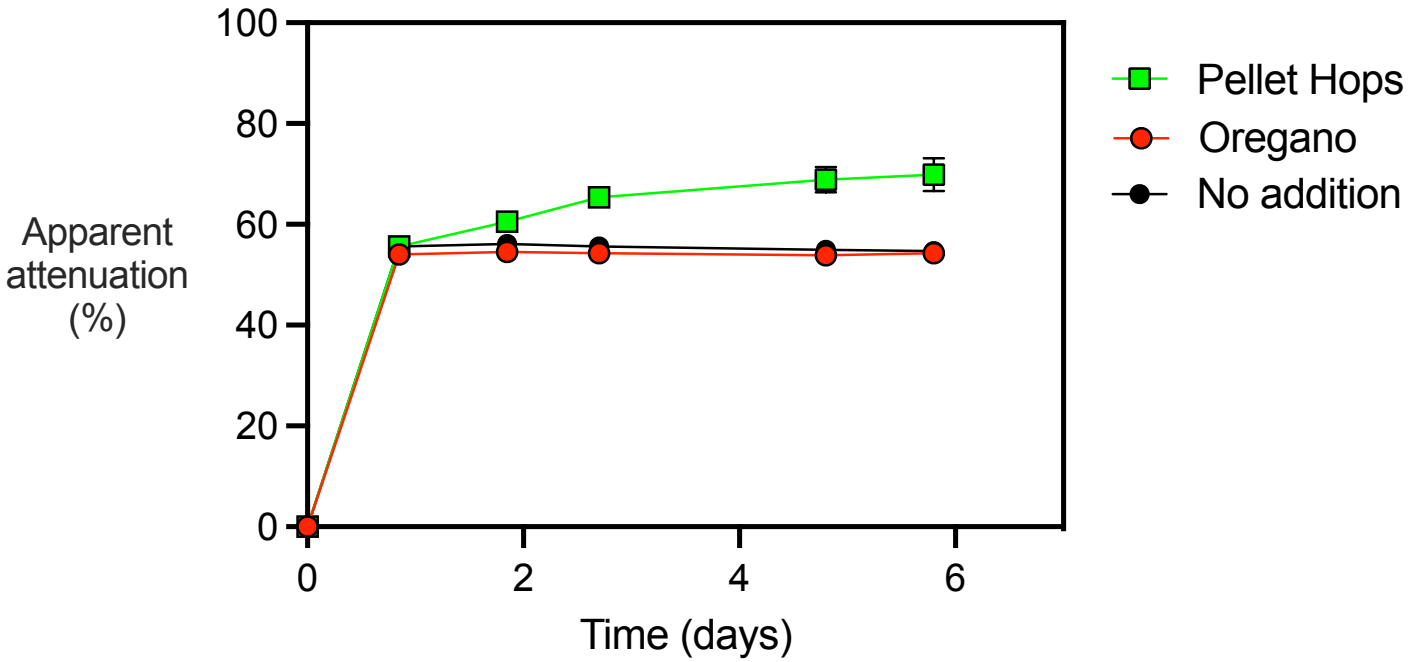
Negative control plant material

Cannabaceae Family	
Aphananthe	*Humulus*
Cannabis	*Lozanella*
Celtis	*Parasponia*
Chaetachme	*Pteroceltis*
Gironniera	*Trema*

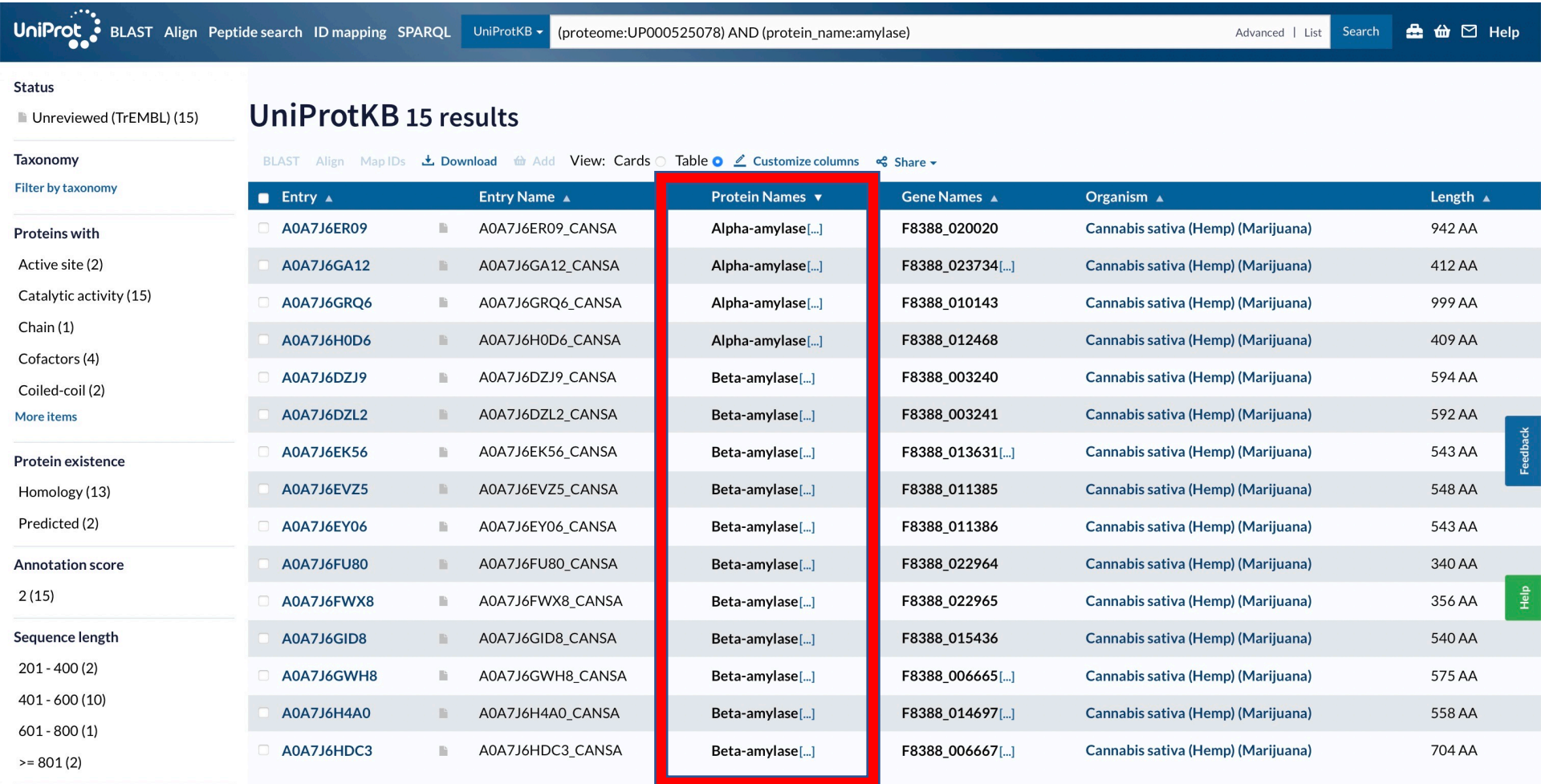
Diastatic power of *Cannabis sativa* flower



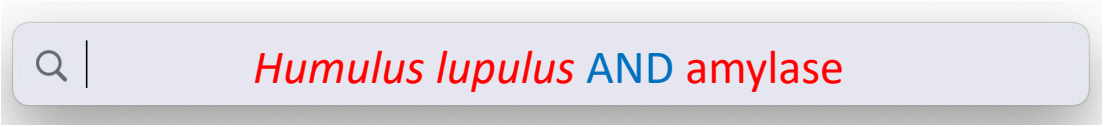
No diastatic power of oregano herb



Cannabis sativa proteome includes 15 amylases



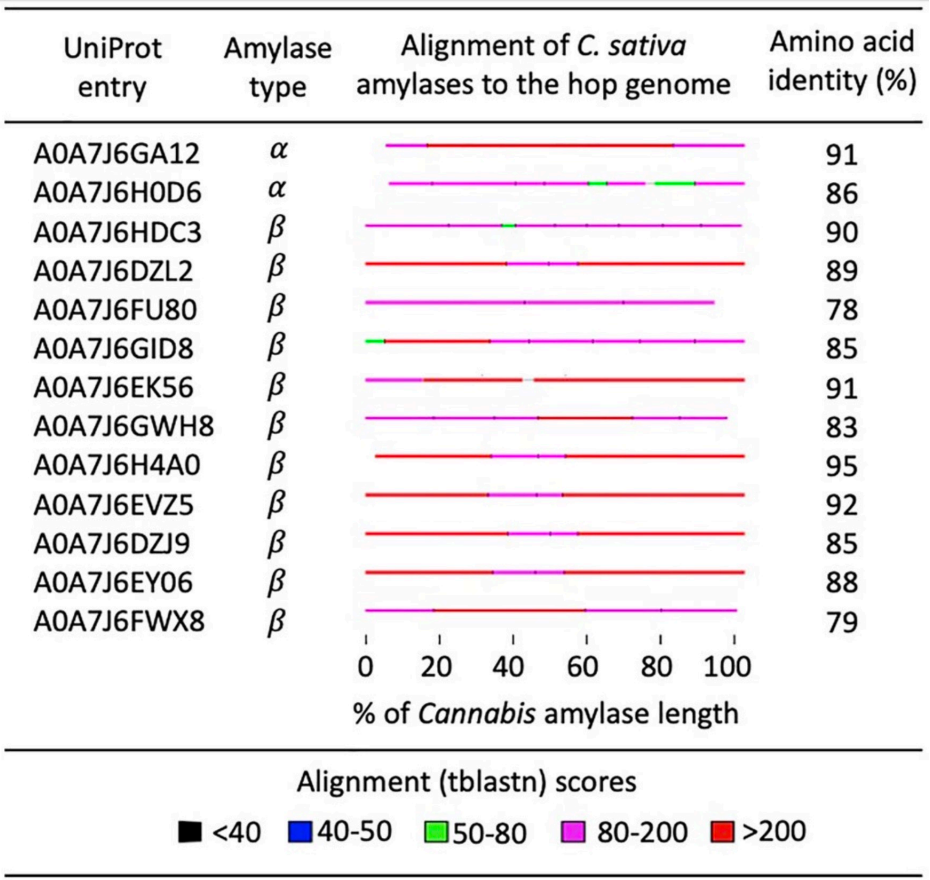
No *H. lupulus* genome or proteome annotation



- Text search for “amylase” not possible
- Use bioinformatics and *Cannabis* amylases to find similar genes in *H. lupulus* genome

Cannabis sativa amylases aligned to hop genome

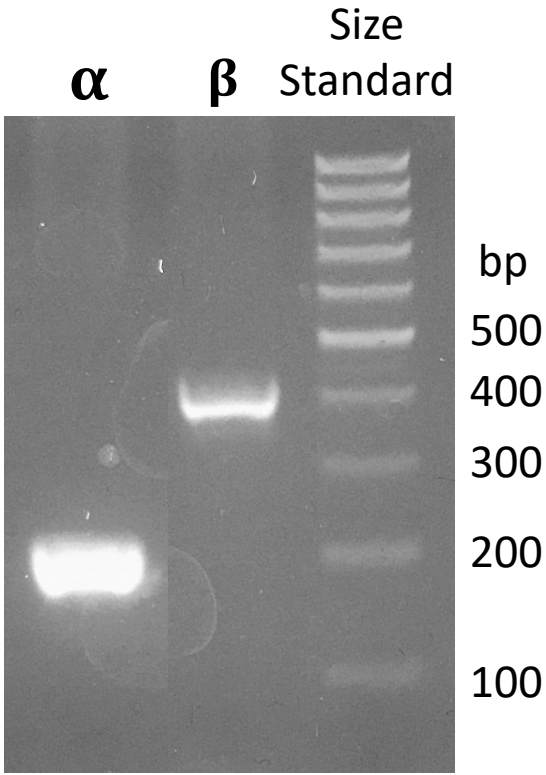
C. sativa amylase proteins



Translated hop genes

Hop amylase gene PCR amplicons

Amplicons match expected ~200 bp and ~400 bp sizes



Confirmed by DNA sequencing

plants

MDPI

Article

Freezing Tolerance and Expression of β-amylase Gene in Two *Actinidia arguta* Cultivars with Seasonal Changes

Kiwi fruit

Shihang Sun [1,2], Jinbao Fang [1,*], Miaomiao Lin [1,*], Xiujuan Qi [1], Jinyong Chen [1], Ran Wang [1], Zhi Li [1], Yukuo Li [1] and Abid Muhammad [1]

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Research article

Establishment of CRISPR/Cas9 mediated targeted mutagenesis in hop (*Humulus lupulus*)

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ABSTRACT

The CRISPR/Cas9-based targeted genome editing has emerged as a versatile technique, widely employed in plant genome engineering, both to decipher gene function and as an alternative to classical breeding technique for traits improvement in plants. However, to date, no such platform has been developed for hop (*Humulus lupulus* L.), which is an economically important crop producing valuable secondary metabolites utilized in the brewing and pharmaceutical industries. Here, we present the first report on the successful establishment of efficient CRISPR/Cas9-based genome editing using the visible endogenous marker gene *phytoene desaturase* (*PDS*)

Take-home message

- Two sources of amylases identified
  - Exogenous bacterial and fungal amylases
  - Endogenous hop amylases
- Direct evidence of microbial involvement still lacking
- Transcription and expression of hop amylase genes not yet demonstrated
- Hop amylases potential targets for blocking hop creep

Resources

Cottrell MT. 2022. A search for diastatic enzymes endogenous to *Humulus lupulus* and produced by microbes associated with pellet hops Driving “hop creep” of dry hopped beer.

J Am Soc Brew Chem

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Q&A