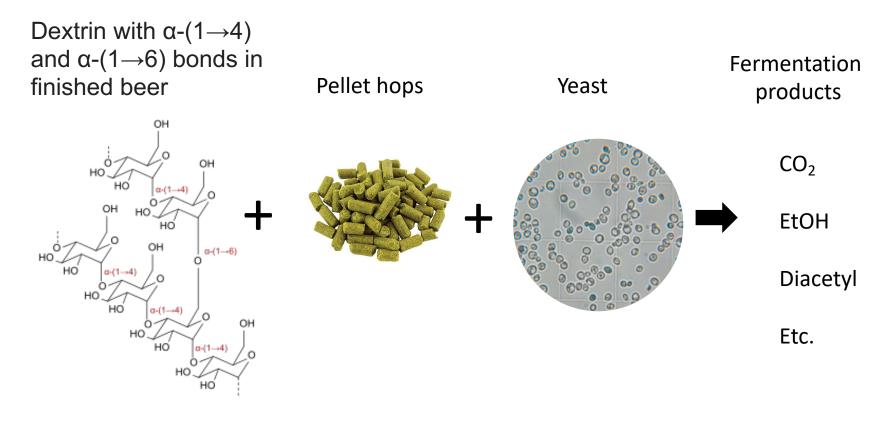
A Search for Diastatic Enzymes Endogenous to *Humulus lupulus* and Produced by Microbes Associated with Pellet Hops Driving "Hop Creep" of Dry Hopped Beer

Matthew T. Cottrell Quality Manager and Microbiologist Heavy Seas Beer, Baltimore, MD



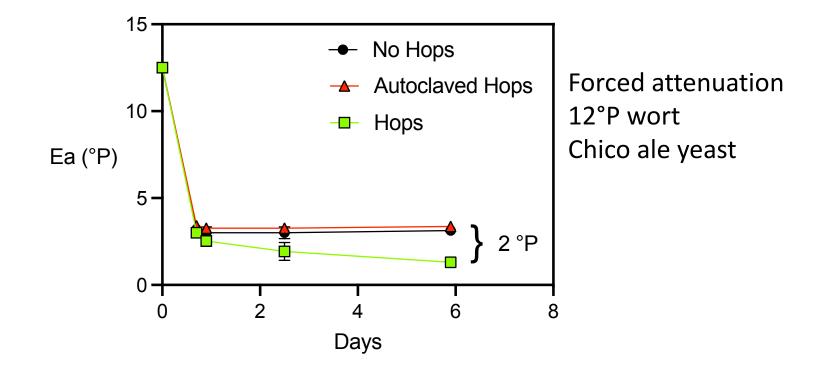
#### "Hop creep" is the diastatic power of hops



www.yakimachief.com

braukaiser.com

#### Pellet hops produce 2°P over attenuation



#### **Exogenous** Fungi on hops

### Endogenous

Hop cone with lupulin glands



David Gent, USDA Agricultural Research Service, Bugwood.org



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

#### Some questions to address

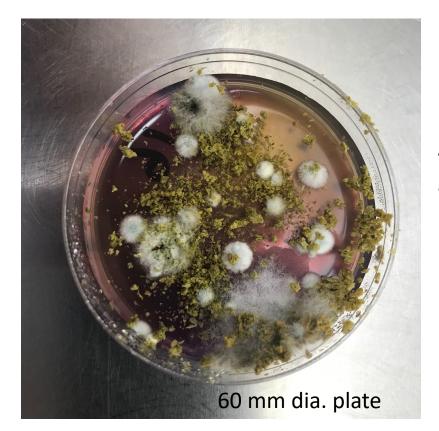
- What are the sources of amylases driving hop creep?
  - Do microbes play a role?
    - Amylase-producing microbes on pellet hops
    - Bacteria
    - Fungi
  - Does *Humulus lupulus* (hops) produce amylases?
- Understanding the source presents options for control

## **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?

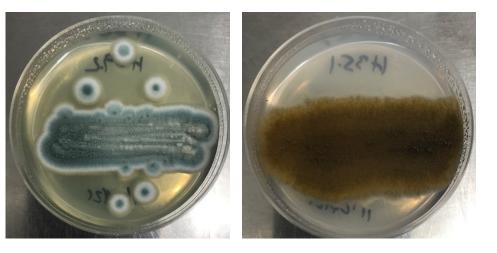
#### Agar plate inoculated with 0.1 g of Cascade pellet hops



~18 fungal colonies after seven days at 30°C

#### Fungi isolated from pellet hops

#### Penicillium sp. Alternaria sp.



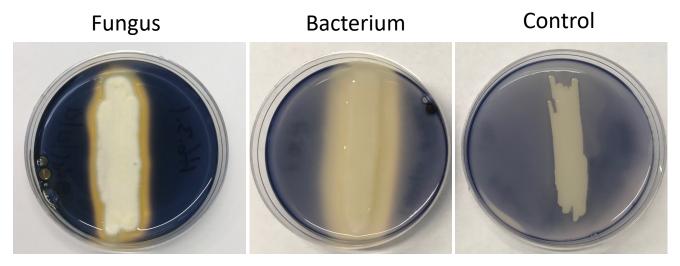
Azacca Cascade Centennial Citra Galaxy Mosaic

63%

37%

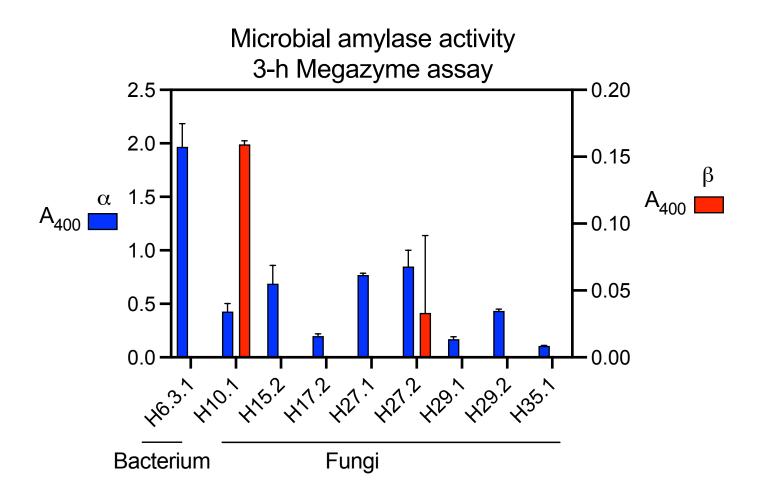
#### Percent of fungus isolates

#### Starch degrading fungi and bacteria



8 of 40 fungi 1 of 28 bacteria

Clearing of iodine staining indicates starch degradation



### Do bacteria and fungi drive hop creep?

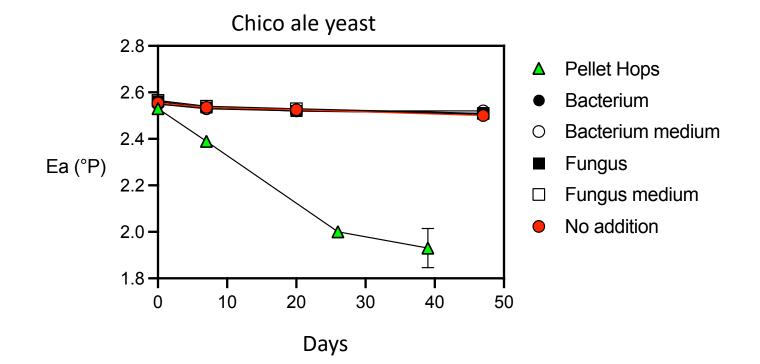
- Add microbes to wort and beer
  - Look for over attenuation and continued fermentation
    - Extract consumption
    - Alcohol production
- Controls with hops or no addition

### Do bacteria and fungi drive hop creep?

- Add microbes to wort and beer
  - Look for over attenuation and continued fermentation
    - Extract consumption
    - Alcohol production
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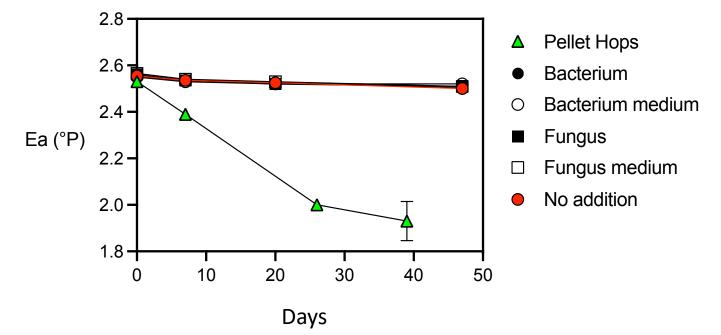
No evidence that these microbes drive hop creep

#### Continued fermentation of Pale Ale

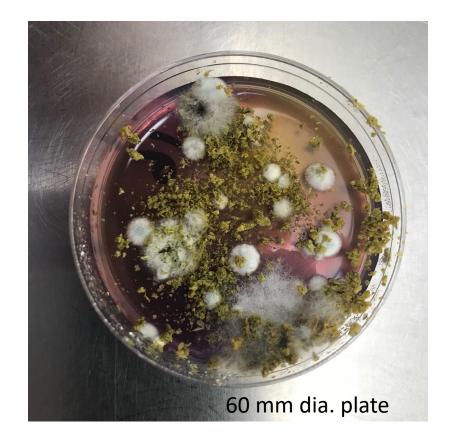


#### Continued fermentation of Pale Ale

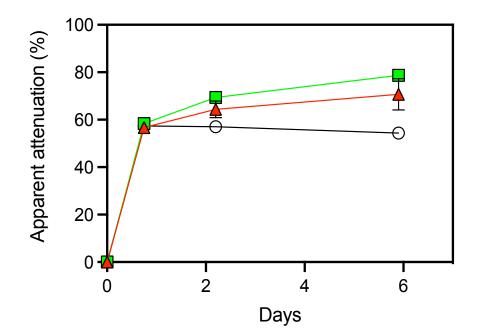
Spent bacterial and fungal media contained amylases!



#### Moldy pellet hops



# Moldy pellet hops no more diastatic than fresh pellet hops



- Pellet hops
- Moldy pellet hops
- O No addition

### Search for endogenous hop amylases

### Search for endogenous hop amylases

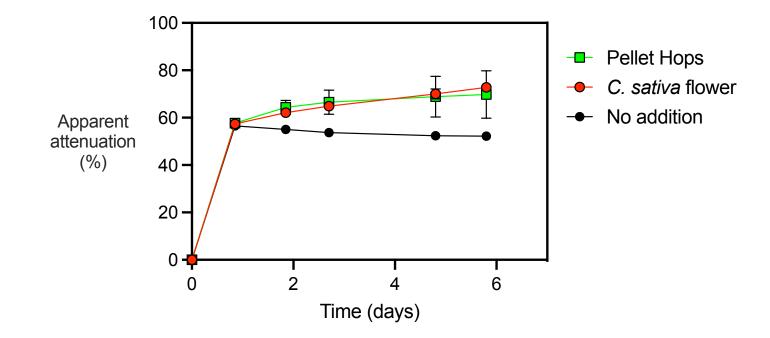
- Success seemed unlikely
  - Starch metabolism not featured in hop physiology
  - No published literature on hop amylases
- Questions before proceeding
  - Is hop creep specific to hops?
  - Non-specific effect of any plant material?

#### Negative control plant material

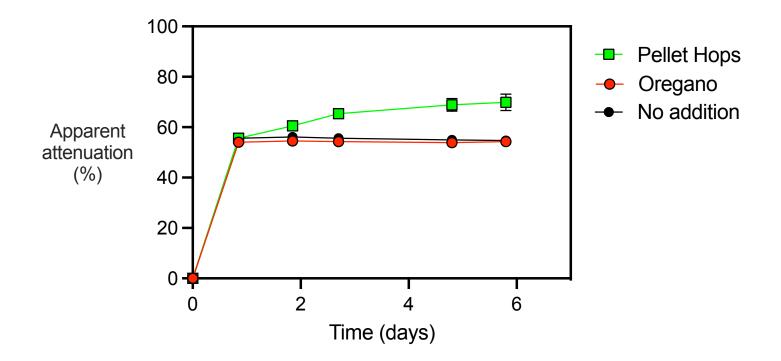
Cannabaceae Family

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

UniProt BLAST Align Peptide search ID mapping SPARQL UniProtKB - (proteome:UP000525078) AND (protein\_name:amylase)

Advanced | List Search

🚔 🏠 🗹 Help

#### Status

Unreviewed (TrEMBL) (15)

#### **UniProtKB** 15 results

#### Taxonomy

BLAST	Align	Map IDs	🛨 Download	🔂 Add	View: Cards	Table 🔾	0	Customize columns	ŝ	Share
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Filter by taxonomy	Entry A		Entry Name 🔺	Protein Names 🔻	Gene Names 🔺	Organism 🔺	Length 🔺
Proteins with	A0A7J6ER09	lì	A0A7J6ER09_CANSA	Alpha-amylase[]	F8388_020020	Cannabis sativa (Hemp) (Marijuana)	942 AA
Active site (2)	A0A7J6GA12	III.	A0A7J6GA12_CANSA	Alpha-amylase[]	F8388_023734[]	Cannabis sativa (Hemp) (Marijuana)	412 AA
Catalytic activity (15)	A0A7J6GRQ6	lì	A0A7J6GRQ6_CANSA	Alpha-amylase[]	F8388_010143	Cannabis sativa (Hemp) (Marijuana)	999 AA
Chain (1)	A0A7J6H0D6		A0A7J6H0D6_CANSA	Alpha-amylase[]	F8388_012468	Cannabis sativa (Hemp) (Marijuana)	409 AA
Cofactors (4) Coiled-coil (2)	A0A7J6DZJ9	- Ili	A0A7J6DZJ9_CANSA	Beta-amylase[]	F8388_003240	Cannabis sativa (Hemp) (Marijuana)	594 AA
Protein existence Homology (13) Predicted (2)	A0A7J6DZL2		A0A7J6DZL2_CANSA	Beta-amylase[]	F8388_003241	Cannabis sativa (Hemp) (Marijuana)	592 AA
	A0A7J6EK56	lì	A0A7J6EK56_CANSA	Beta-amylase[]	F8388_013631[]	Cannabis sativa (Hemp) (Marijuana)	Eedback Pack 27
	A0A7J6EVZ5		A0A7J6EVZ5_CANSA	Beta-amylase[]	F8388_011385	Cannabis sativa (Hemp) (Marijuana)	548 AA
	A0A7J6EY06	lì	A0A7J6EY06_CANSA	Beta-amylase[]	F8388_011386	Cannabis sativa (Hemp) (Marijuana)	543 AA
Annotation score	A0A7J6FU80	III.	A0A7J6FU80_CANSA	Beta-amylase[]	F8388_022964	Cannabis sativa (Hemp) (Marijuana)	340 AA
2 (15)	A0A7J6FWX8	li	A0A7J6FWX8_CANSA	Beta-amylase[]	F8388_022965	Cannabis sativa (Hemp) (Marijuana)	356 AA 🚽
Sequence length	A0A7J6GID8		A0A7J6GID8_CANSA	Beta-amylase[]	F8388_015436	Cannabis sativa (Hemp) (Marijuana)	540 AA
201 - 400 (2)	A0A7J6GWH8	lli	A0A7J6GWH8_CANSA	Beta-amylase[]	F8388_006665[]	Cannabis sativa (Hemp) (Marijuana)	575 AA
401 - 600 (10)	A0A7J6H4A0		A0A7J6H4A0_CANSA	Beta-amylase[]	F8388_014697[]	Cannabis sativa (Hemp) (Marijuana)	558 AA
601 - 800 (1) >= 801 (2)	A0A7J6HDC3	lì	A0A7J6HDC3_CANSA	Beta-amylase[]	F8388_006667[]	Cannabis sativa (Hemp) (Marijuana)	704 AA

#### No *H. lupulus g*enome or proteome annotation



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

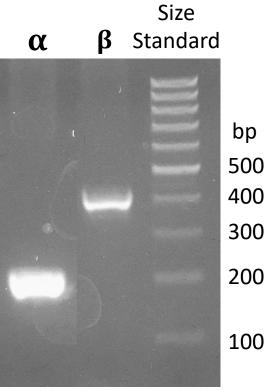
#### Cannabis sativa amylases aligned to hop genome

UniProt entry	Amylase type	am	-		of <i>C. s</i> e hop			Amino acid dentity (%)	
A0A7J6GA12	α	-	-			-		91	
A0A7J6H0D6	α	-				-		86	
A0A7J6HDC3	β	-						90	
A0A7J6DZL2	β	-						89	
A0A7J6FU80	β	-					-	78	Translated
A0A7J6GID8	β							85	
A0A7J6EK56	β	_					_	91	hop genes
A0A7J6GWH8	β	-					_	83	
A0A7J6H4A0	β	_						95	
A0A7J6EVZ5	β	_						92	
A0A7J6DZJ9	β	-						85	
A0A7J6EY06	β	_						88	
A0A7J6FWX8	β	_					_	79	
		Ó	20	40	60	80	100		
		% 0	f Can	nabis	amyla	se le	ngth		
	Alig	nme	ent (tb	lastn	score	es			
	<40 40	)-50	5	0-80	8	0-200	>2	00	

*C. sativa* amylase proteins

#### Hop amylase gene PCR amplicons

Amplicons match expected ~200 bp and ~400 bp sizes



Confirmed by DNA sequencing





#### Article

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

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Research article

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

Praveen Awasthi, Tomáš Kocábek, Ajay Kumar Mishra<sup>\*</sup>, Vishnu Sukumari Nath, Ankita Shrestha, Jaroslav Matoušek

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#### ARTICLE INFO

Keywords: CRISPR/Cas9 Genome editing Hop Phytoene desaturase Transformation and T7E1 assay

#### 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



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

doi.org/10.1080/03610470.2022.2084327

