Biotransformation, Thiols, and Yeast: Oh My! - NanoCon Virtual

Brittney Christianson, December 2021

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Primary Points:

1. Basic Understanding of Biotransformation
2. Overview of Thiols
3. Thiol Precursors
4. Enzymes and Yeast Influence Re: Thiols
5. Practical Take Home

Biotransformation: What does it mean?

Biotransformation

- What does biotransformation mean?

  In the most basic terms, biotransformation is the alteration or chemical modification of a compound by an organism

- In brewing terminology:

  "Yeast derived impact or modification on hoppy aromas" - Tom Shellhammer

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Yeast Impact on Hoppy Aromas

1. Potential reduction of aromas
    - Stripping of compounds during fermentation
        - CO_2 production/stripping
        - Adsorption

- Masking of aromas?
- Although not biotransformation per say, but still important to mention that this happens!

Yeast Impact on Hoppy Aromas

2. Esterification

- The production of esters during fermentation
- Esters are formed from alcohols and acids
- Range from fruity to floral in aromas

Ex: Geraniol → geranyl acetate (roses)

Ex: Hop degradation products → ethyl esters (fruity esters)

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Yeast Impact on Hoppy Aromas

3. Glycosides

What are Glycosides?

- Sugar bound molecules, non-volatile
- Come from hops
- B-glucosidase enzyme needed to hydrolyze bond
- Free form = aromatic

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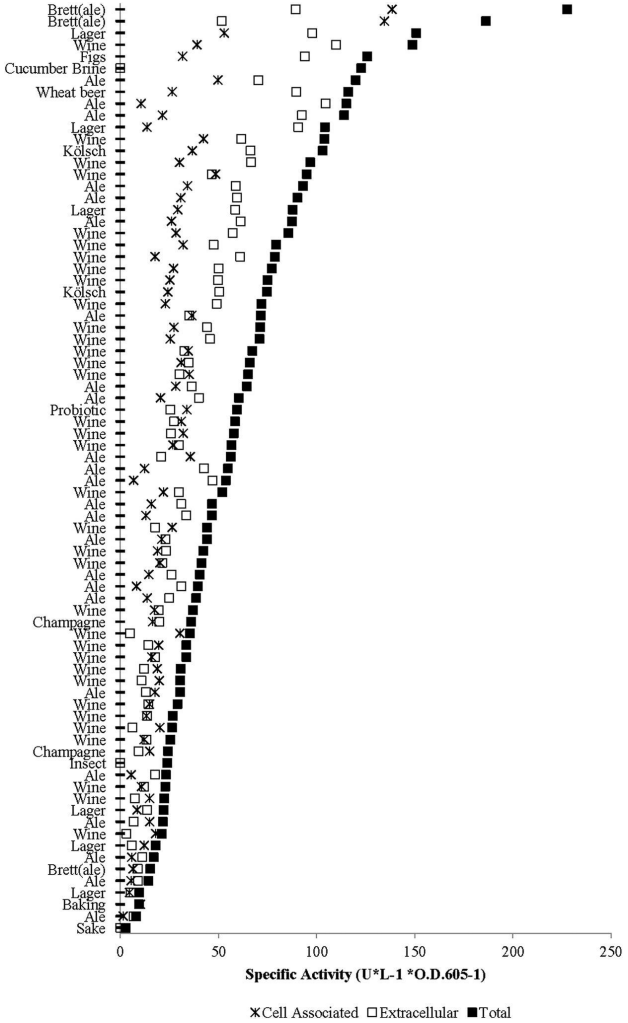
Glycoside research (done by the researchers at OSU)

- Brewing yeast exhibits a wide range of glucosidase hydrolysis activity
- Maximum hydrolysis occurred within 3 days of primary ferm
- BUT, found that this B-glucosidase activity made small contributions to overall aroma



Sharp, D et al. 2017.

Yeast Impact on Hoppy Aromas

4. Thiols

Pungent, sulfur-containing compounds - can smell at ppt levels! (yes, that's parts per trillion)

Bound to amino acids or Free forms are available

Bound thiols = non-volatile/flavorless

Free forms = aromatic, tropical

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Other Influencing Factors:

- Yeast strain selection
- Cell count
- Temperature
- Hop Variety
- Point of hop addition
- Contact time

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But really, what are thiols and why is everyone talking about them??

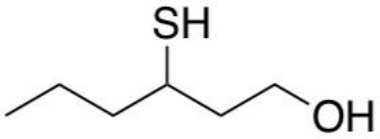
Thiols Overview

Thiols are found in a lot of tropical fruits

Also what distinguishes those new school hops, Sauv Blanc from New Zealand, etc.

Grapefruit, Passion Fruit, Guava

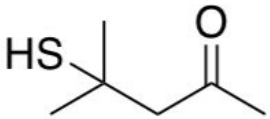
3MH



Grapefruit, Rhubarb

55ng/L

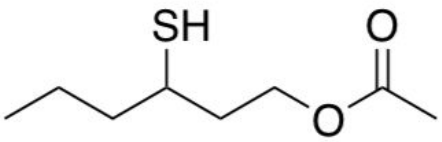
4MMP



Boxtree, black currant

1.5ng/L

3MHA*



Passionfruit, Guava

4ng/L

*acetate ester of 3MH and a relevant player in tropical free aromatics

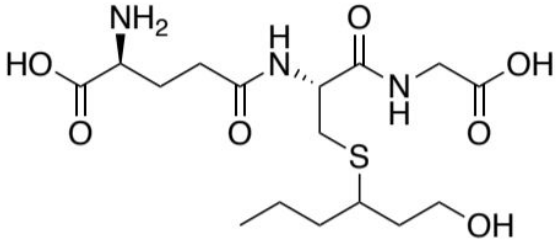
(Roland, et al. World Brewing Congress. Denver 2016)

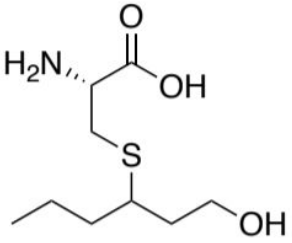
Where do thiols come from: Precursors

Barley and hops have thiol precursors

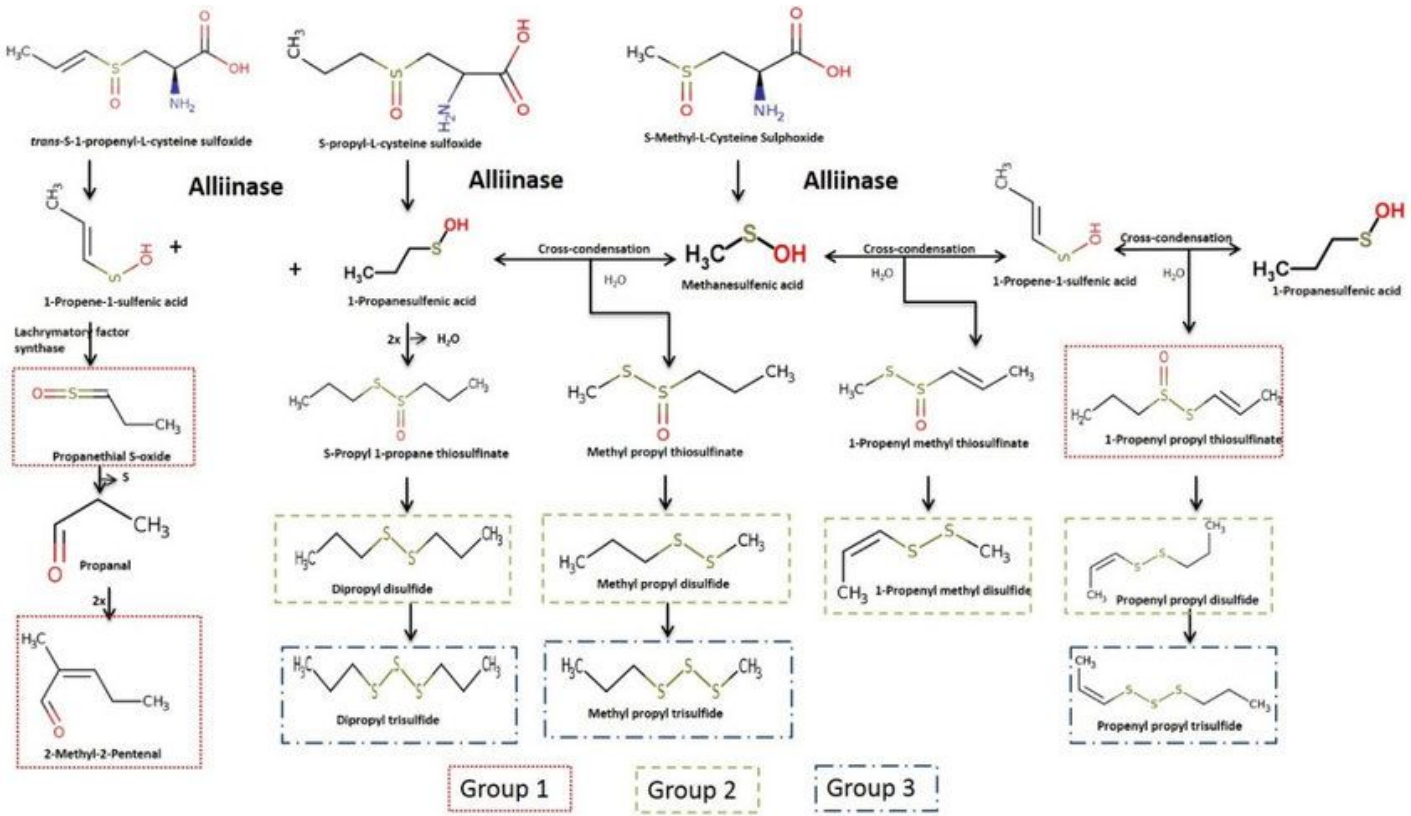
Most common 3MH precursors are**: Cysteinylated** and **Glutathionylated** thiol precursors (often written seen as Cys-3MH or Glut-3MH)

Most yeast strains are only able to release 3MH from the Cys-3MH precursor, so they leave all of the thiol forming potential of Glut-3MH untapped

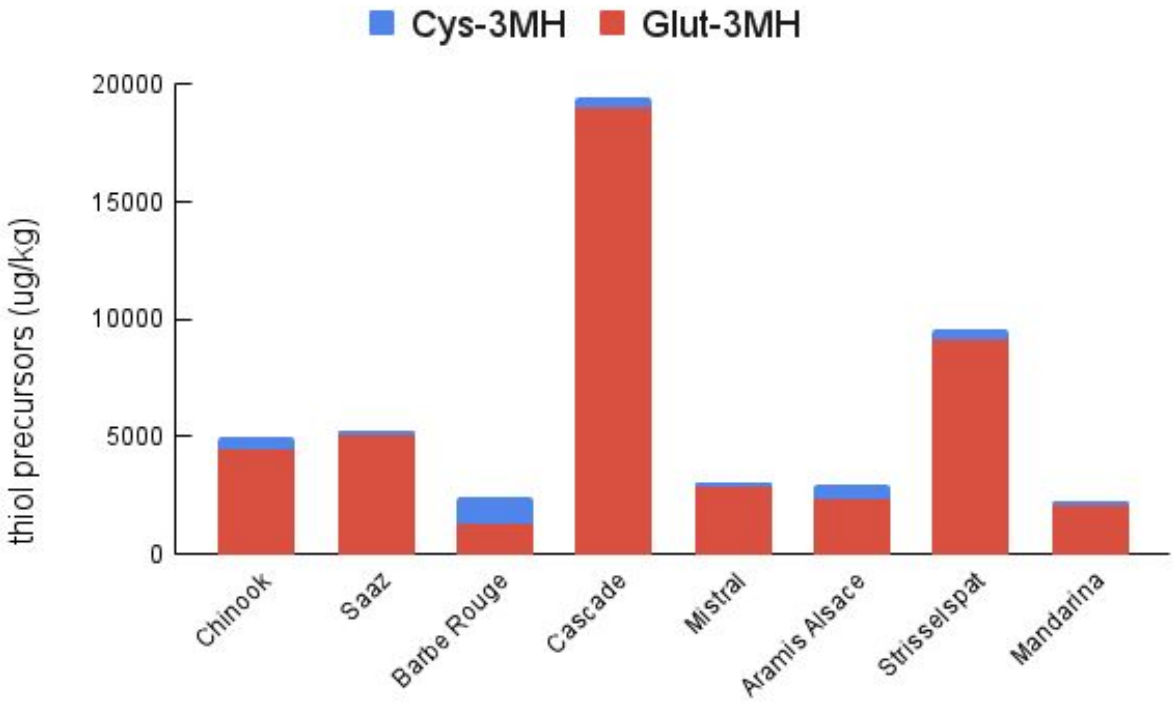




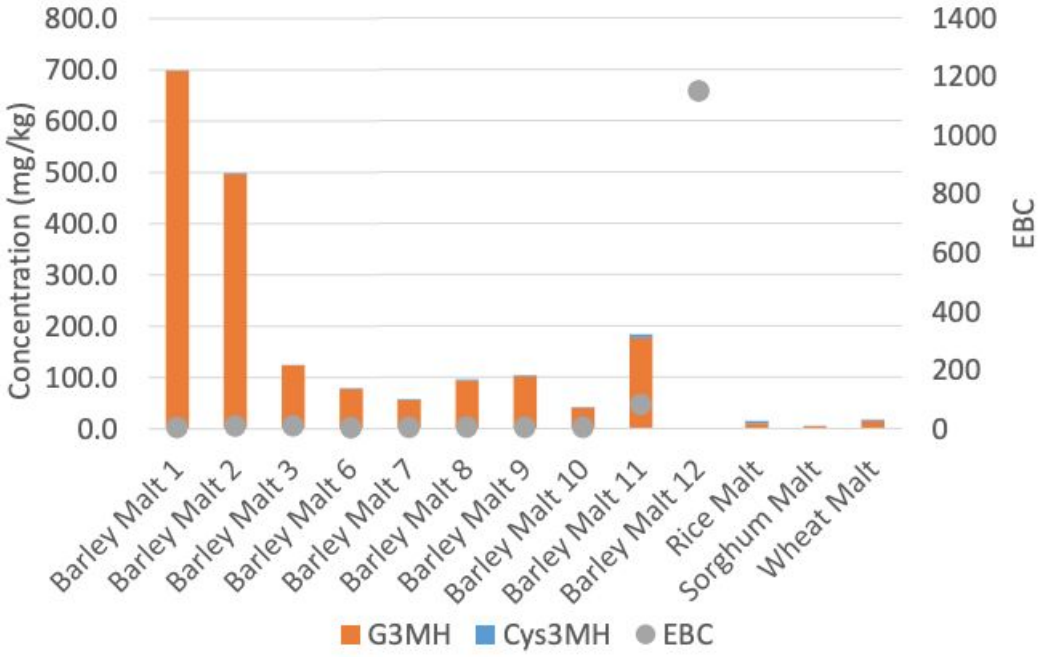
Garlic/onion flavors are not related to beta-lyase activity



Where do thiols come from: Precursor data



Adapted from Roland et al., *Flavour Fragr J.*, 2016



Roland et al., WBC Poster, 2016

Yeast Enzymes

To release a bound thiol → free, aromatic form we need a specific enzyme to do the job: in this case, β-lyase aka C-S lyase

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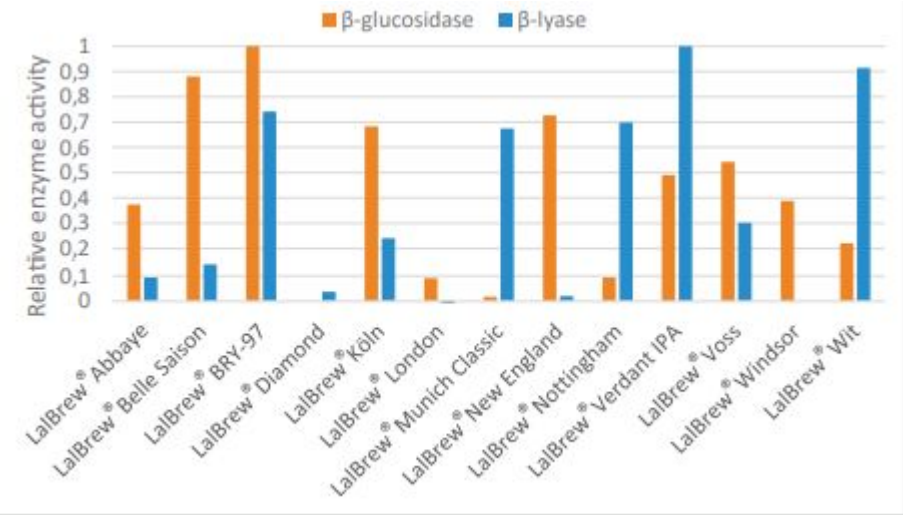
Yeast and enzymatic activity

Different yeast strains have different enzymatic activity of b-lyase

- Check in with your yeast suppliers to see if they've measured their strains enzymatic activity

Example: Lallemand Brewing Biotransformation Best Practices

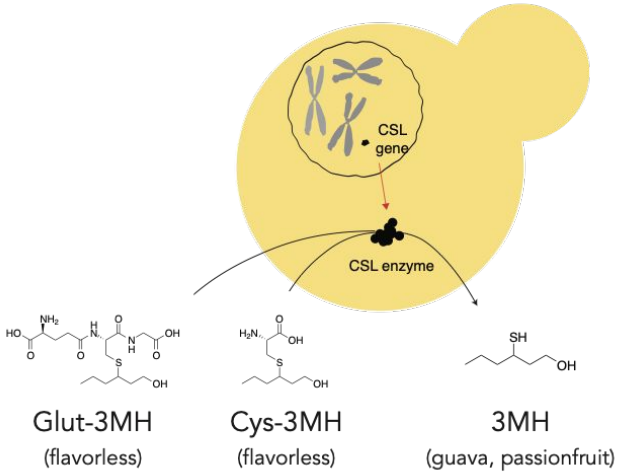


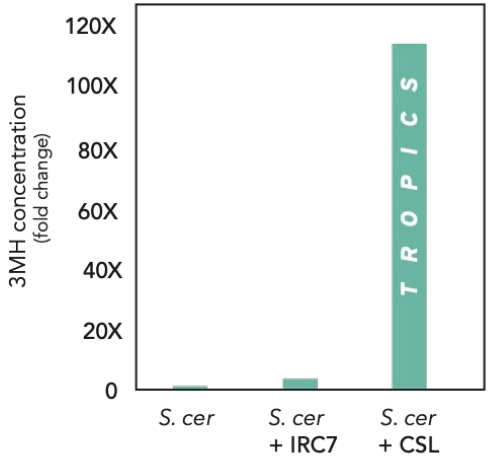
GE Yeast - Thiols

There are also yeasts on market that have been genetically engineered to encourage the transformation of precursors to aromatic thiols

2 methods so far:

- Gene expression (turning up IRC7)
- Expression of active CSL





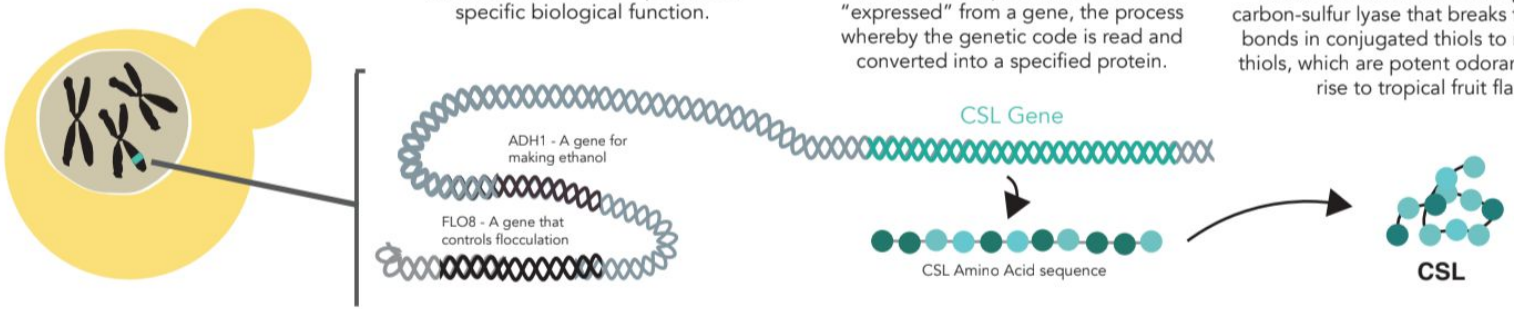
How does this work?

Inside every yeast cell are chromosomes made of DNA.

Encoded in the DNA are thousands of genes. Each gene contains the information needed to perform a specific biological function.

Genes contain information for making a specific sequence of amino acids that will form a protein. Proteins are "expressed" from a gene, the process whereby the genetic code is read and converted into a specified protein.

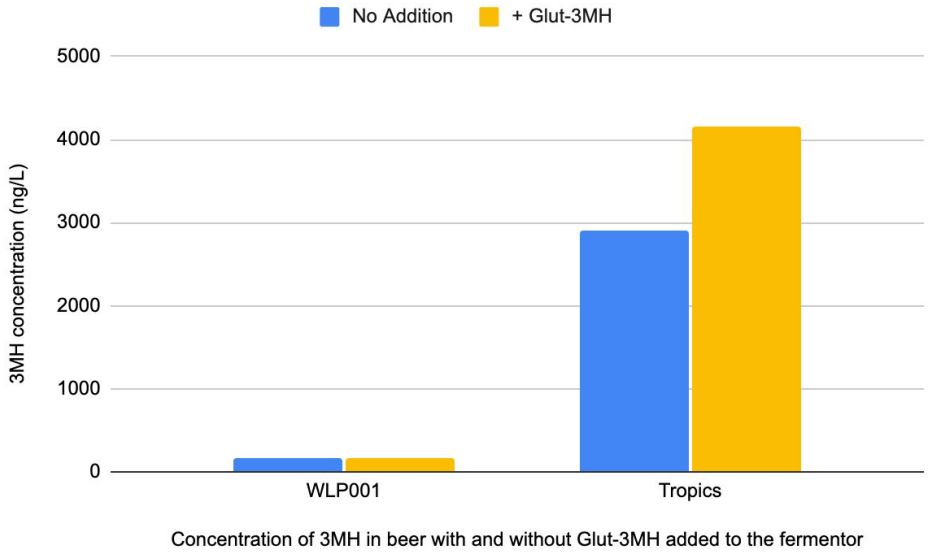
Proteins perform cellular functions. The CSL gene encodes amino acids that fold into the CSL protein, a carbon-sulfur lyase that breaks the chemical bonds in conjugated thiols to release free thiols, which are potent odorants that give rise to tropical fruit flavor



Addition of Glut-3MH increases 3MH production

-Experiment at BY using pure Glut-3MH added to the fermentor.

-Requires an active CSL



Concentration of 3MH in beer with and without Glut-3MH added to the fermentor

How to use this information for your brewery/ experimentation?

How to increase free thiols:

1. Control Precursor
    - Lighter kiln malts
    - Hop Variety: hops with free thiols vs bound thiols
    - Add hops to whirlpool or mash hopping to extract more
    - Phantasm Powder

Note: Directly related to precursors, not temp, pressure, underpitching, stress, etc.

How to increase free thiols:

2. Control Enzyme

- Exogenous enzyme additions
- Increasing yeast gene expression
- Overexpression of highly active CSL

In Summary:

- Thiols an thiol precursors are new and interesting area of study in the brewing industry
- Thiols provide tropical fruity aromas to beer, specifically looking at 3MH, 4MMP, 3MHA
- Hops and barley both contain thiol precursors, mainly Glut-3MH, but also Cys-3MH available
- Yeast have different levels of b-lyase enzymatic activity, engineered yeast is able to increase the production of this enzyme or increase the activity of the enzyme itself - both can release bound thiols more efficiently
- Experimentation focusing on precursors and enzyme

Thank You

Brittney Christianson
brittney@berkeleyyeast.com