The Future of Brewing Yeast

Lance Shaner

OMEGA YEAST

Educational and Professional Background

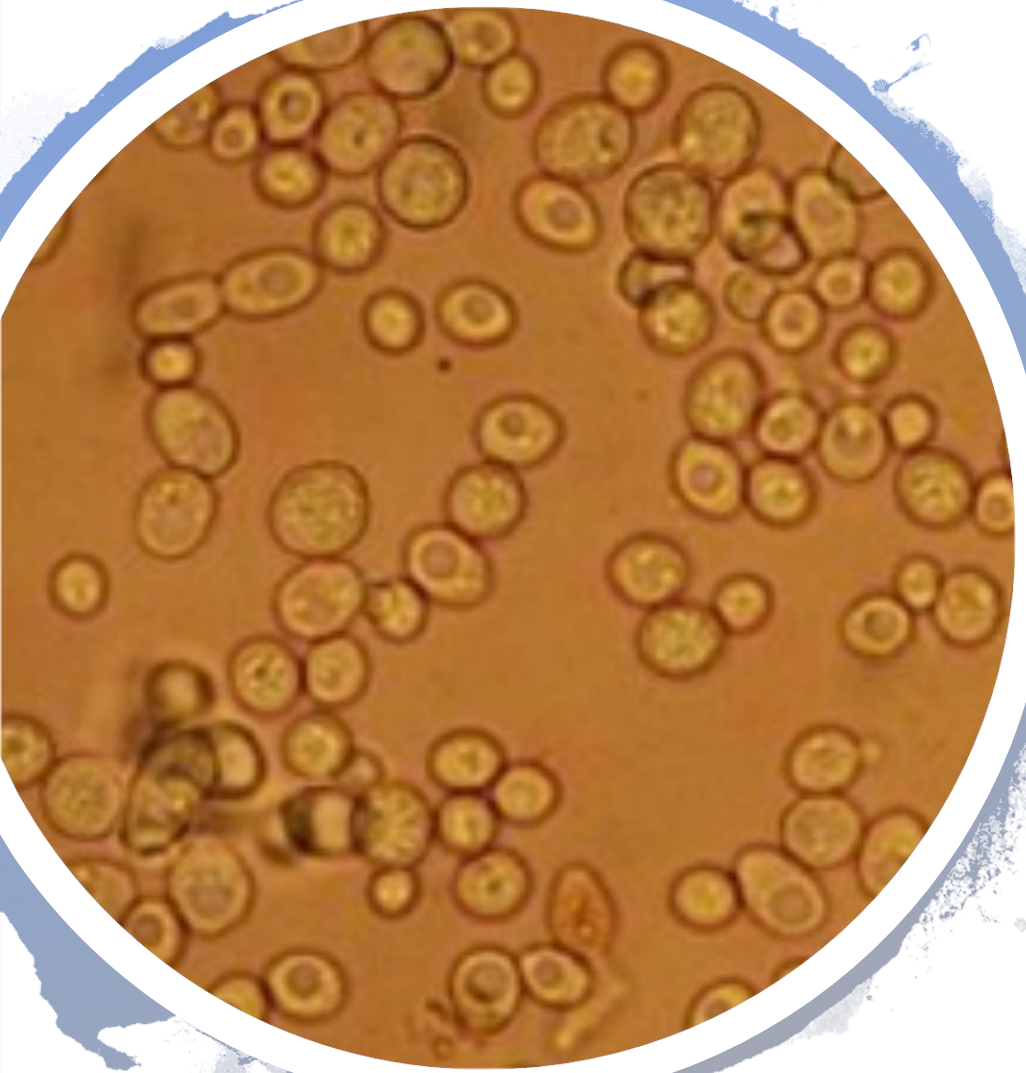
- B.S. in Microbiology from U. of Ill. at Urbana-Champaign (’01)
- Ph.D. in Microbiology & Molecular Genetics from UT-Houston Graduate School of Biomedical Sciences
- J.D. from University of Houston School of Law (‘09)
- Patent Attorney at Marshall Gerstein & Borun LLC (‘09-’13)
- Co-founder at Omega Yeast Labs LLC (‘13-present)

OMEGA YEAST

Outline of the Presentation

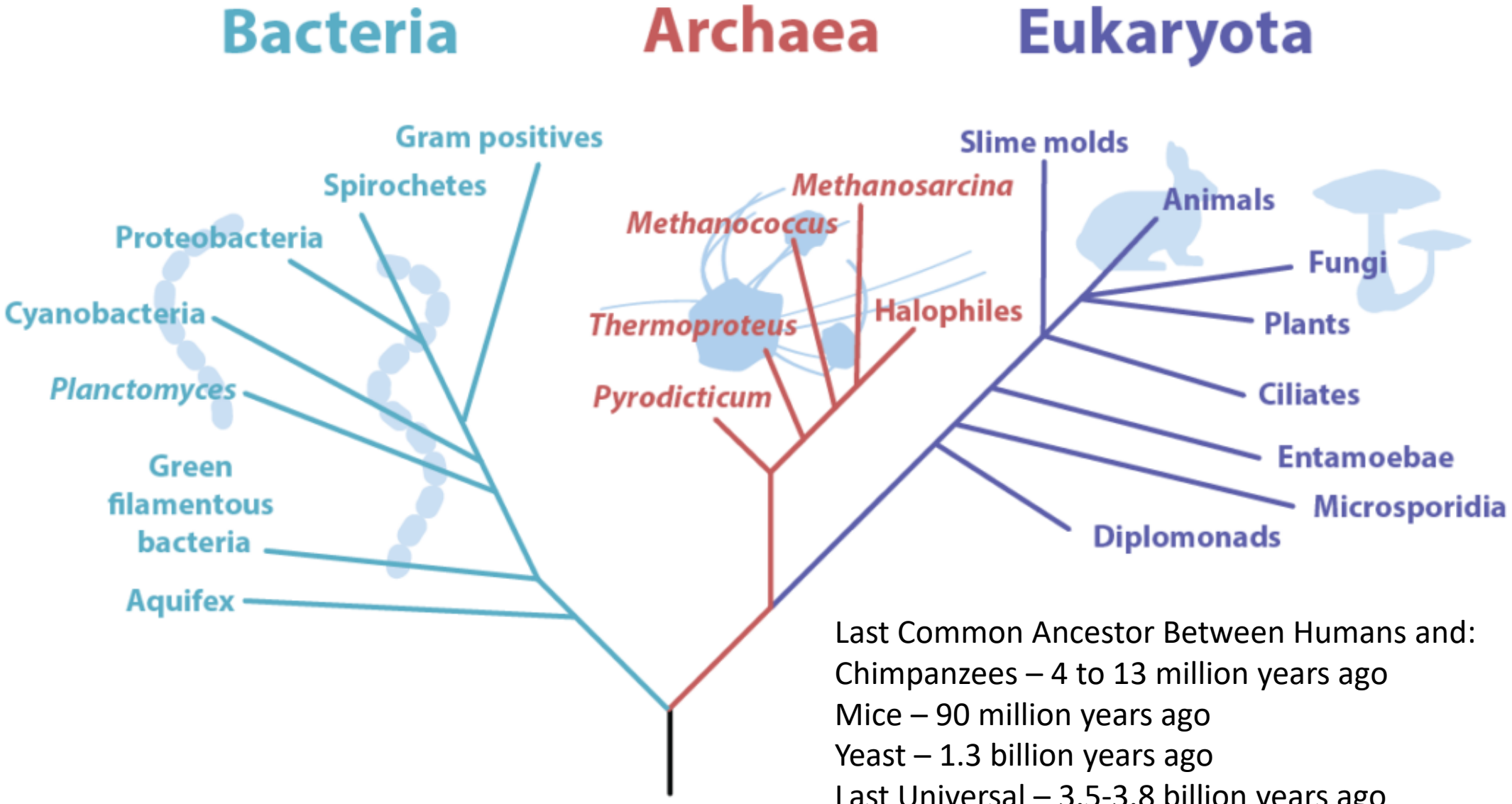
1. Domesticated and Industrialized Ale/Lager Yeasts, i.e., the Status Quo
2. Yeasts Preserved Through Tradition
3. “Foraged” or “Wild” Yeasts
4. Mating Yeast to Make Hybrid Strains
5. Genetically Engineered Yeasts
    1. Cisgenic
    2. Transgenic

OMEGA YEAST



What is Yeast?

- Single-celled fungus
- Being Eukaryotes, yeast are more closely related to humans than bacteria
- Two main species used to make beer:
  - Ale yeast (*Saccharomyces cerevisiae*)
  - Lager yeast (*Saccharomyces pastorianus*)
- Reproduce asexually by “budding”
- Role in beer is to consume sugars and produce alcohol and CO2 (and other flavor/aroma compounds)



Last Common Ancestor Between Humans and:
Chimpanzees – 4 to 13 million years ago
Mice – 90 million years ago
Yeast – 1.3 billion years ago
Last Universal – 3.5-3.8 billion years ago

Ale Yeast

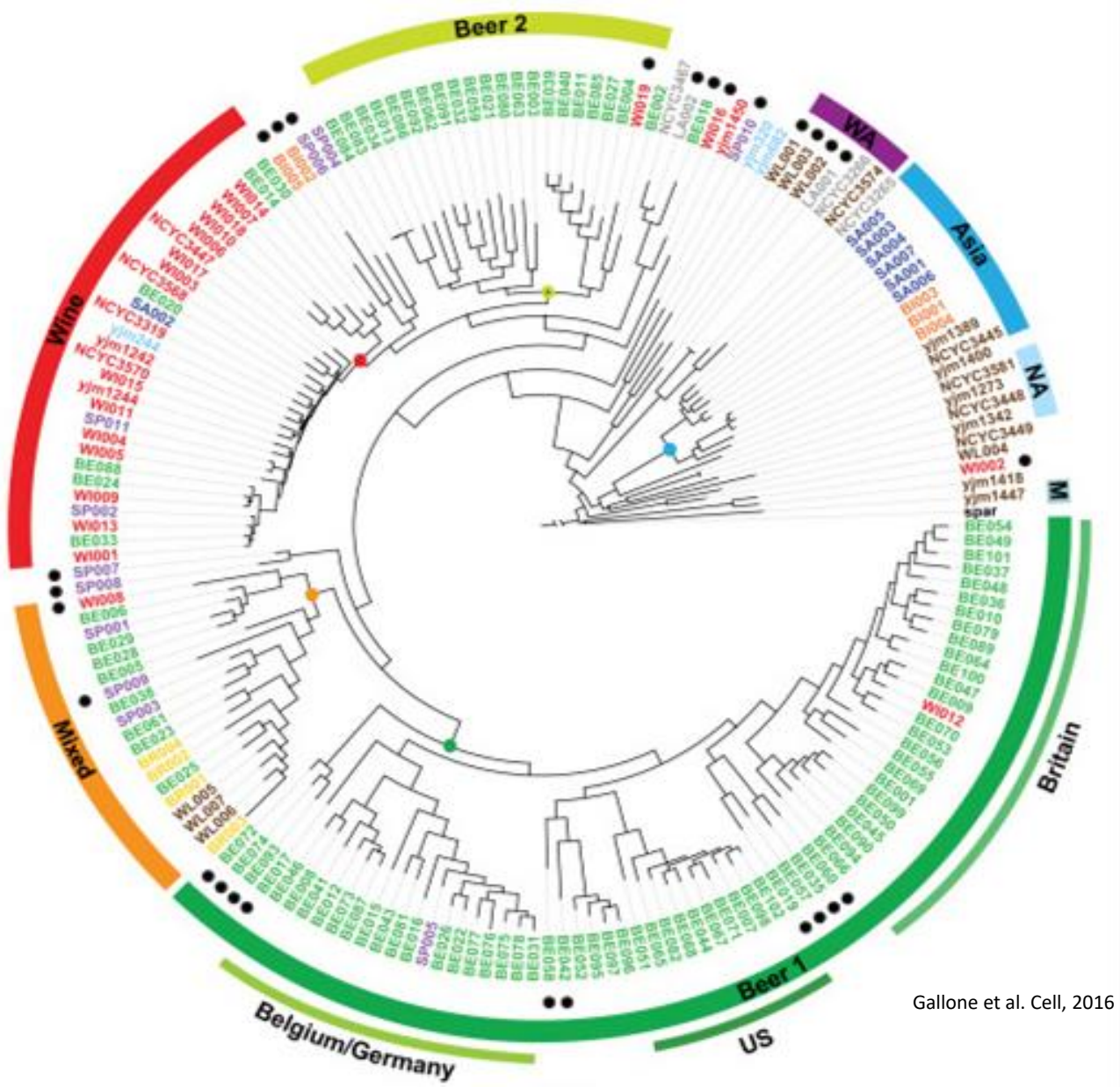
- *S. cerevisiae*
- “Top fermenting”
- Fermentation temperatures around 65-75F, ferment quickly
- More yeast-derived flavors compared to lager yeast
  - Esters (fruity flavors/aroma)
  - Phenolics (clove, pepper) – only in some ale yeasts (w/ functional *FDC1* gene)

Lager Yeast

- *S. pastorianus* (naturally occurring hybrid of *S. cerevisiae* and *S. eubayanus*)
- Genetic evidence points to 17th Century German origin
- “Bottom Fermenting”
- Cold fermentation temperatures (48-55F)
- Low amount of yeast-derived flavors
  - Clean, crisp, lets malt and hops steal the show
- By far more Lager produced world-wide than Ale.

OMEGA YEAST

Domesticated and Industrialized Brewing Yeast



Gallone et al. Cell, 2016

* Diversity through random mutation and human selection of desired traits

Examples:
Speed of fermentation increased by duplication of maltose utilization genes.

POF- (lack of 4-vinylguaiacol produced by FDC1 gene product).

High flocculation for clear beer and easy yeast cropping.

OMEGA YEAST

Yeasts Preserved Through Tradition

Jovaru

Norwegian Kveik

OMEGA YEAST

Norwegian Kveik

- Norwegian dialect word for “yeast”
- Traditional Norwegian farmhouse cultures passed on for generations
- Brought to the attention of the brewing world by blogger Lars Marius Garshol (Larsblog)
- Generally speaking, the cultures are high temp tolerant, flocculent, non-phenolic and high alcohol tolerant.
- Norwegian brewers regularly push temps to 100F!
- Genetics suggest kveik are a result of hybridization event between domesticated ale yeast and wild *S. cerevisiae*.

Hallmarks of Norwegian Farmhouse Brewing Tradition

- Juniper-infused mash water, long boils, little hop character
- Rapid fermentation (1-2 days) of high gravity (~19P) wort
- Yeast stored dry on kveikstokker (yeast logs)
- Kveikstokker used to inoculate next batch by dipping in 86-104F (30-40C) wort(!)
- In one region of Norway, they scream into the fermenter as yeast is being pitched

OMEGA YEAST

Omega Yeast Kveik Offerings

- HotHead® Ale (OYL-057) - Highly flocculent strain with an astoundingly wide temperature range (62-98F) and little change in flavor across the range. Clean enough for both American and English styles. It has a unique honey-like aroma with overripe mango which is complementary to modern, fruity hops.
- Voss Kveik (OYL-061) – From the Gjernes farmstead, orange-citrus notes present throughout its wide temperature range (68-98F). Relatively clean across its fermentation temperature range and pairs well with citrusy, fruity hops.
- Hornindal Kveik (OYL-091) - From the farmstead of Terje Raftevold, Hornindal presents fruity flavors and aromas of pineapple, mango and tangerine, which complement fruit-forward hops. Add even more dimension to “C” hops with a high fermentation temperature, intensifying aroma and fermentation speed.

OMEGA YEAST

Kveik FAQ

- Will kveik infect all of my equipment?
  - No. It's just Sacch yeast. Not diastatic.
- How do I make a kveik beer?
  - There's no such thing. "Kveik" means "yeast". That's like saying "How do I make a yeast beer?"
- What styles can I make using kveik?
  - Anything where you would use an English ale yeast – IPA, NEIPA, APA, porter, stout, barleywine, imperial stout, cream ale, etc.
- How do I pronounce "kveik"?
  - K (combination of w and v) ike
  - Ask a Norwegian.

OMEGA YEAST

Jovaru Strain

- From the Jovaru Alus Brewery in Jovarai, Lithuania
- Aldona Udriene, brewer
- Strain obtained by Aldona's grandfather, apparently from a local forest 100+ years ago.
- Strain is *STA1+*, phenolic
- ITS sequencing shows "unknown Saccharomyces species." Intriguing!
- Whole genome sequencing underway.

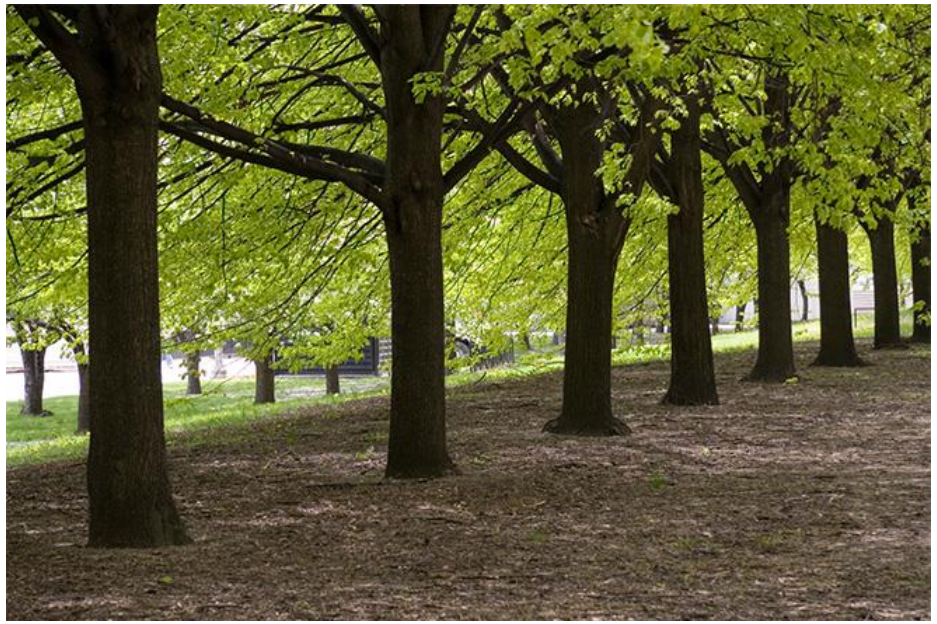
- Available as Jovaru Lithuanian Farmhouse Ale (OYL-033).
- Flavor profile: citrusy, lemon pitch, black pepper
- Suitable for Belgian-style ales

Thoughts on “Traditional” Strains

- Lars Marius Garshol is a treasure.
- It seems unlikely that there are *too* many heirloom strains out there.
- The advent of pure cultures likely caused a yeast genocide.
- Traditional strains like kveik and Jovaru can serve as breeding stock for crossing with existing brewing strains to increase diversity.

“Wild” and “Foraged” Yeasts

Yeast can be found virtually anywhere…



OMEGA YEAST

“Wild” and “Foraged” Yeasts

Lactic Acid Yeast (LAY)

- *Lachancea thermotolerans*
- *Lachancea fermentati*
- *Wickerhamomyces anomalus*

Other yeast species groups are experimenting with:

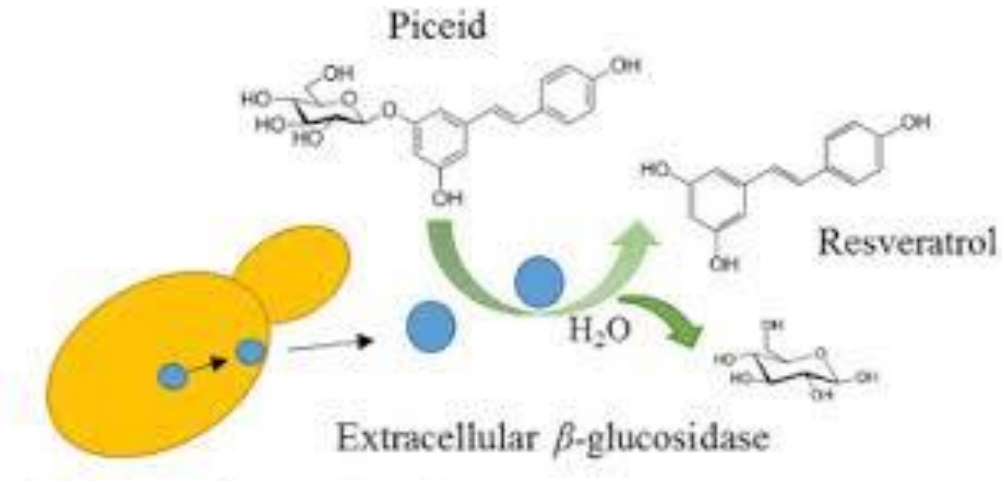
Brettanomyces spp
Torulaspora spp
Kluyveromyces spp
Hansienaspora spp
Schizosaccharomyces spp
Debaryomyces spp

OMEGA YEAST

Advantages and Disadvantages of Working with “Wild” Yeasts

Advantages

- “Biotransformation”
- Novel flavors/aromas
- Sour beer with high IBU



Disadvantages

- Slow
- Disgusting flavors/aromas
- Low floc
- Non-ideal temp ranges
- Low alcohol tolerance
- Inability to ferment maltose
- Phenolic

OMEGA YEAST

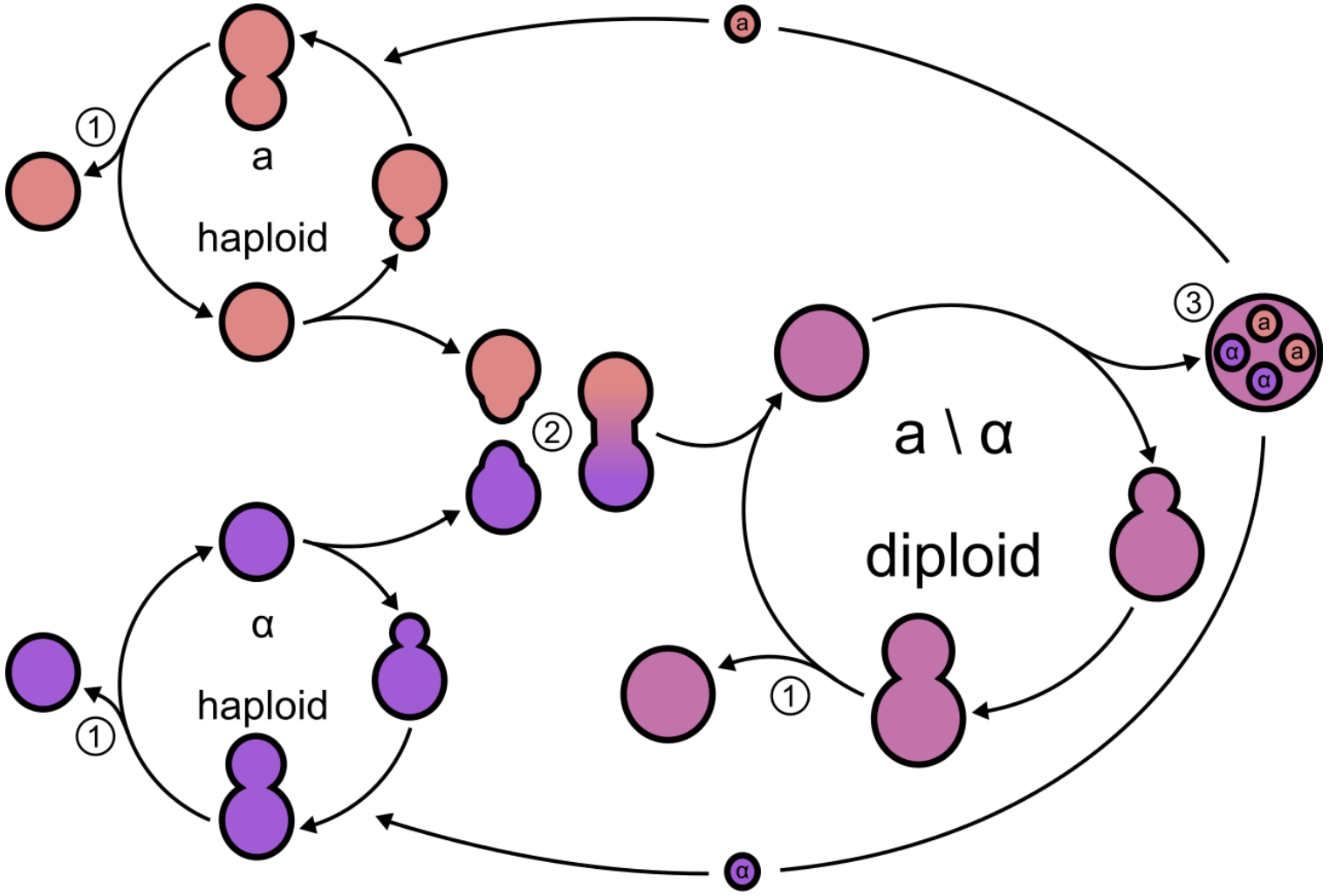
Hybrid Strains

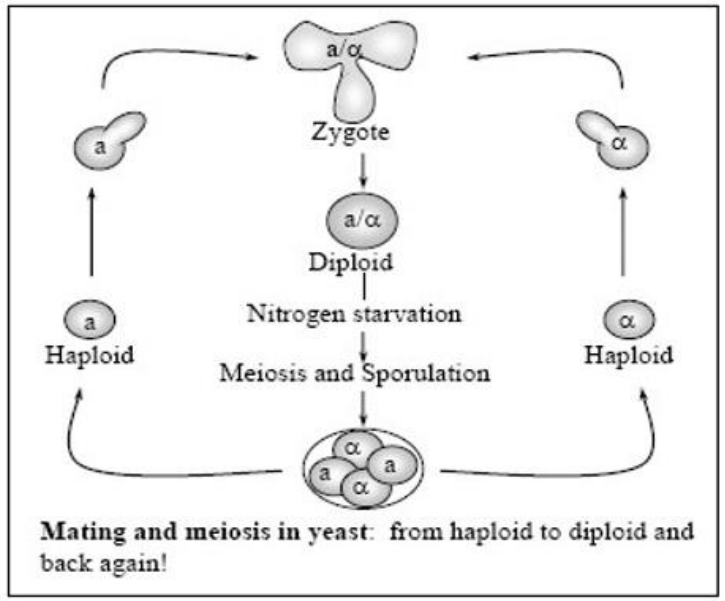
Hybrid can mean a couple of things in the yeast context:

1. Interspecies hybrid
   A. Lager yeast – *Saccharomyces cerevisiae* x *Saccharomyces eubayanus*
   B. Some Trappist strains – *Saccharomyces cerevisiae* x *Saccharomyces kudriavzevii*
2. Intraspecies hybrid
   A. *Saccharomyces cerevisiae* x *Saccharomyces cerevisiae*
   Saisonstein's Monster (OYL-500) – hybrid of French Saison and Dupont Saison
   Gulo Ale (OYL-501) – hybrid of French Saison and Irish Ale

OMEGA YEAST

Life Cycle of Yeast





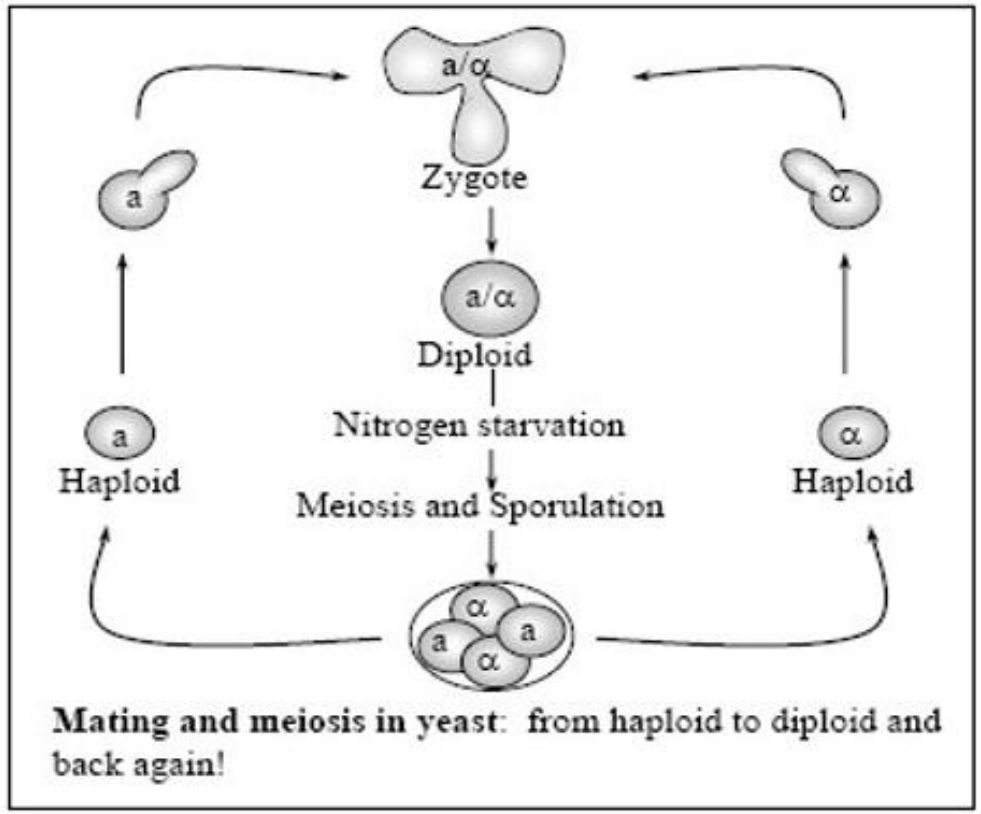
Mating and meiosis in yeast: from haploid to diploid and back again!

Why Hybrids?

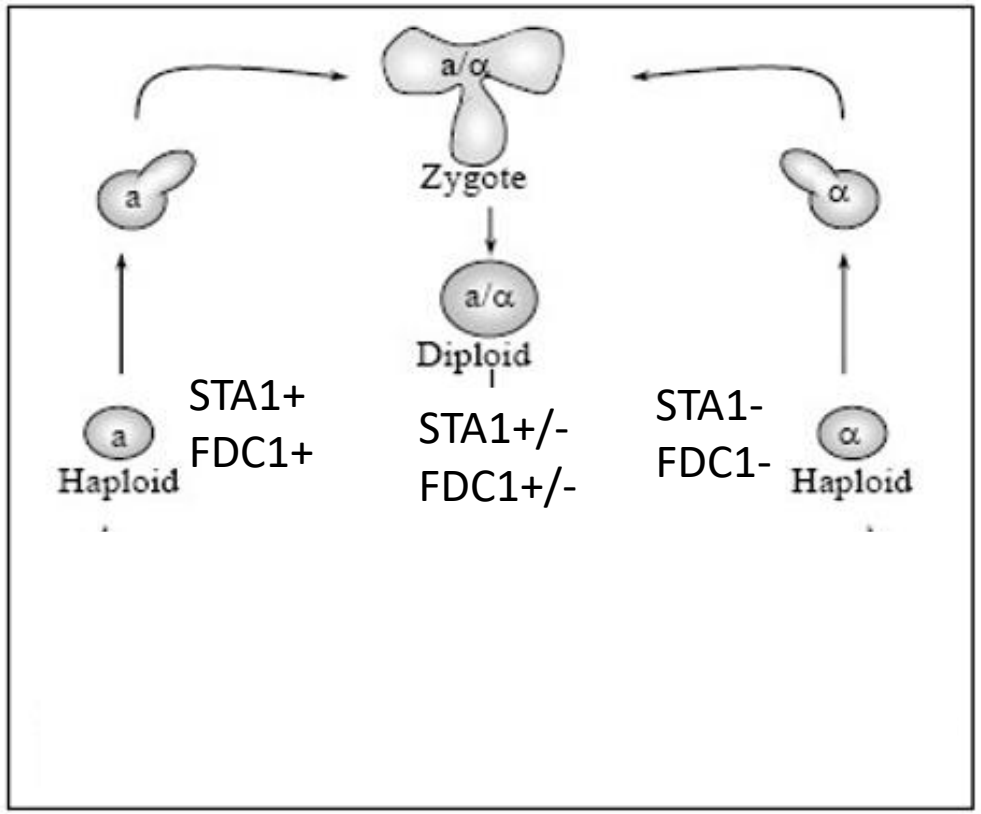
- Combine desirable traits from 2 strains
- Remove undesirable traits from a strain
- Discover novel flavor combinations

Gulo Ale Strategy

Sporulate parental strains



Mate parental strains

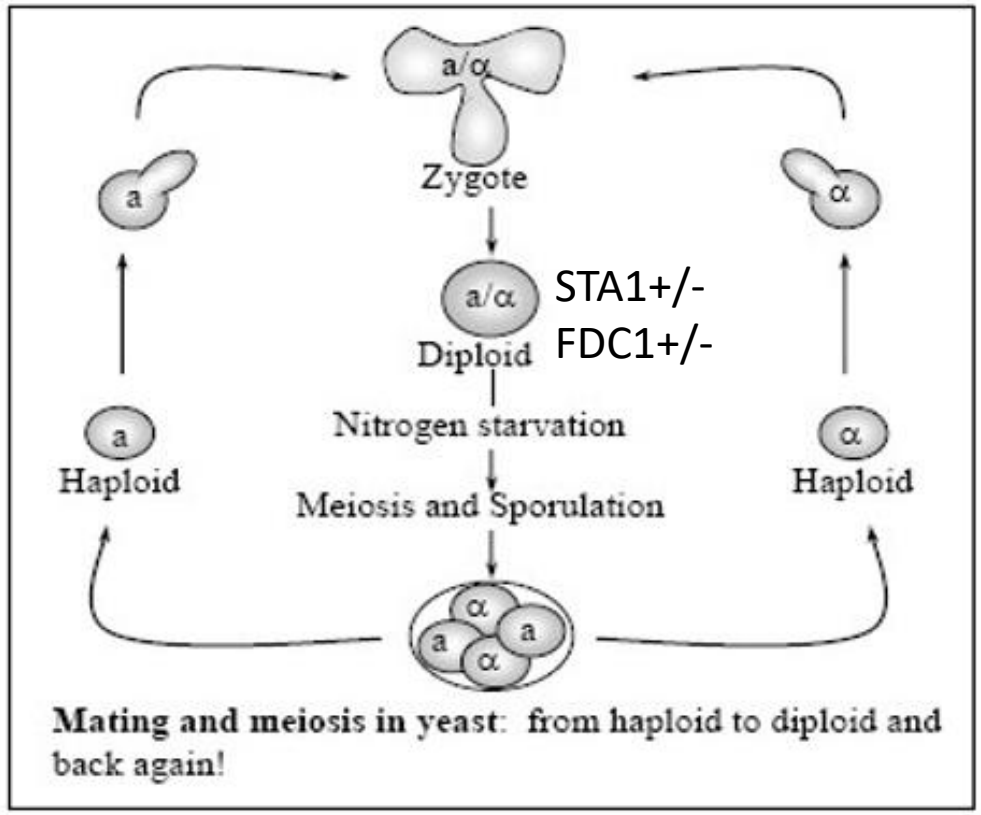


Irish Ale = STA1-/STA1-, FDC1-/FDC1-
French Saison = STA1+/STA1+, FDC1+/FDC1+

OMEGA YEAST

Gulo Ale Strategy (cont.)

Re-sporulate hybrids



*Haploids spontaneously revert to diploids.

Progeny should have the following distribution:

¼ = STA1+/FDC1+
¼ = STA1-/FDC1-
¼ = STA1+/FDC1- (Gulo Ale)
¼ = STA1-/FDC1+

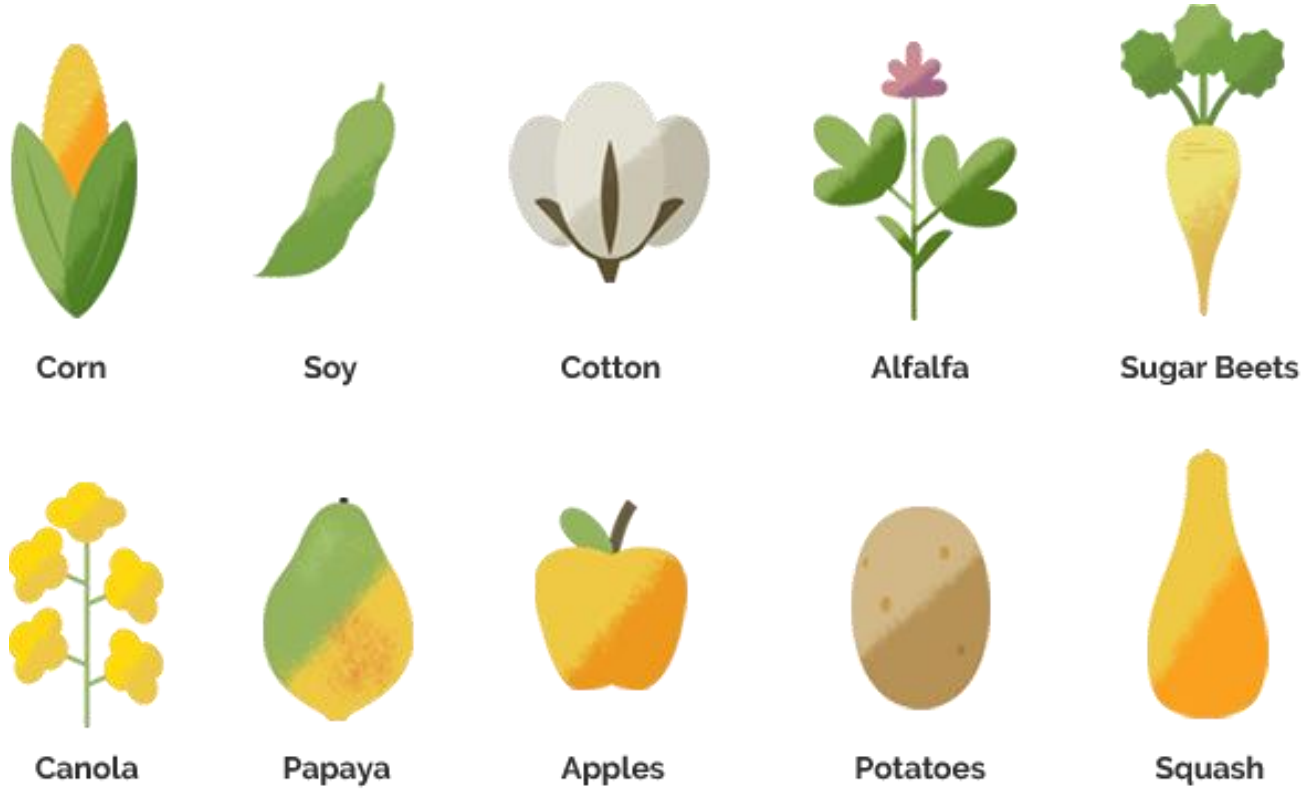
Screen for STA1 by PCR, FDC1 by sniffing ferulic acid plates.

OMEGA YEAST

Genetically Modified Organisms

- Definition of a Genetically Modified Organism (GMO):

- Any organism whose genetic material has been altered using genetic engineering techniques.



OMEGA YEAST

Genetically Modified Yeast

Transgenic Modification = genes from one species transferred into another species

ARTICLE

DOI: 10.1038/s41467-018-03293-x OPEN

Industrial brewing yeast engineered for the production of primary flavor determinants in hopped beer

Charles M. Denby[1,2], Rachel A. Li[2,3,4], Van T. Vu[5], Zak Costello[2,4,6], Weiyin Lin[1,2], Leanne Jade G. Chan[2,4], Joseph Williams[7], Bryan Donaldson[8], Charles W. Bamforth[7], Christopher J. Petzold[2,4], Henrik V. Scheller[2,3,9], Hector Garcia Martin[2,4,6] & Jay D. Keasling[1,2,4,5,10,11]

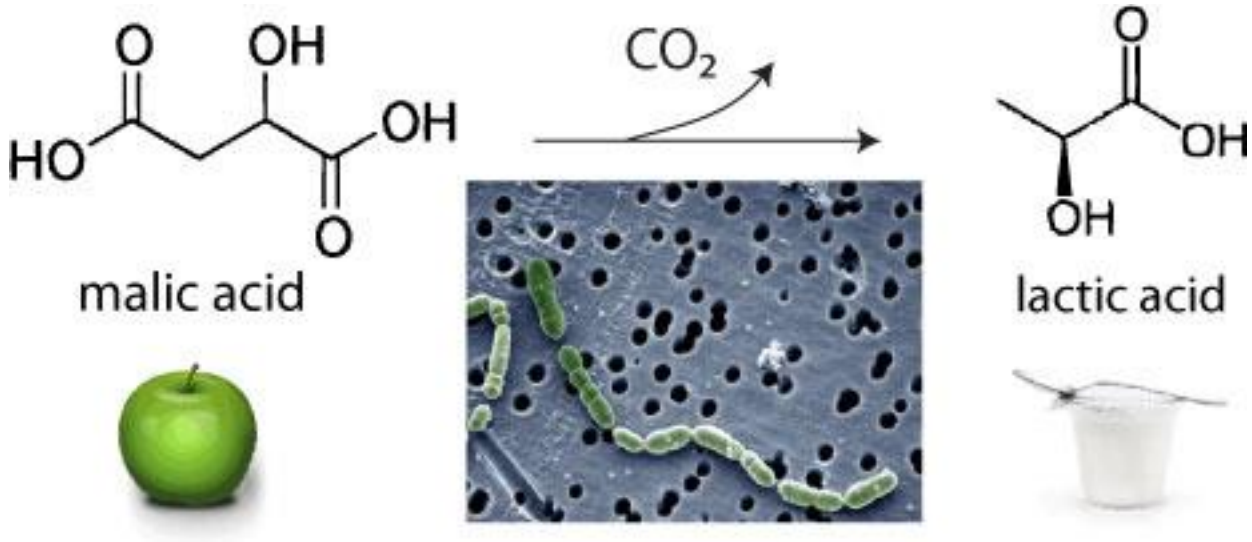
* Genes derived from basil and mint introduced into brewing yeast to make the aromatic monoterpenes, linalool and gerianol.

OMEGA YEAST

FDA-Approved GM Wine Yeast

Springer Oenologie – ML01

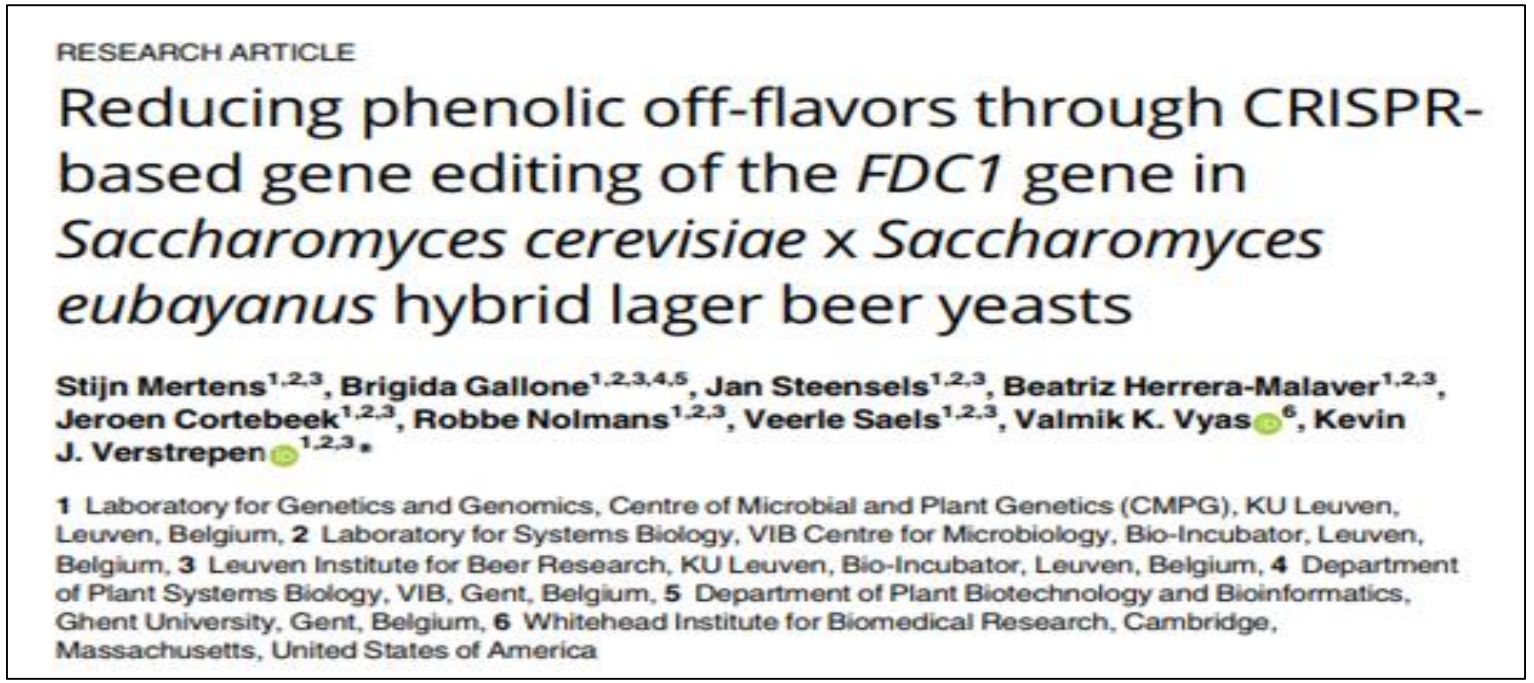
- Harbors 2 transgenes that allow yeast to perform malo-lactic fermentation
  - mae1 gene from *Schizosaccharomyces pombe* – malate transporter
  - mleA gene from *Oenococcus oeni* – malic acid decarboxylase



OMEGA YEAST

Genetically Modified Yeast

Cisgenic Modification = genes are artificially transferred between organisms that could otherwise be conventionally bred



RESEARCH ARTICLE

Reducing phenolic off-flavors through CRISPR-based gene editing of the *FDC1* gene in *Saccharomyces cerevisiae* x *Saccharomyces eubayanus* hybrid lager beer yeasts

Stijn Mertens[1,2,3], Brigida Gallone[1,2,3,4,5], Jan Steensels[1,2,3], Beatriz Herrera-Malaver[1,2,3], Jeroen Cortebeek[1,2,3], Robbe Nolmans[1,2,3], Veerle Saels[1,2,3], Valmik K. Vyas[6], Kevin J. Verstrepen[1,2,3]*

1 Laboratory for Genetics and Genomics, Centre of Microbial and Plant Genetics (CMPG), KU Leuven, Leuven, Belgium, **2** Laboratory for Systems Biology, VIB Centre for Microbiology, Bio-Incubator, Leuven, Belgium, **3** Leuven Institute for Beer Research, KU Leuven, Bio-Incubator, Leuven, Belgium, **4** Department of Plant Systems Biology, VIB, Gent, Belgium, **5** Department of Plant Biotechnology and Bioinformatics, Ghent University, Gent, Belgium, **6** Whitehead Institute for Biomedical Research, Cambridge, Massachusetts, United States of America

Non-phenolic ale and lager yeast have a frameshift mutation in *FDC1*, encoding a Ferulic Acid Decarboxylase. Same mutation can be introduced precisely into POF+ yeast strains.

OMEGA YEAST

Ethical/Safety Concerns with GMOs

1. GMOs are “unhealthy.”
   A. Critics cite increase in allergies, reproductive disorders, autism.
   B. No connection proven. And relevance depends on genetic change made.
2. GMOs “contaminate” genes of non-GMO organisms.
   A. Example – BT/Roundup Ready corn pollen can pollinate non-GMO corn and pass trait
   B. Not really relevant to brewing yeast
3. GMOs increase herbicide use.
   A. Specious and not relevant to brewing yeast.
4. Unintended side effects with process of creating GMO.
   A. Not as relevant with modern genetic tools. Very precise changes can be made and whole genome can be cheaply sequenced to verify desired change was induced.
5. GMOs harm the environment.
   A. Very much case by case.
   B. Argument for some GMOs helping the environment (e.g., yeast making hops aromas).

OMEGA YEAST

OMEGA YEAST


OMEGA YEAST