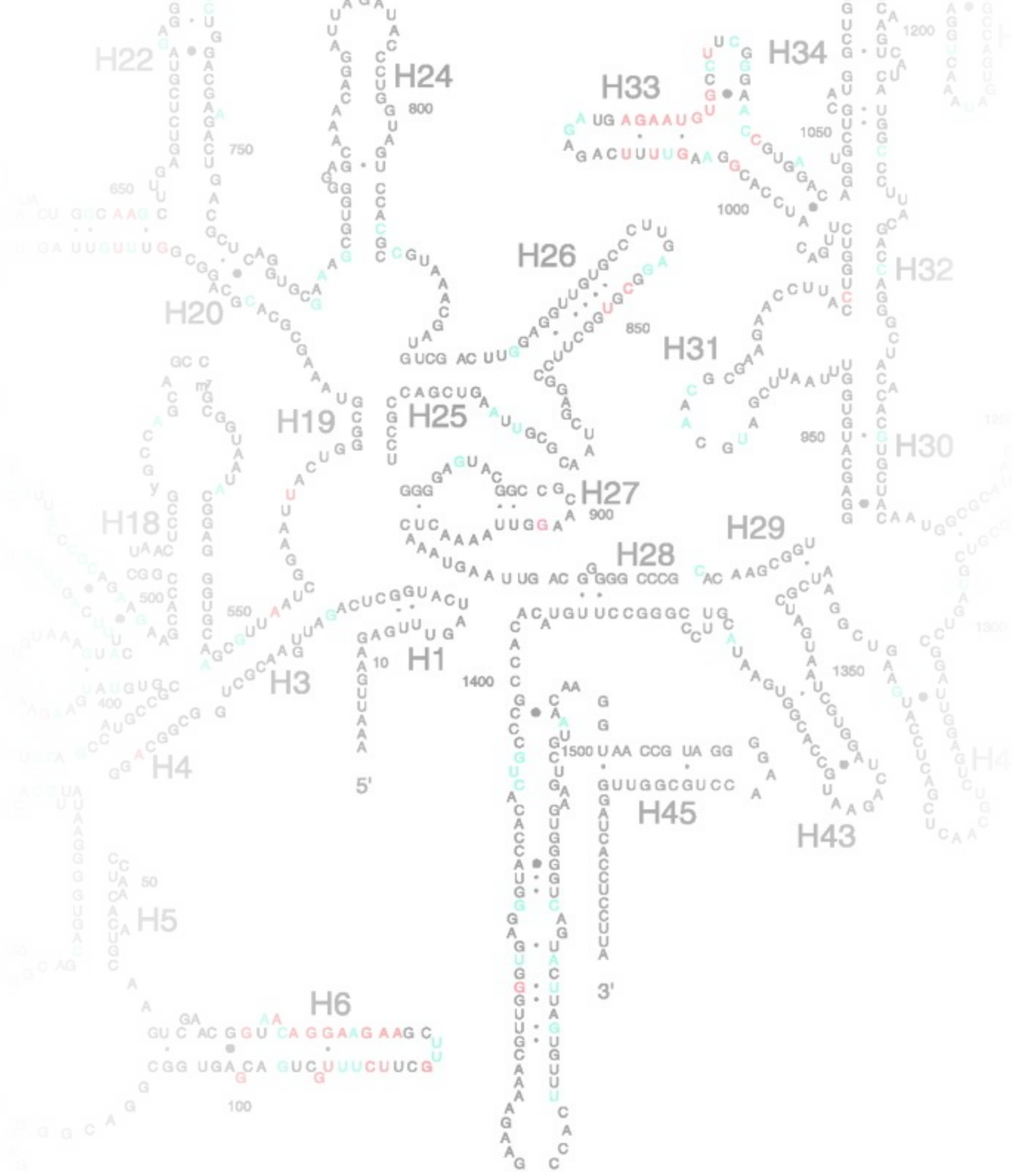
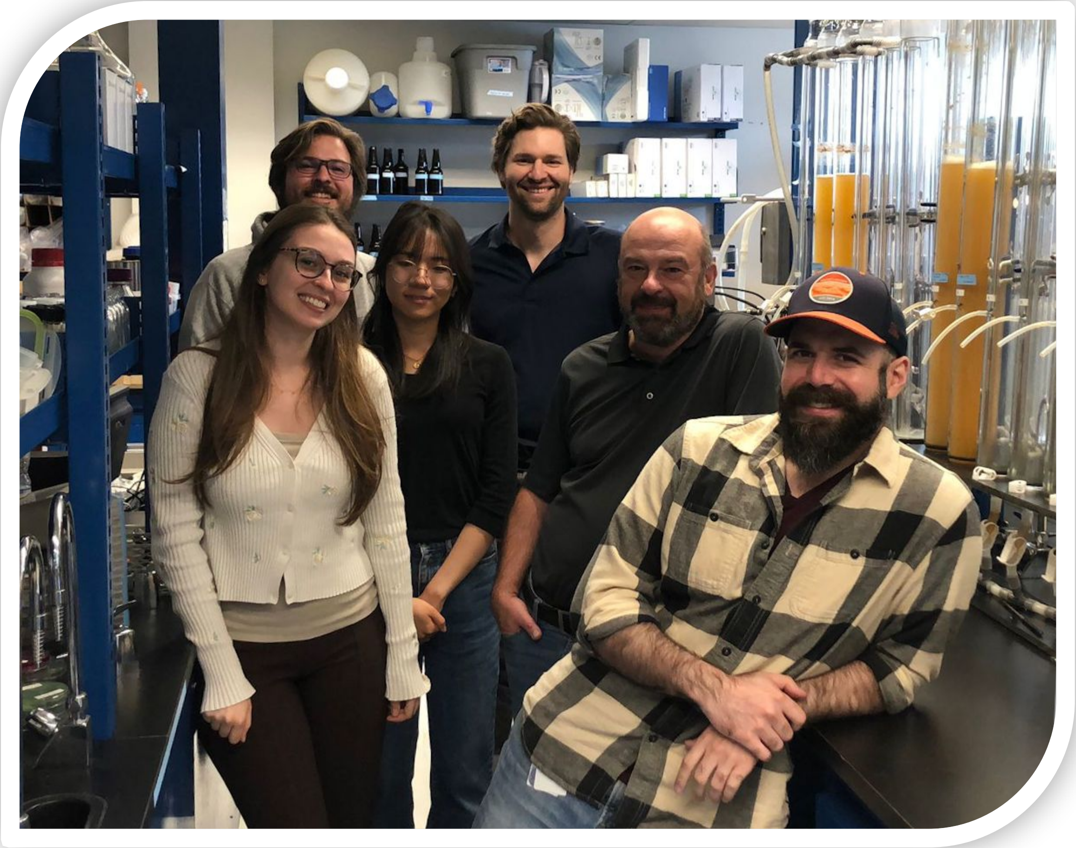
HARNESSING MODERN BIOTECHNOLOGY:

Conceptual development of high-value strains to address industry challenges



INTRODUCTION



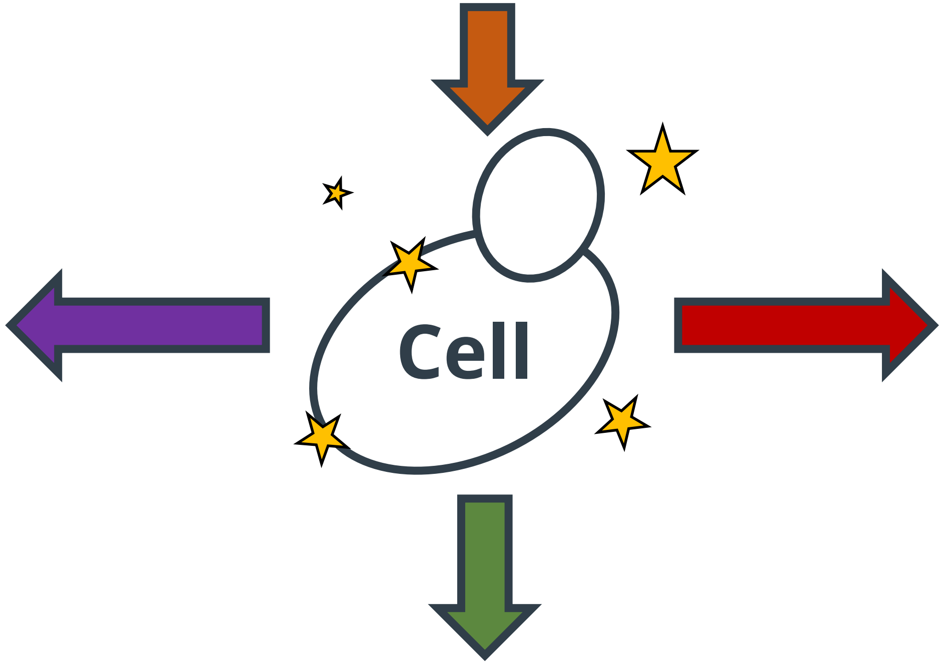
Lallemand Inc. Corporate BRW Team, Montreal, QC, Canada (2022)

- Who I am:
  - Avi Shayevitz
  - R&D Scientist for Lallemand Inc. (4 years)
  - Primary focus on brewing yeast development, brewing process aids, and life cycle analyses
  - Interconnected team of different research units

INTRODUCTION: WHAT WE DO

Nutrients

Carbon, nitrogen, minerals, vitamins



Metabolic Products

(ethanol, secondary metabolites)

Biomass/Cell Components

(whole yeast cells, chitin, nucleotides)

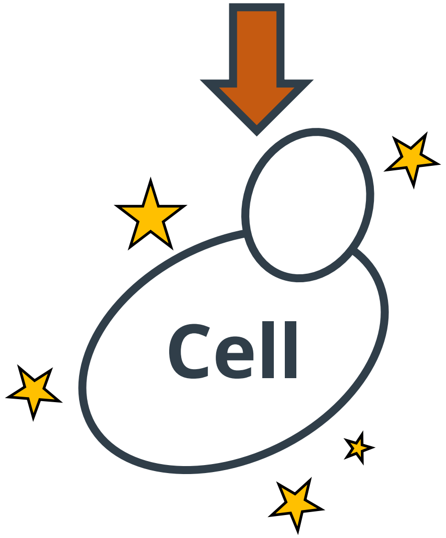
Extracellular Macromolecules

(membrane-bound proteins, chitin/call wall components)

INTRODUCTION: INDUSTRIAL APPLICATIONS

Nutrients

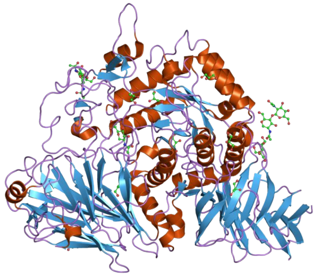
Carbon, nitrogen, minerals, vitamins



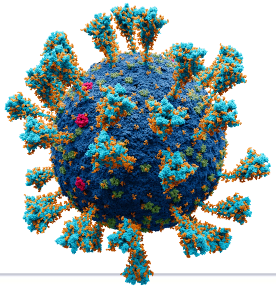
Metabolic Products

(ethanol, secondary metabolites)

- Insecticides
- Pharmaceuticals



- Protein
- Growth factors



- Interferons

INTRODUCTION: EARLY WORK INTO MORE EFFICIENT BREWERY PROCESSES

The Genetic Modification of Brewing Yeast with Recombinant DNA[1]

Edward Hinchliffe and Christine J. Daubney, *Research Department, Bass Public Limited Company, Burton-on-Trent, England*

intermittant process problems. One enzyme that has received considerable attention in recent years is β-glucanase, which can be used to obtain process benefits in wort production, fermentation, and conditioning (3). For example, an endo-1,3-1,4-β-glucanase from the Gram-positive bacterium *Bacillus subtilis* has been shown to produce a quantitative improvement in beer filtration performance (11,12). In view of the cost of adding commercial

INTRODUCTION: EARLY WORK INTO MORE EFFICIENT BREWERY PROCESSES

European Food Research and Technology
November 2012, Volume 235, Issue 5, pp 951–961 | Cite as

Construction of recombinant industrial brewer's yeast with lower diacetyl production and proteinase A activity

Authors   Authors and affiliations

Jun Lu, Jian Dong, Deguang Wu, Yefu Chen, Xuewu Guo, Yu Shi, Xi Sun, Dongguang Xiao

INTRODUCTION: EARLY WORK INTO MORE EFFICIENT BREWERY PROCESSES

Article | OPEN ACCESS | Published: 20 March 2018

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

Charles M. Denby, Rachel A. Li, Van T. Vu, Zak Costello, Weiyin Lin, Leanne Jade G. Chan, Joseph Williams, Bryan Donaldson, Charles W. Bamforth, Christopher J. Petzold, Henrik V. Scheller, Hector Garcia Martin & Jay D. Keasling

Nature Communications **9**, Article number: 965 (2018) | Download Citation

INTRODUCTION: MODIFICATION OF COMPLEX METABOLIC PATHWAYS

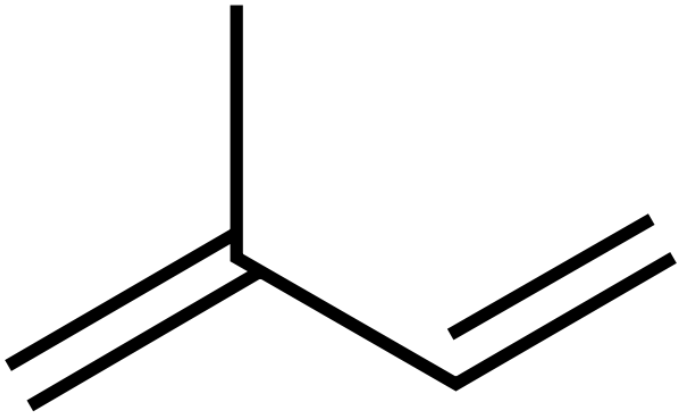
Complete biosynthesis of cannabinoids and their unnatural analogues in yeast

Xiaozhou Luo, Michael A. Reiter, Leo d'Espaux, Jeff Wong, Charles M. Denby, Anna Lechner, Yunfeng Zhang, Adrian T. Grzybowski, Simon Harth, Weiyin Lin, Hyunsu Lee, Changhua Yu, John Shin, Kai Deng, Veronica T. Benites, George Wang, Edward E. K. Baidoo, Yan Chen, Ishaan Dev, Christopher J. Petzold & Jay D. Keasling

Nature **567**, 123–126 (2019) | Download Citation

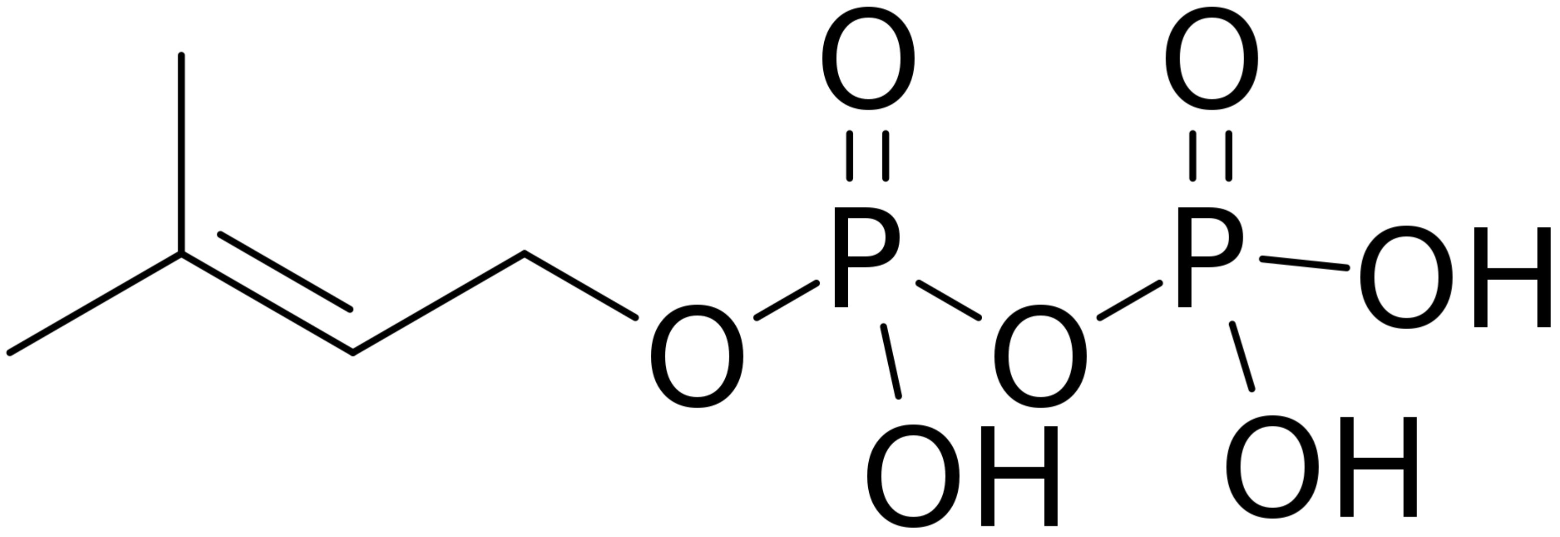
LALLEMAND

BIOENGINEERING: SIMPLIFIED WORKFLOW



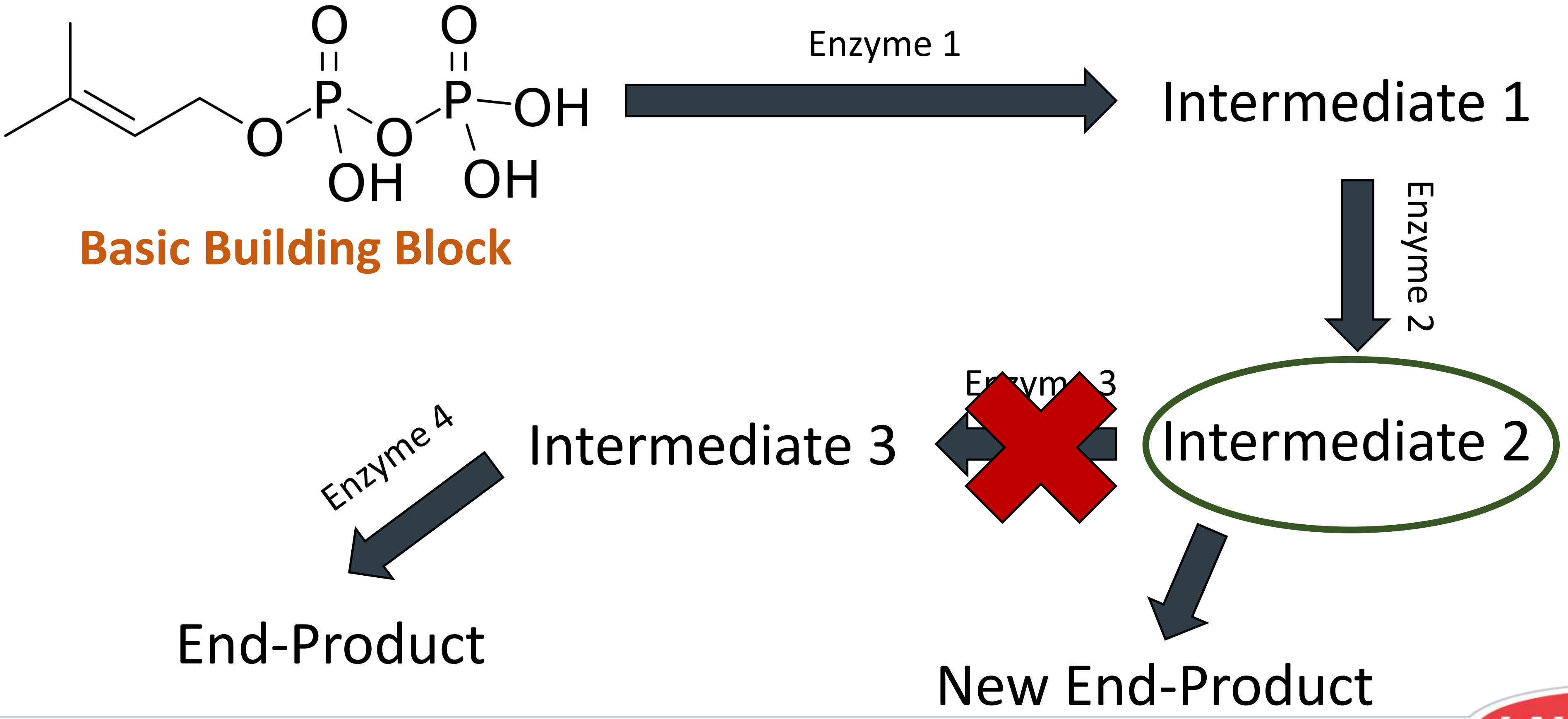
Isoprene – A Universal Biological Compound

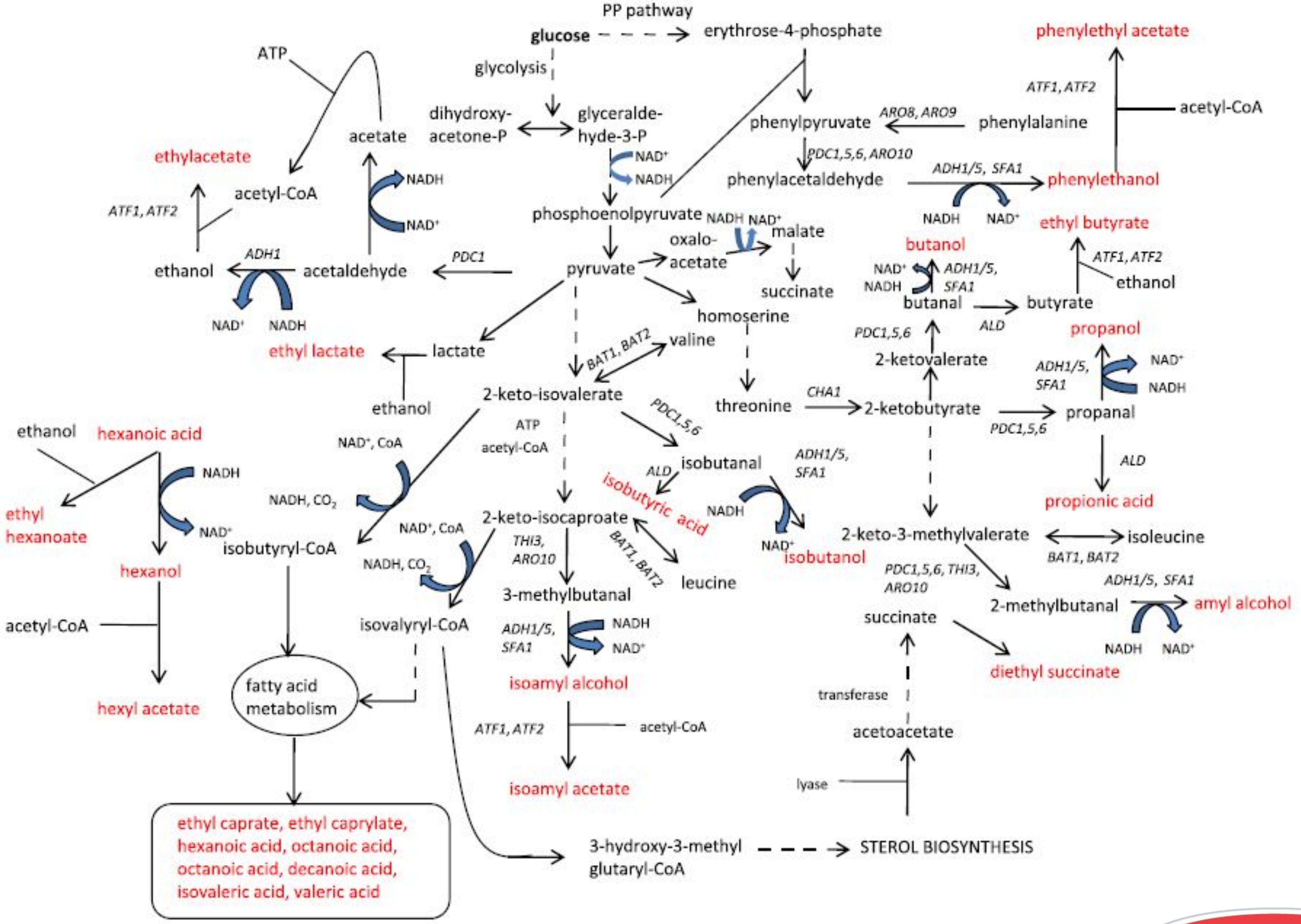
BIOENGINEERING: SIMPLIFIED WORKFLOW



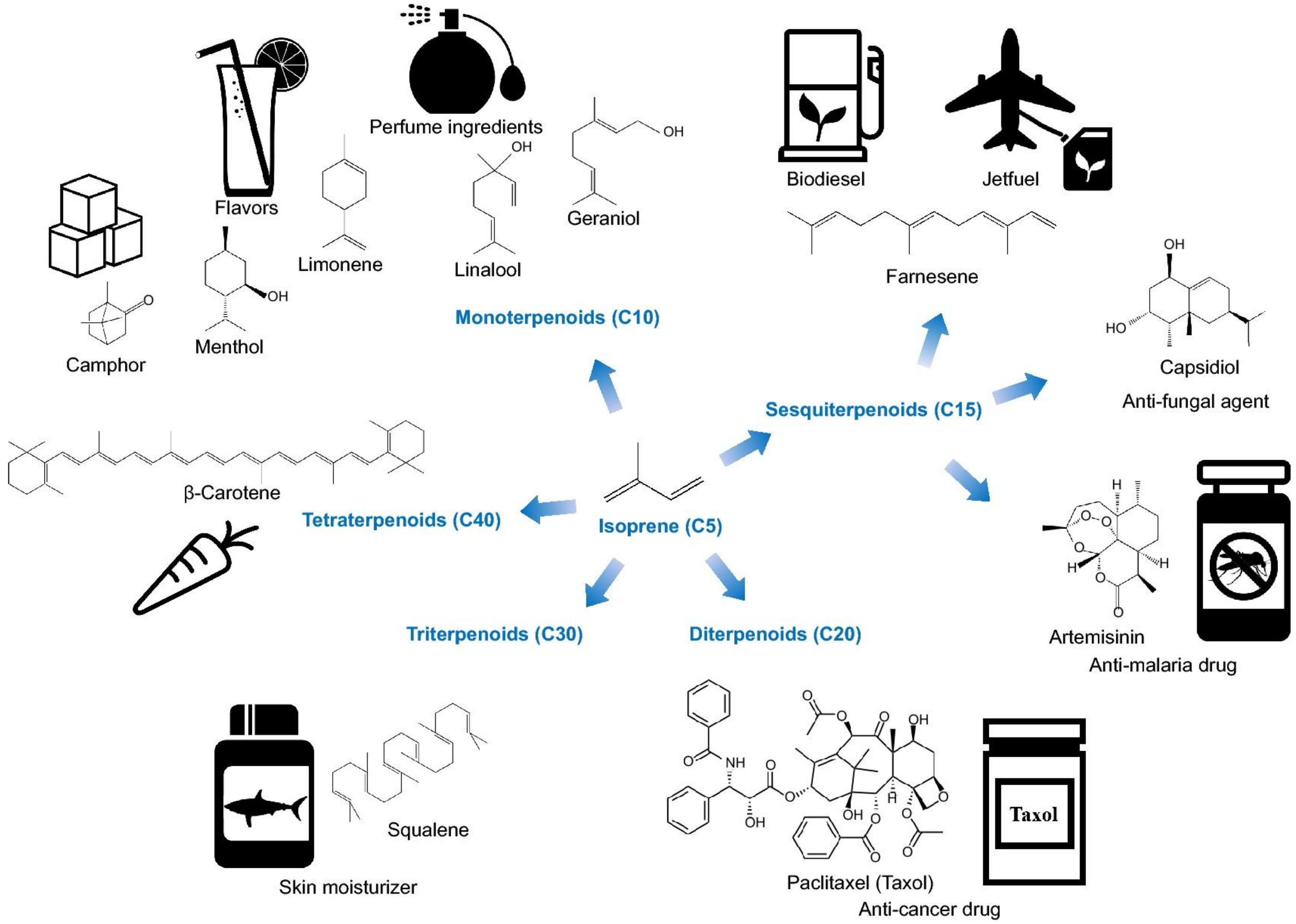
Dimethylallyl pyrophosphate: Terpene-Building Compound

BIOENGINEERING: SIMPLIFIED WORKFLOW




PP pathway
glucose
erythrose-4-phosphate
phenylethyl acetate
ATP
glycolysis
ATF1, ATF2
acetyl-CoA
dihydroxy-acetone-P
glyceraldehyde-3-P
phenylpyruvate
ARO8, ARO9
phenylalanine
acetate
ethylacetate
NAD+
NADH
PDC1,5,6, ARO10
acetyl-CoA
phenylacetaldehyde
ADH1/5, SFA1
phenylethanol
ATF1, ATF2
phosphoenolpyruvate
oxalo-acetate
malate
NADH
NAD+
ethyl butyrate
butanol
ADH1
PDC1
ethanol
acetaldehyde
pyruvate
succinate
ADH1/5, SFA1
ATF1, ATF2
ethanol
butanal
butyrate
homoserine
ALD
ethyl lactate
lactate
valine
BAT1, BAT2
PDC1,5,6
propanol
2-ketovalerate
ethanol
2-keto-isovalerate
threonine
CHA1
2-ketobutyrate
propanal
ethanol
hexanoic acid
NAD+, CoA
ATP
acetyl-CoA
PDC1,5,6
isobutanal
ADH1/5, SFA1
ALD
NADH, CO_2
isobutyric acid
propionic acid
ethyl hexanoate
2-keto-isocaproate
2-keto-3-methylvalerate
isoleucine
isobutyryl-CoA
NAD+, CoA
THI3, ARO10
BAT1, BAT2
isobutanol
hexanol
NADH, CO_2
leucine
PDC1,5,6, THI3, ARO10
3-methylbutanal
2-methylbutanal
amyl alcohol
acetyl-CoA
isovalryl-CoA
ADH1/5, SFA1
succinate
diethyl succinate
isoamyl alcohol
hexyl acetate
fatty acid metabolism
transferase
ATF1, ATF2
acetyl-CoA
acetoacetate
isoamyl acetate
lyase
ethyl caprate, ethyl caprylate, hexanoic acid, octanoic acid, octanoic acid, decanoic acid, isovaleric acid, valeric acid
3-hydroxy-3-methyl glutaryl-CoA
STEROL BIOSYNTHESIS

BIOENGINEERING: SIMPLIFIED WORKFLOW



Terpene Pathways:

- Of particular interest
- Highly versatile
- Wide range of metabolic byproducts
- Conserved/universal

FEMS Yeast Research, Volume 17, Issue 8, December 2017, fox080, https://doi.org/10.1093/femsyr/fox080

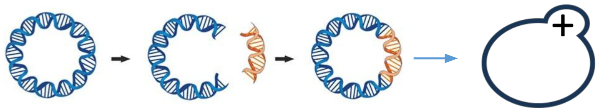
OPTIMIZATION ORIENTED GOALS

Workflow dynamics:

- Assessing industry need
- Identify appropriate phenotype
- Cross-discipline/intra-industry coordination
- Viability
- Execution

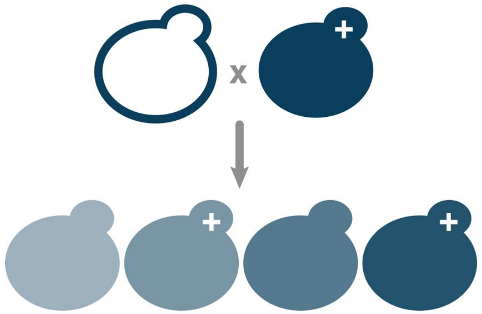
HOW CAN THIS BE ACCOMPLISHED?

Genetic Engineering



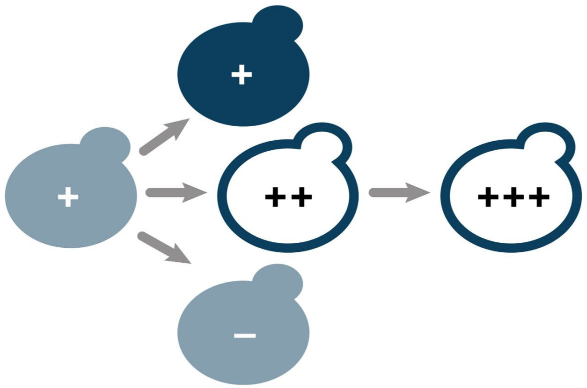
non GMO

Selective Breeding/Hybridization



non GMO

Adaptive Evolution



LALLEMAND

HOW CAN THIS BE ACCOMPLISHED?

Generating new yeast for modern applications:

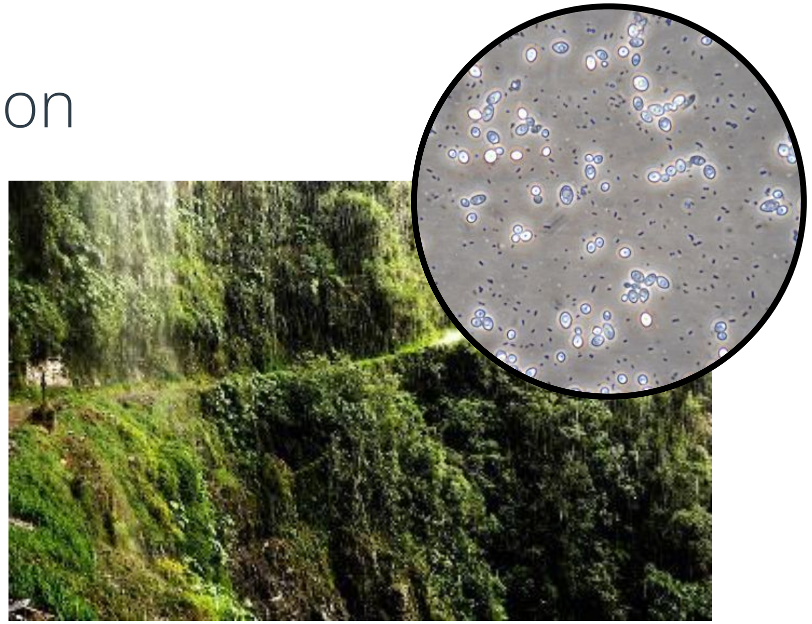
- Organism discovery
  - New genes!
- Breeding new strains using classical genetics
  - Mixing genes!

- Direct manipulation of genome with biotechnology
  - Custom genes!

SEARCHING FOR THAT NOVELTY

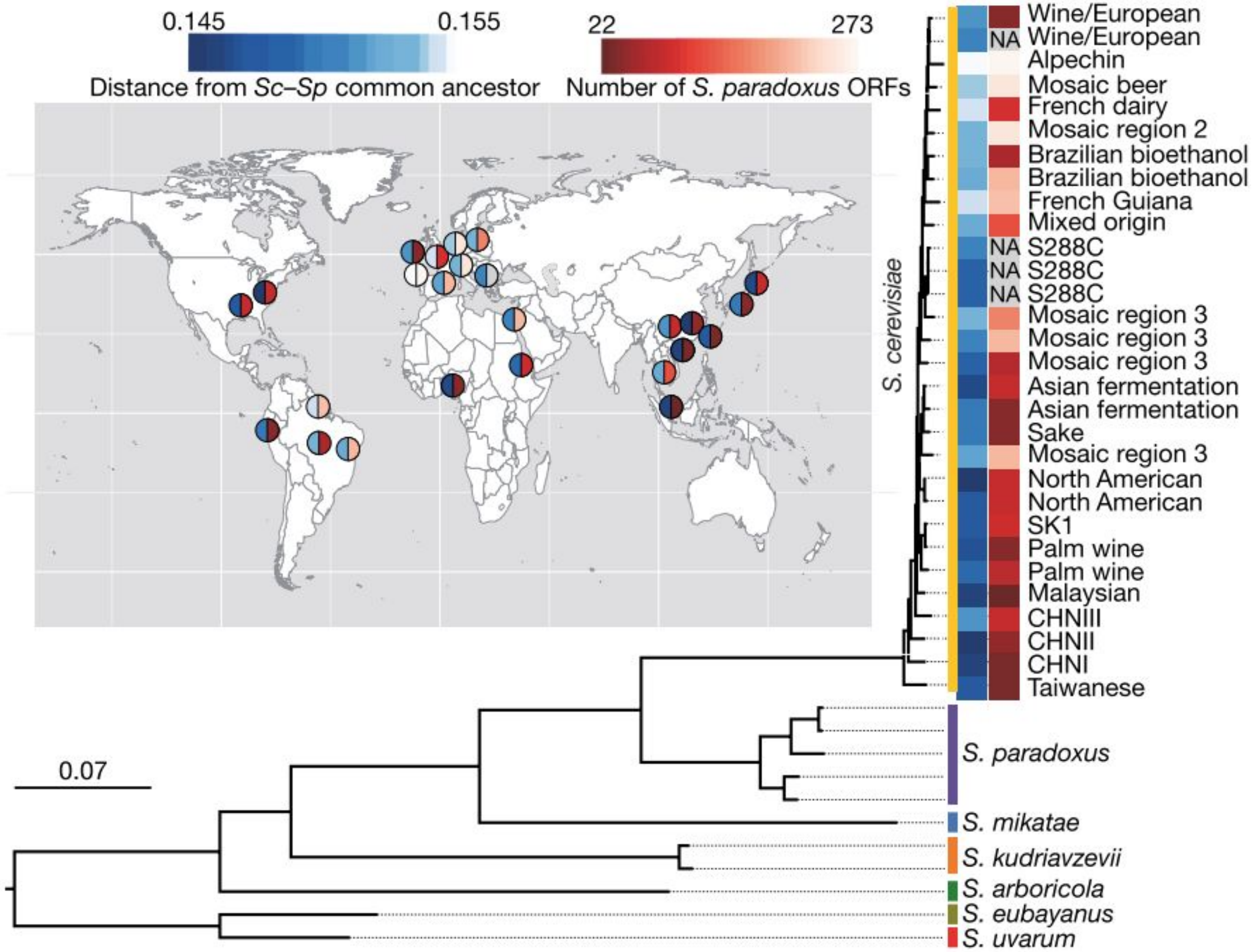
Organism discovery:
Prospecting for novel genetic material

- Exploring new species for fermentation



FOLLOWING THE CLUES

Fig. 2 | Chinese origin of *S. cerevisiae*. Maximum-likelihood rooted tree of the *Saccharomyces* complex, based on the alignment of 2,018 concatenated conserved genes. Heat maps display the distance from the last common ancestor of *S. cerevisiae* (*Sc*)–*S. paradoxus* (*Sp*) (white–blue), and the number of introgressed *S. paradoxus* ORFs (white–red). The map shows the geographical origins of the strains.



Peter J. et al. 2018. Genome evolution across 1,011 Saccharomyces cerevisiae isolates Species-wide genetic and phenotypic diversity. Nature. 556:339–347.

HOW CAN THIS BE ACCOMPLISHED?

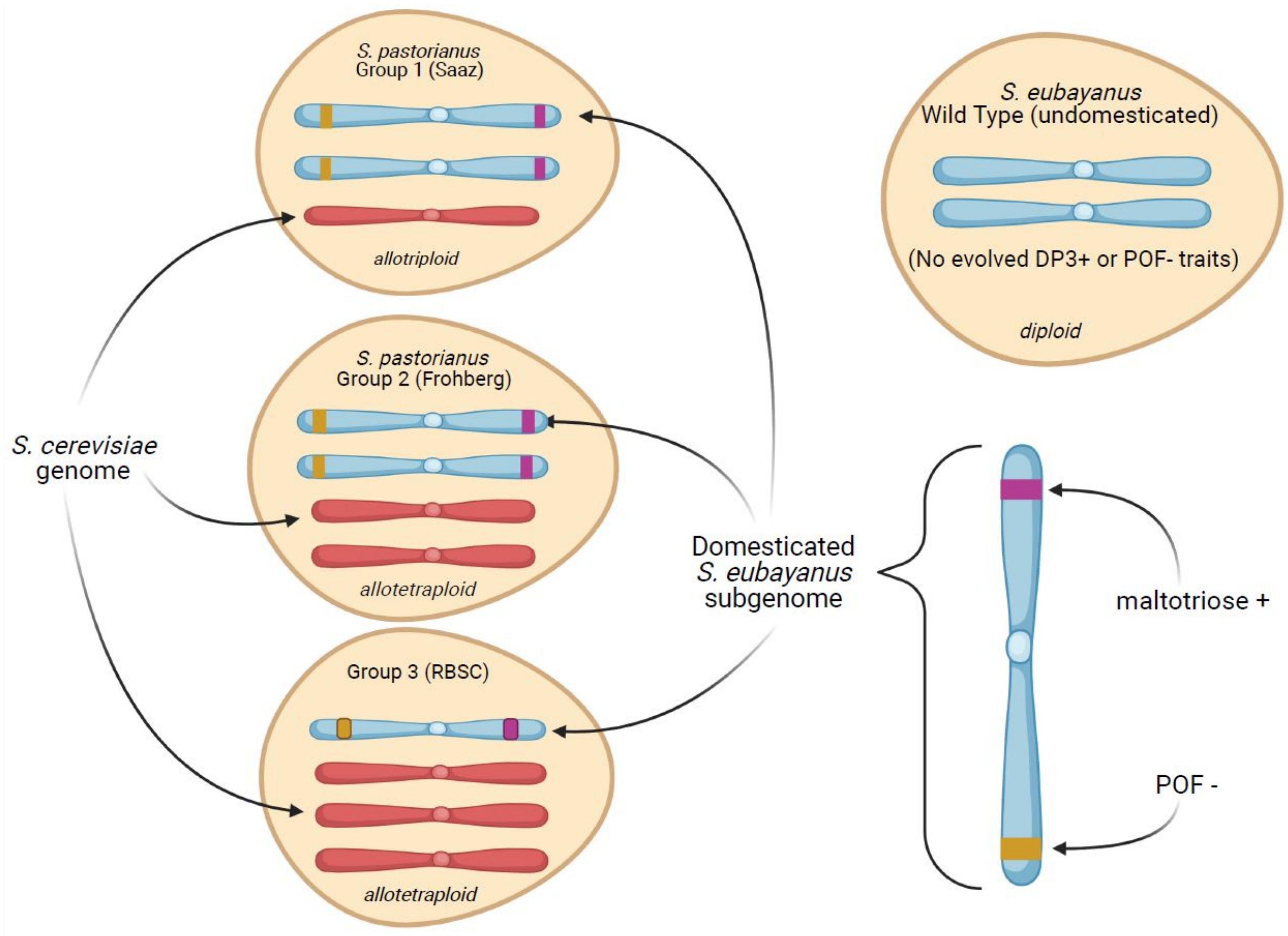
Hybridization:

- Well accepted method as an alternative to direct genetic engineering
- Excellent method of artificially enriching genetic diversity
- Can happen when in nature when conditions are right

However…

- Relies on mating-competent meiotic segregants
- Extensive characterization of parent organisms for optimum results

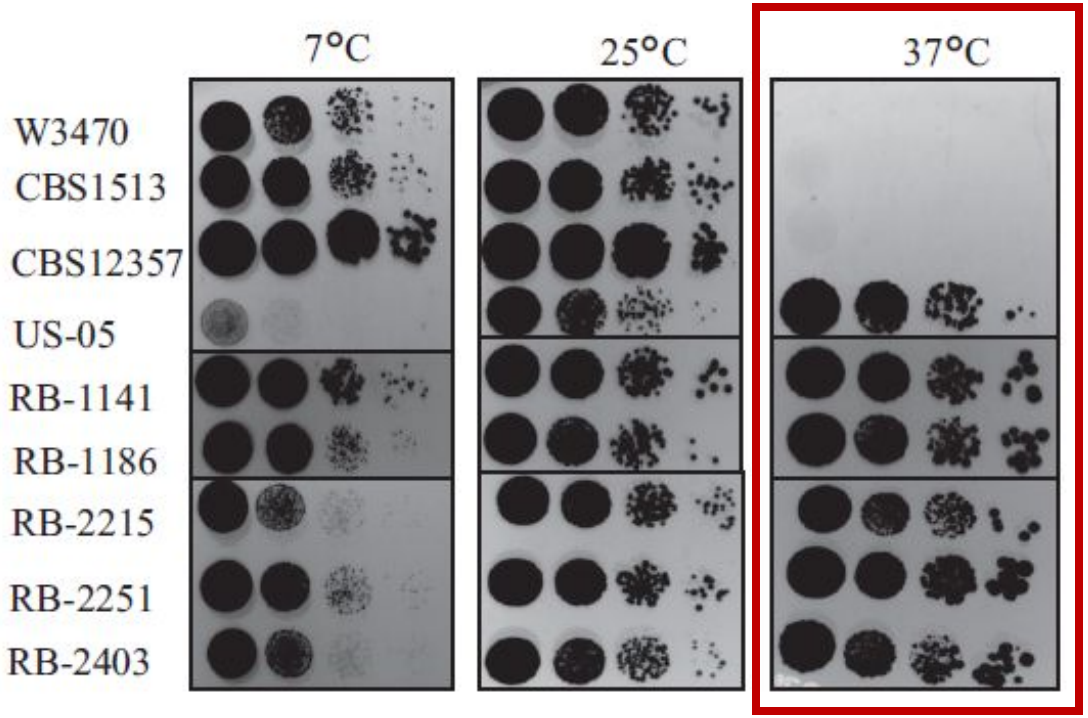
HYBRIDIZATION SCHEMATIC: REAL WORLD APPLICATIONS



Adapted from Turgeon et al. 2021

- *"Group 3"* – proposed by Turgeon *et al*. (2021) taxonomic subgrouping of brewing yeast into new family of artificially derived lager yeast
- Genetically distinct from Group 1 and 2 lager yeast with higher proportion of *S. cerevisiae* genome while retaining classical traits defining domesticated *S. eubayanus.*

HYBRIDIZATION SCHEMATIC: REAL WORLD APPLICATIONS

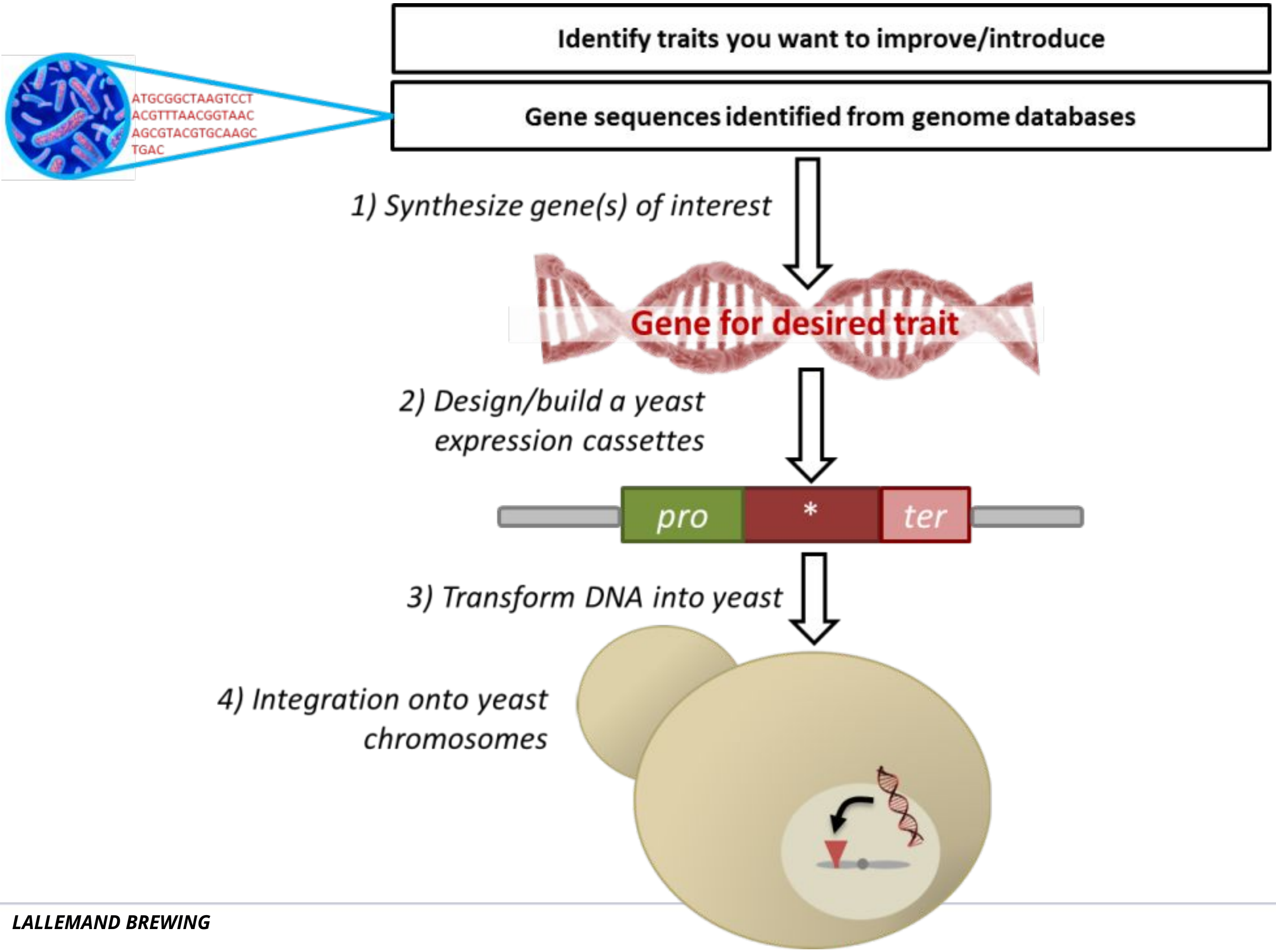


Significantly improved hybrid survival

FIG 1 Temperature tolerance of novel hybrids compared to that of group I (CBS1513), group II (W3470), *S. eubayanus* (CBS12357), and *S. cerevisiae* (US-05) controls at 7°C, 25°C, and 37°C. Each spot represents a 10-fold serial dilution (10^4 to 10^1 cells/spot) on YEG medium.

Adapted from Turgeon et al. 2021

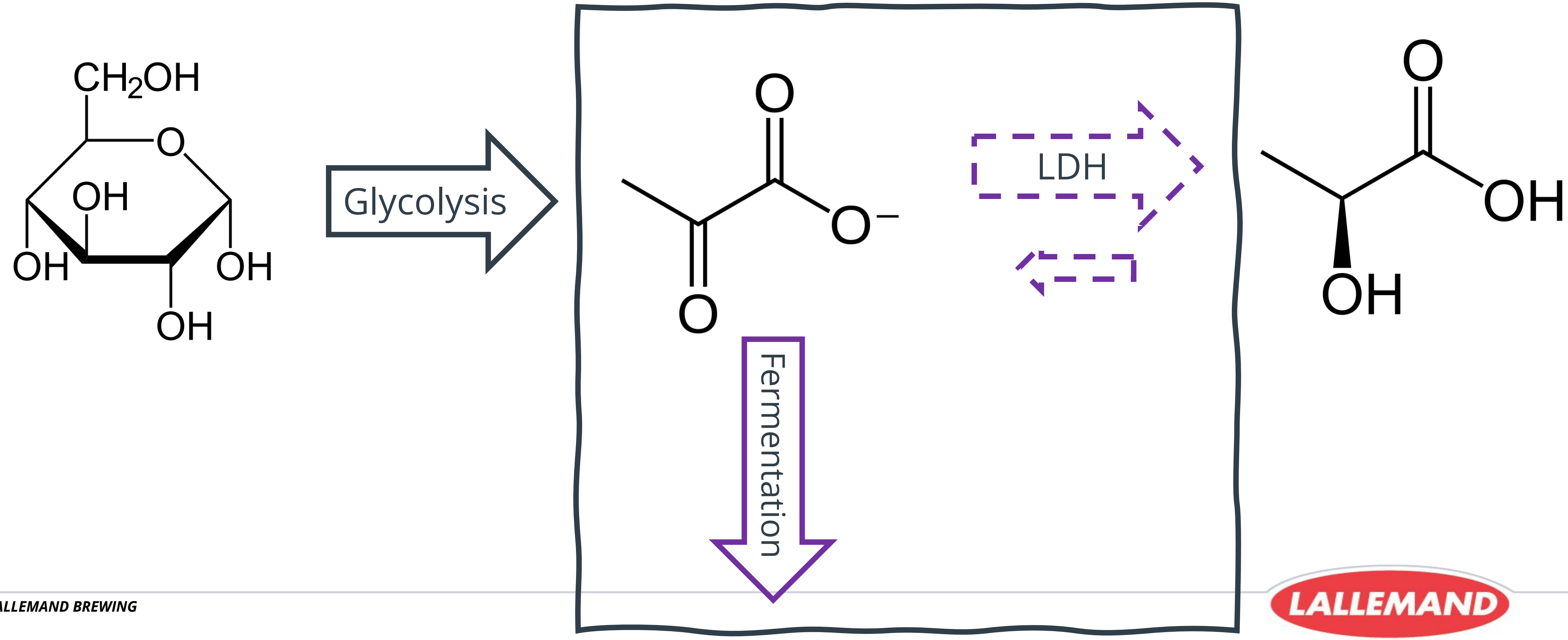
BIOENGINEERING: SIMPLIFIED WORKFLOW



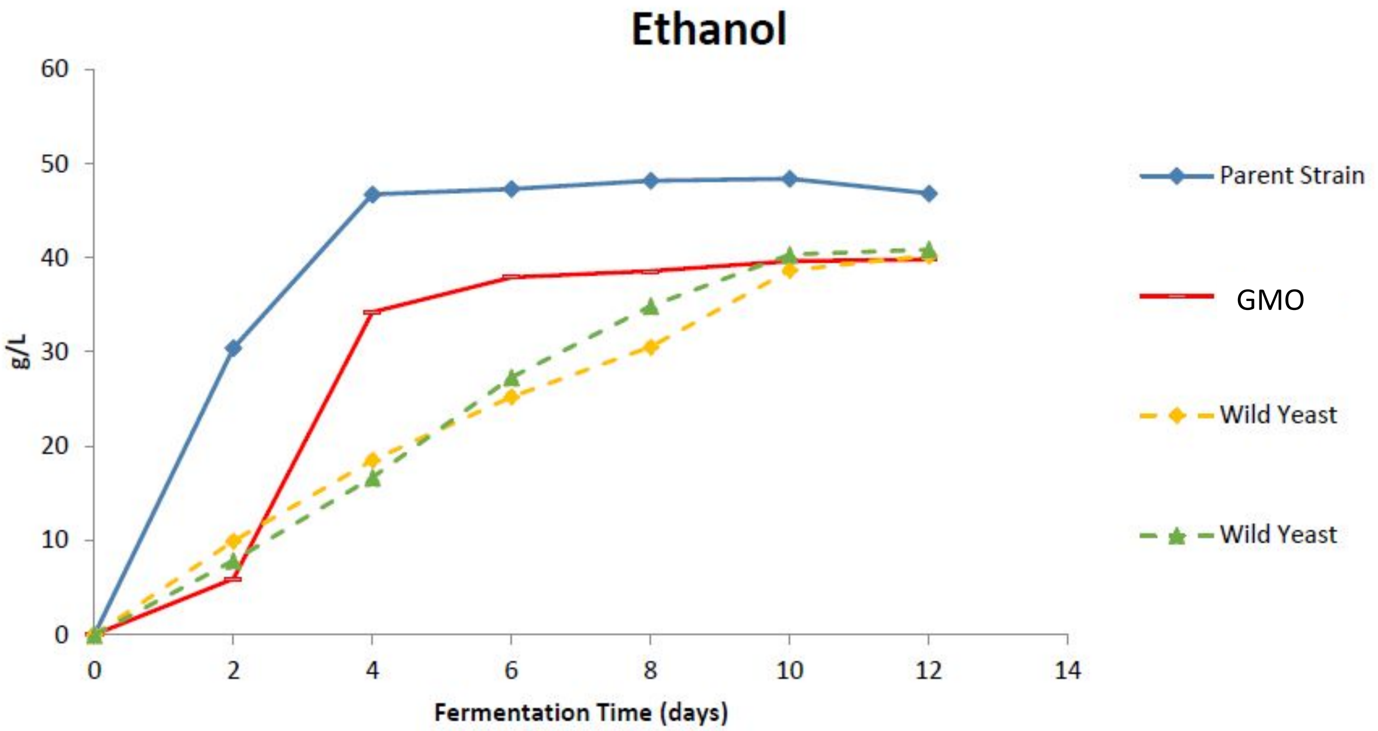
- *Truncated development scheme*

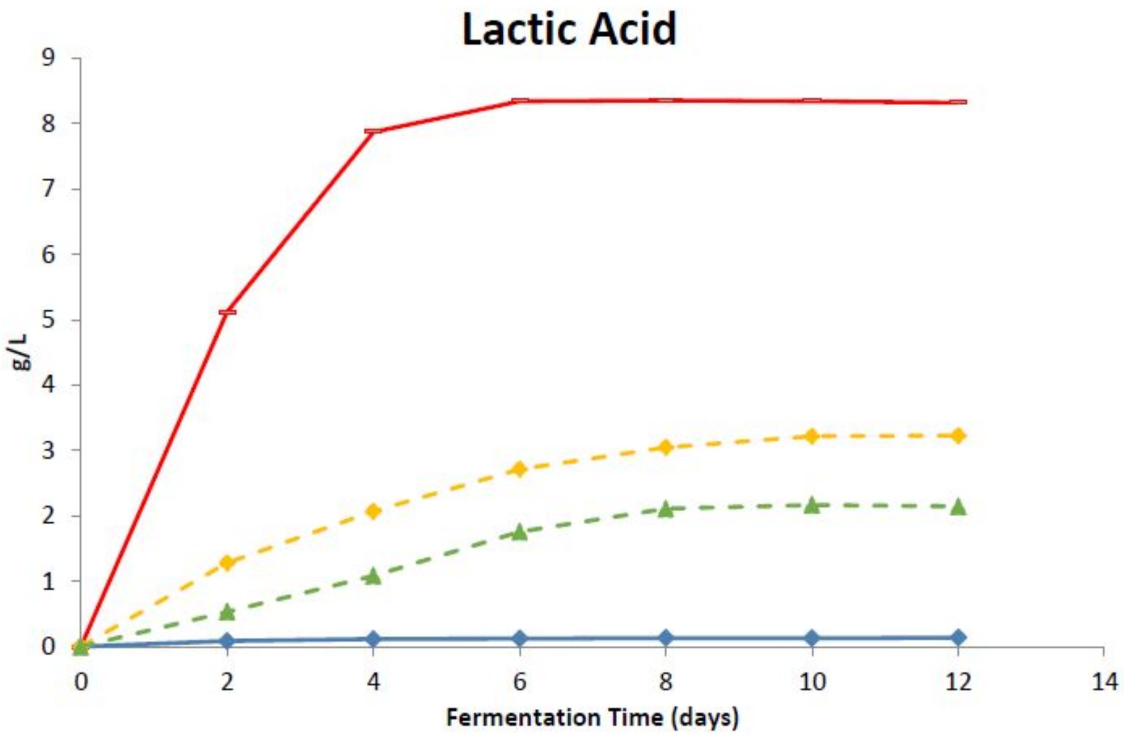
BIOENGINEERING: REAL WORLD APPLICATIONS

Proof-of-concept modification turned brewing product:

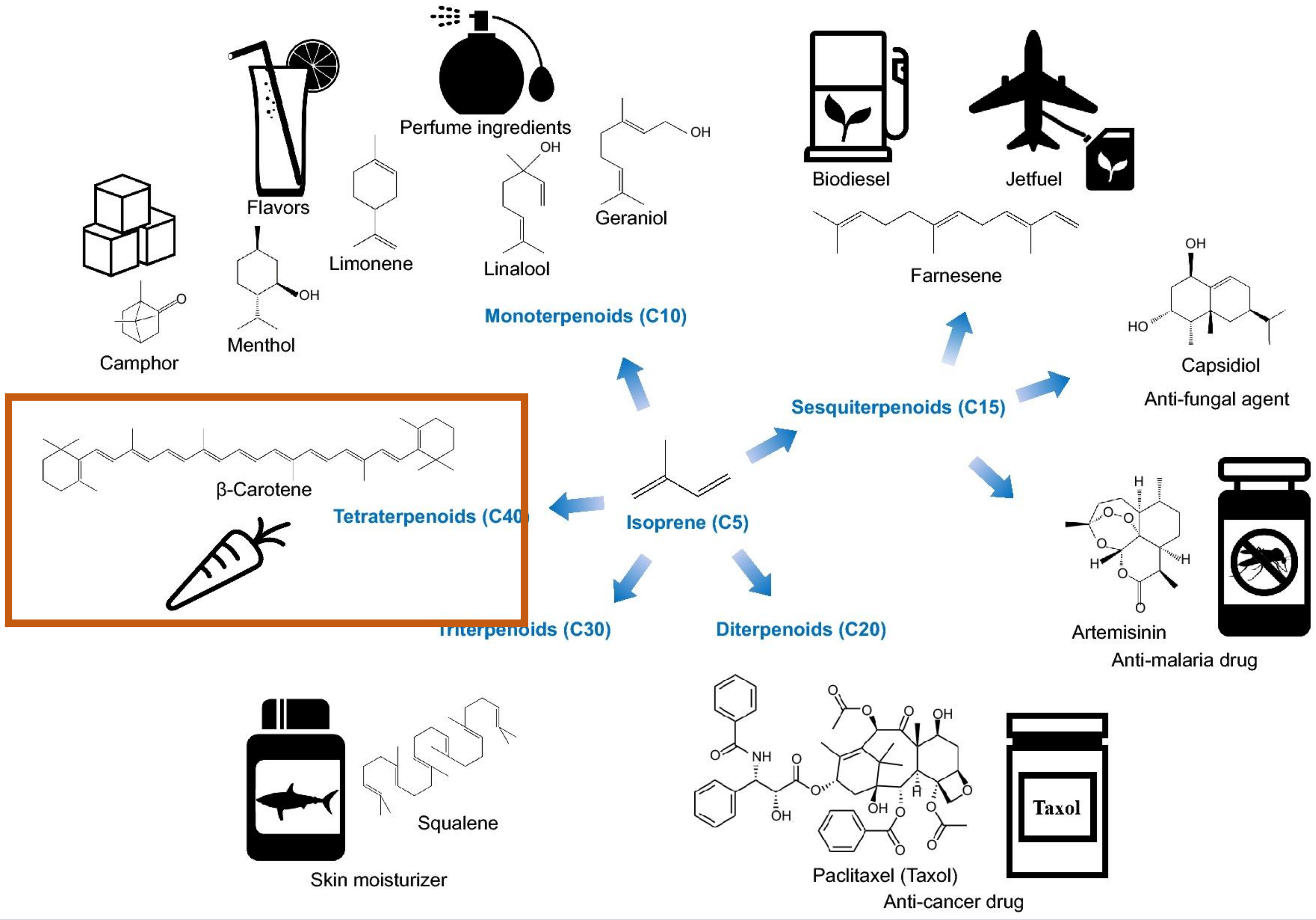


BIOENGINEERING: REAL WORLD APPLICATIONS



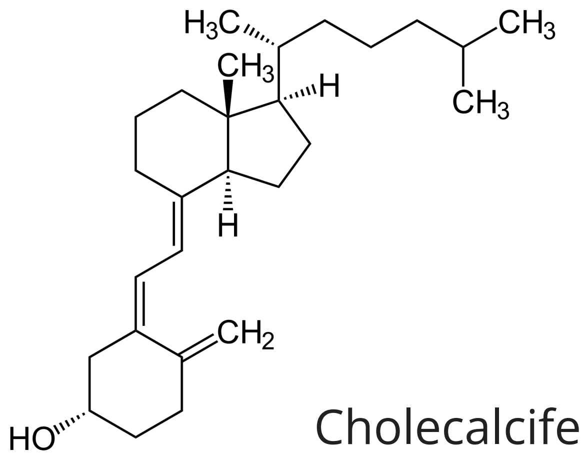


BIOENGINEERING: REAL WORLD APPLICATIONS



Exploration of other pathways:

- Further proof of concept technology



Cholecalcifero
l

FEMS Yeast Research, Volume 17, Issue 8, December 2017, fox080, https://doi.org/10.1093/femsyr/fox080

BIOENGINEERING: REAL WORLD APPLICATIONS

Lee *et al. Microb Cell Fact* (2016) 15:49
DOI 10.1186/s12934-016-0446-2

Microbial Cell Factories

RESEARCH — Open Access

CrossMark

Heterologous production of raspberry ketone in the wine yeast *Saccharomyces cerevisiae* via pathway engineering and synthetic enzyme fusion

Danna Lee[1], Natoiya D. R. Lloyd[1], Isak S. Pretorius[2] and Anthony R. Borneman[1,3*]

4-[4-hydroxyphenyl]butanoe-2-one
“berry ketone”

- Value-added compounds
- Flavor enhancement
- Synergistic applications
- Niche brewing applications

FEMS Yeast Research, Volume 17, Issue 8, December 2017, fox080, https://doi.org/10.1093/femsyr/fox080

LALLEMAND

SPECIAL THANKS TO:

- The Lallemand Brewing team
- Bailey Carignan, Mascoma LLC
- Chaz Rice, Mascoma LLC
- Jessica Swanson, Renaissance BioSciences
- Zachari Turgeon, Renaissance BioSciences

QUESTIONS

Avi Shayevitz

R&D Scientist | Montreal, QC Canada

Lallemand Brewing

ashayevitz@lallemand.com

FEMS Yeast Research, Volume 17, Issue 8, December 2017, fox080, https://doi.org/10.1093/femsyr/fox080