



InCider: Selecting your Yeast



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Education:

- **2018 - PhD in Wine Science - University of Adelaide, Wine Microbiology and Microbial Biotechnology Laboratory**
- **2014 - Master's degree in Industrial Biotechnologies - Heineken Netherlands / University of Milan-Bicocca**
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Publications:

- Chen, L., Capone, D. L., **Tondini, F.**, & Jeffery, D. W. (2018). Chiral Polyfunctional Thiols and Their Conjugated Precursors upon Winemaking with Five *Vitis vinifera* Sauvignon blanc Clones. *Journal of Agricultural and Food Chemistry*, 66(18), 4674-4682.
- **Tondini, F.**, Jiranek, V., Grbin, P. R., & Onetto, C. A. (2018). Genome Sequence of Australian Indigenous Wine Yeast *Torulaspora delbrueckii* COFT1 Using Nanopore Sequencing. *Genome Announcements*, 6(17), e00321-18.
- **Tondini, F.**, Lang, T., Chen, L., Herderich, M., & Jiranek, V. (2019). Linking gene expression and oenological traits: comparison between indigenous *Torulaspora delbrueckii* and *Saccharomyces cerevisiae* strains. *International Journal of Food Microbiology*.

Complexity

‘Terroir
expression’

No predictability

No reliability

Osmotic stress

Nutrient

Ethanol toxicity

Saccharomyces

Non-*Saccharomyces*



Assess the risk

Predict the outcome

Which *Yeast* was the most involve in spontaneous Cider fermentation?

The main yeasts found in cider are *Saccharomyces* yeasts. A study of unpasteurized ciders and cider musts obtained from different cider houses from northwestern regions of France reported 15 yeast species among 208 picked isolates

Saccharomyces bayanus was the predominant species from the beginning to the middle steps of the fermentation process, accounting for up to 41% of the picked isolates, whereas *S. cerevisiae* took over the process in the final stages of fermentation.



Family Name:
Saccharomyces

Name:
Cerevisiae

Nationality:
China/Far East Asia

Ø : 3-4 µm

Work:
Ale Beer, Wine

S. arboricolus
S. (eu)bayanus
S. kudriavzevii
S. mikatae
S. paradoxus
S. uvarum

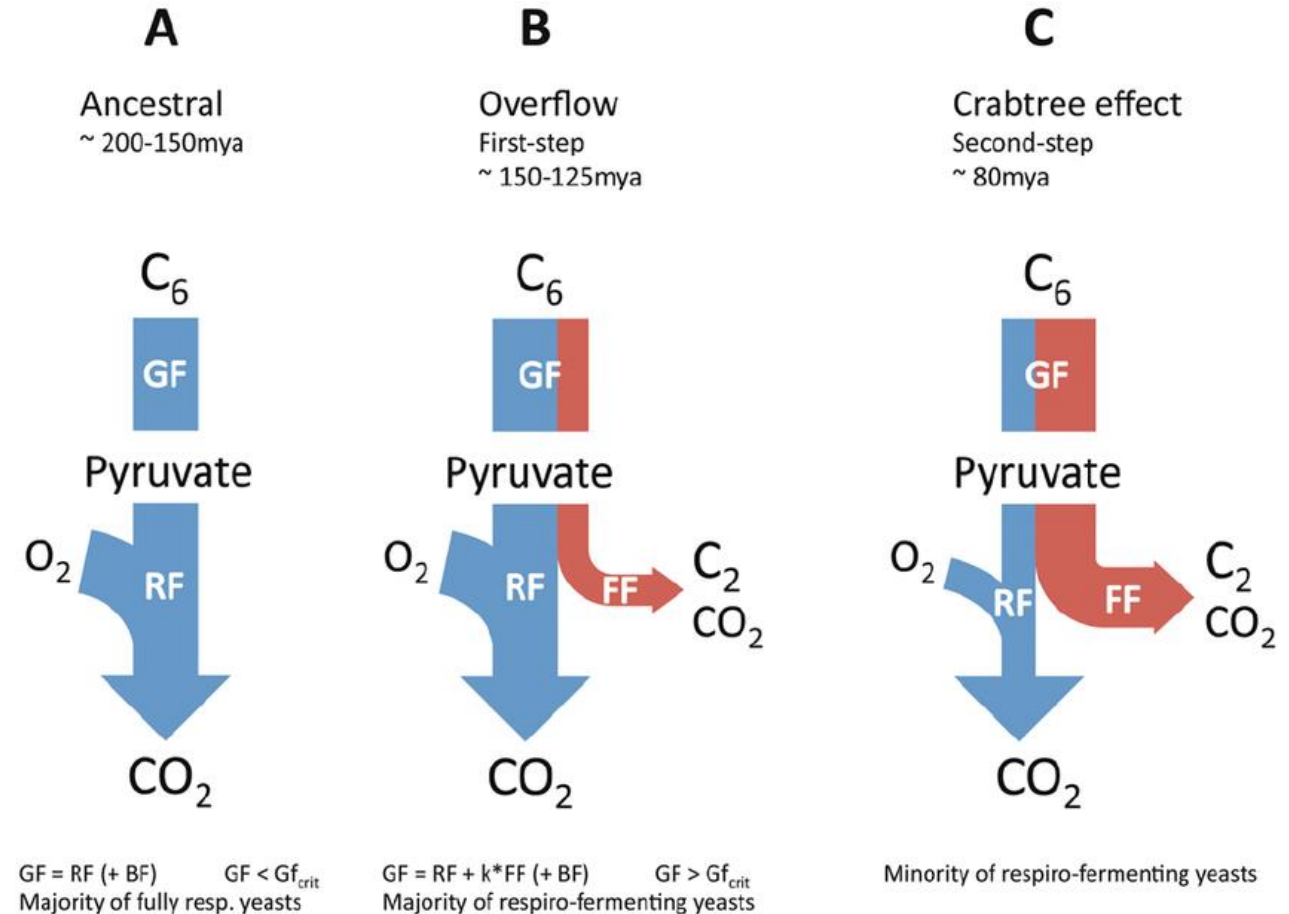
most psychrotrophic species

“The origin and adaptive evolution of domesticated populations of yeast from Far East Asia.” Duan et al. (2018)

‘make-accumulate-consume’

- Crabtree effect: most remarkable characteristics of *S. cerevisiae* and closely related species is their ability to produce and accumulate ethanol
- Overflow in sugar metabolism
- Ethanol inhibits the growth of other microbes.

Ethanol prolongs shelf-life, improves digestibility and acts as a euphoriant



the lager yeasts

S. pastorianus (*S. cerevisiae* × *S. uvarum* × *S. eubayanus*)

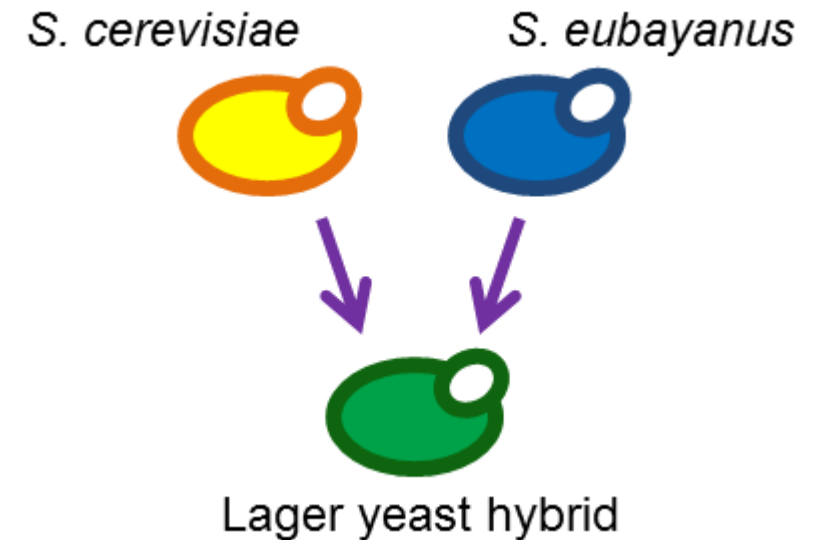
wine, cider and brewing

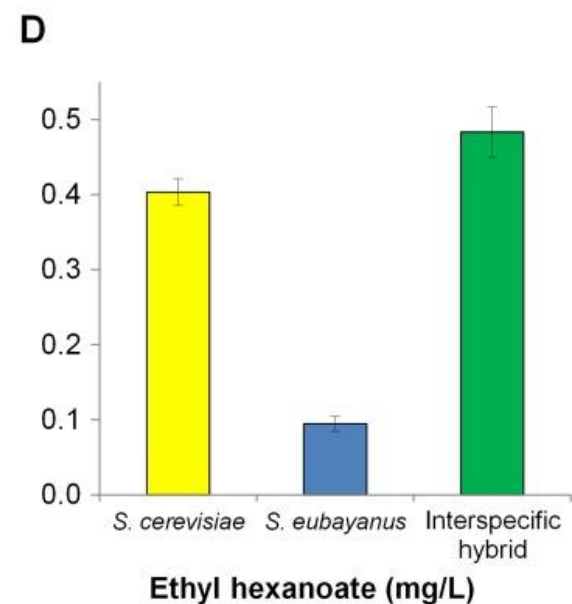
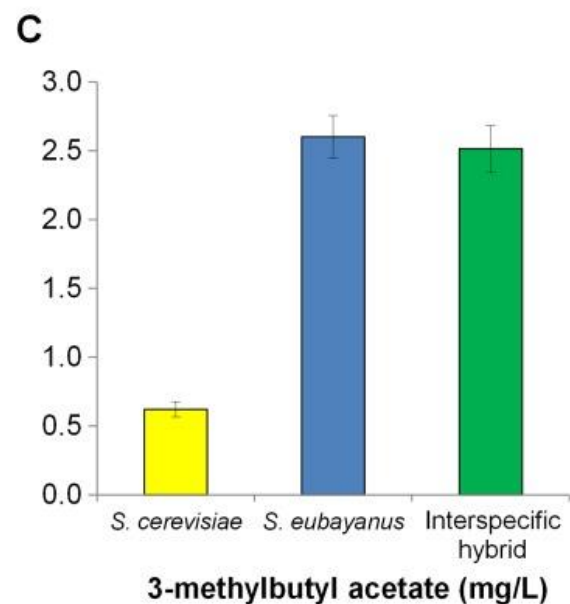
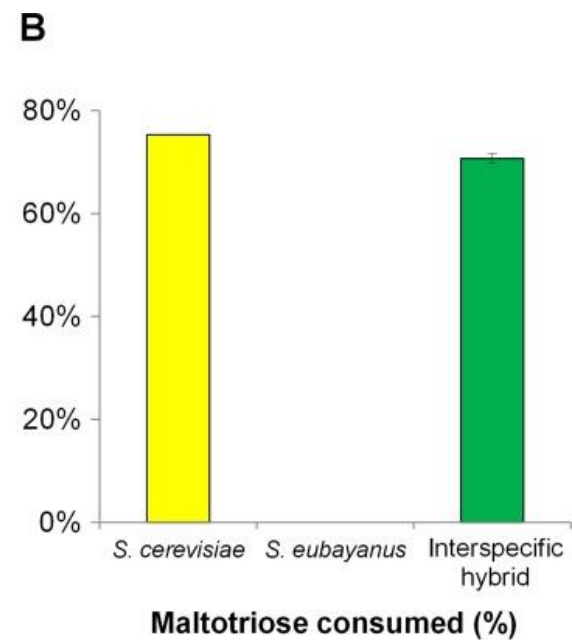
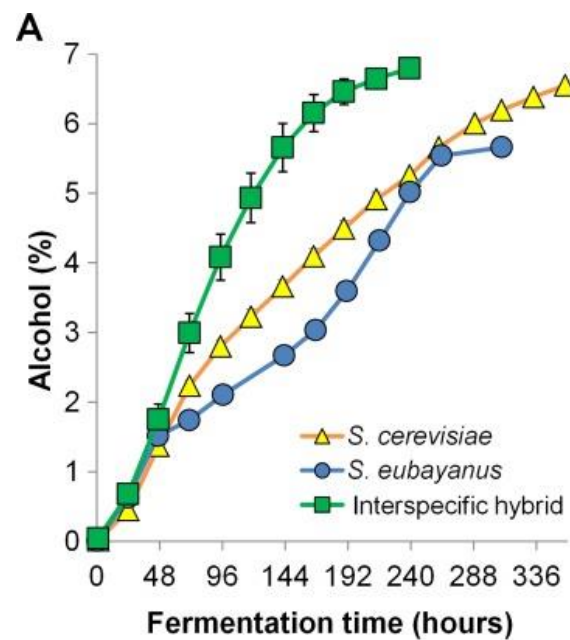
S. cerevisiae × *S. kudriavzevii*

S. bayanus (*S. uvarum* × *S. eubayanus*)

S. cerevisiae × *S. uvarum*

S. cerevisiae × *S. kudriavzevii* × *S. uvarum*





- Man's oldest industrial microorganism
- Man used yeast before the development of a written language
- Ancient Egyptians were using yeast and the process of fermentation to produce alcoholic beverages and to leaven bread over 5,000 years ago



- Early fermentation systems for alcohol production and bread making were formed by natural microbial contaminants
- Microbial flora would have included wild yeasts and lactic acid bacteria that are found associated with cultivated grains and fruits.
- Over the course of time, the use of starter cultures helped to select for improved yeasts by saving a “good” batch of wine, beer or dough for inoculating the next batch
→ DOMESTICATION

DOMESTICATION

- ‘Domestication’ is a term that refers to [artificial selection](#) and breeding of [wild species](#) to obtain cultivated variants with enhanced desirable features that thrive in man-made environments, often at the cost of suboptimal fitness in natural settings.

“Origins, evolution, domestication and diversity of *Saccharomyces* beer yeasts”. Gallone et al. (2018)

- **17th century, Antoni van Leeuwenhoek:** developed high-quality lenses and was able to observe yeast for the first time.
- **1785, Antoine Lavoisier:** French chemist analyzed the mechanism by which sugarcane is transformed into alcohol and carbon dioxide. The experiment provided a clear insight into the basic chemical reactions needed to produce alcohol but nothing about yeast contribution.
- **1857, Louis Pasteur:** demonstrate experimentally that fermented beverages result from the action of living yeast transforming glucose into ethanol.

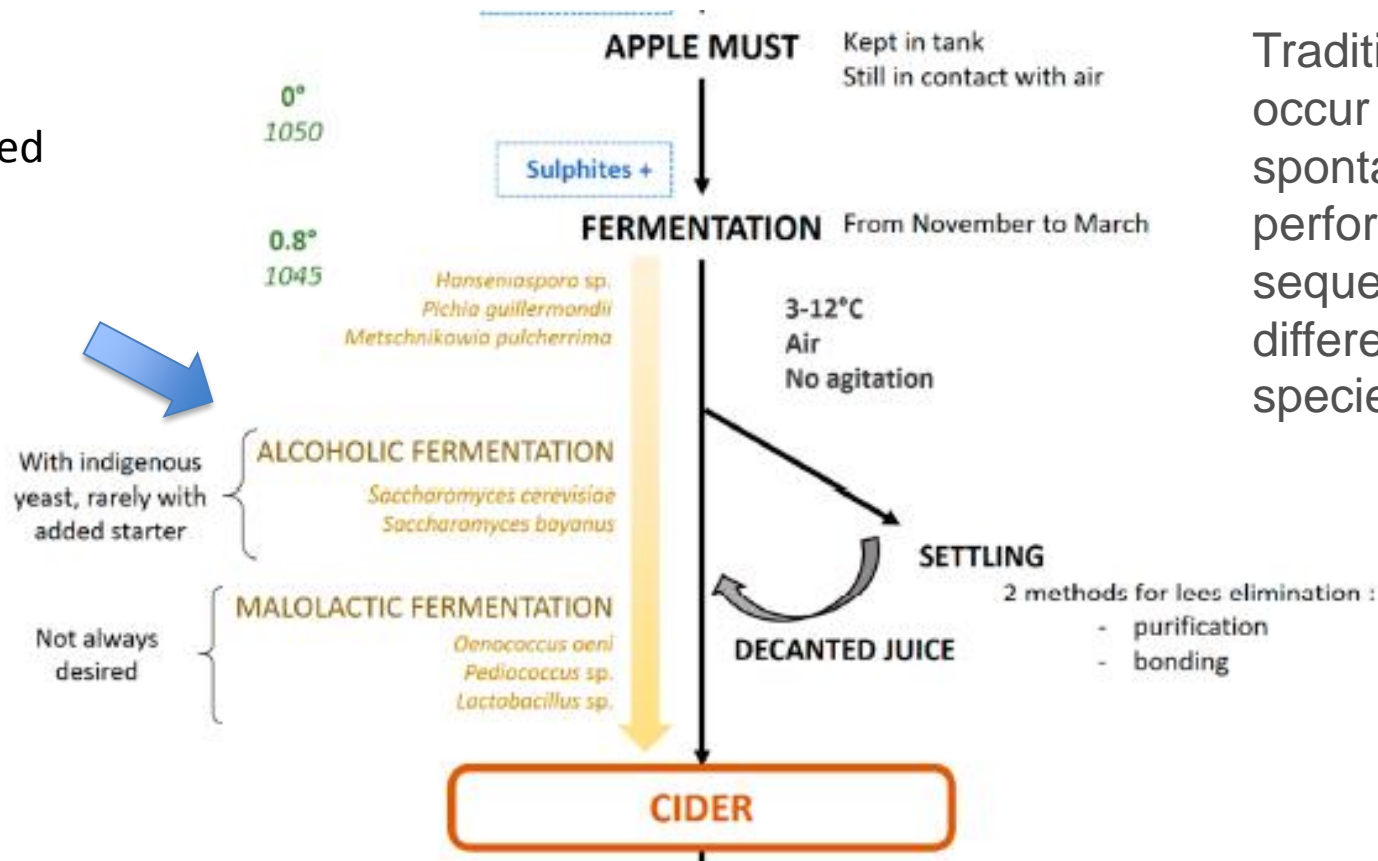


- **1880s, Emile Christian Hansen:** developed the first pure yeast culture and wort inoculation was performed some years later.
- **1890, Müller-Thurgau:** performed the first inoculation of grape must with a pure yeast culture, but only in the late 1970 it became a wine industry common procedure.

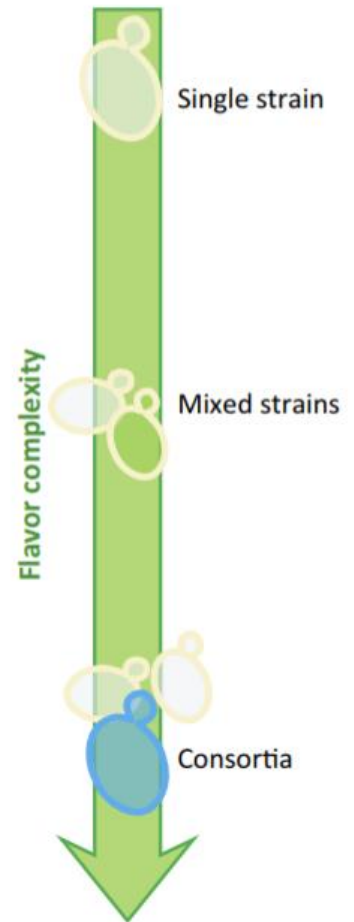
These practices have improved the control and reliability of the fermentation process, limiting microbiological alterations and have largely contributed to increased quality in recent decades.

Although of the entire cider microflora contribute to the cider chemistry, yeasts detain a predominant role, since they promote the AF

Selected yeast



Traditional fermentation occur through a spontaneous process performed by the sequential action of different yeast species/strains



Proper control of fermentation through chemical and nutrient additions, temperature control, and microflora reduction or inoculation allows for a “clean” and consistent fermentation

Debate Still Open

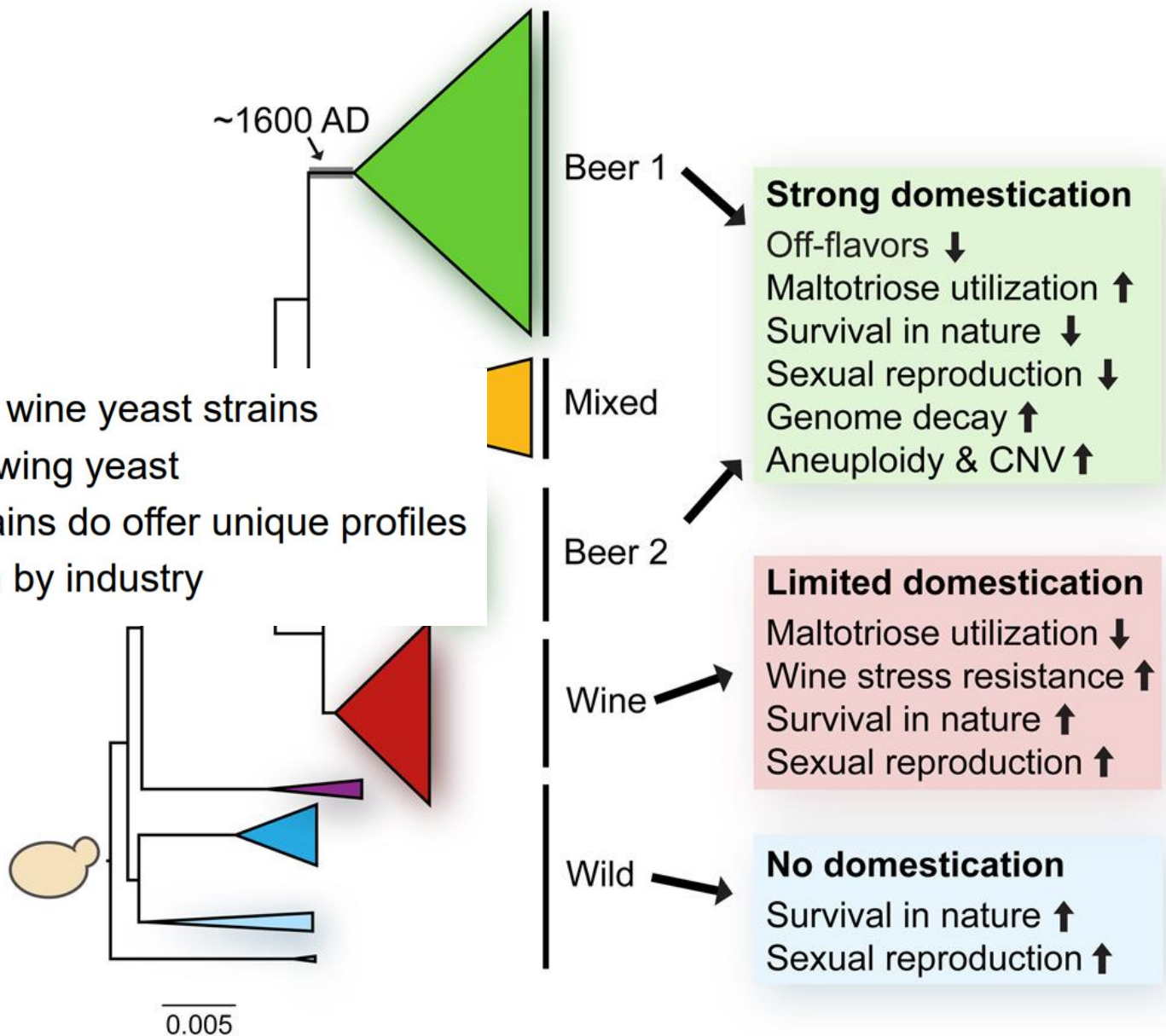
The main objection to the use of selected starter cultures is the standardization of quality, and avoid stuck and sluggish fermentation , with concomitant production of undesired metabolites

vs

Autochthonous yeast starters, reflect the biodiversity of a particular area, which support the idea that indigenous yeast strains can be associated with a “terroir”

Domestication of industrial yeasts

- Very little diversity in wine yeast strains
- More diversity in brewing yeast
- Alternative yeast strains do offer unique profiles
- Phenotypic evolution by industry

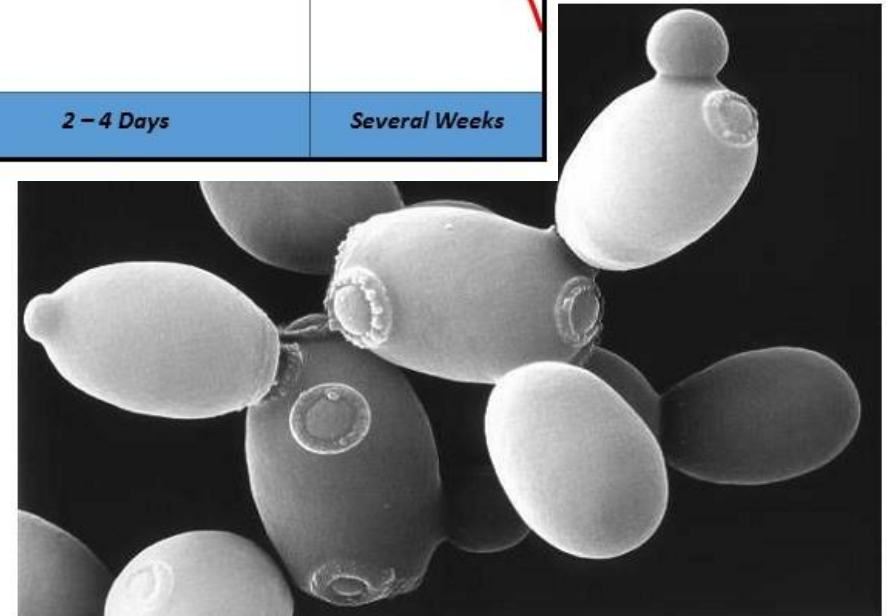
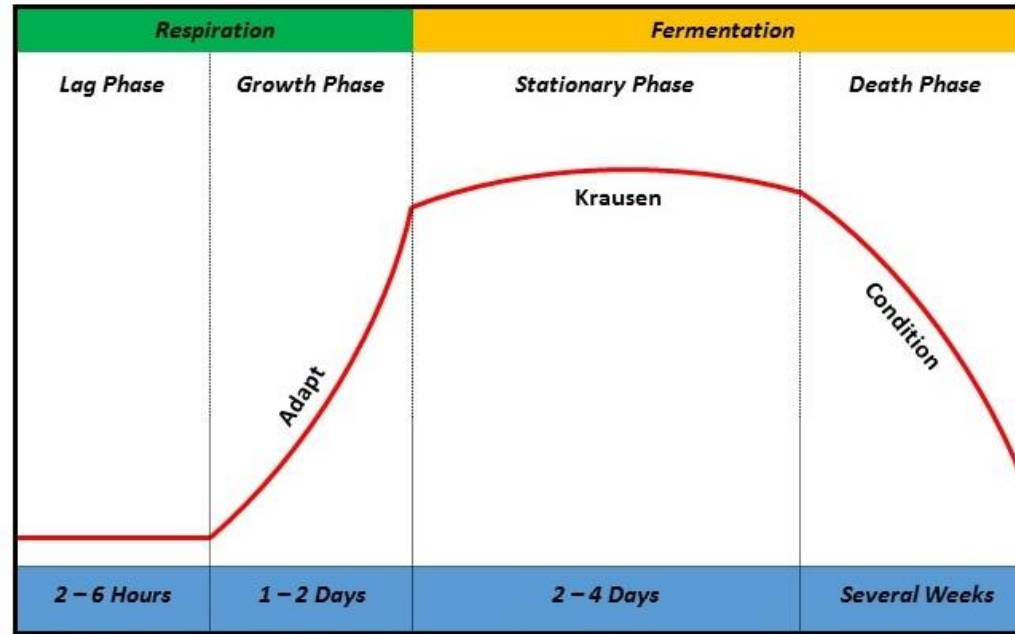


YEAST DIVERSITY
REFLECTS HUMAN
HISTORY

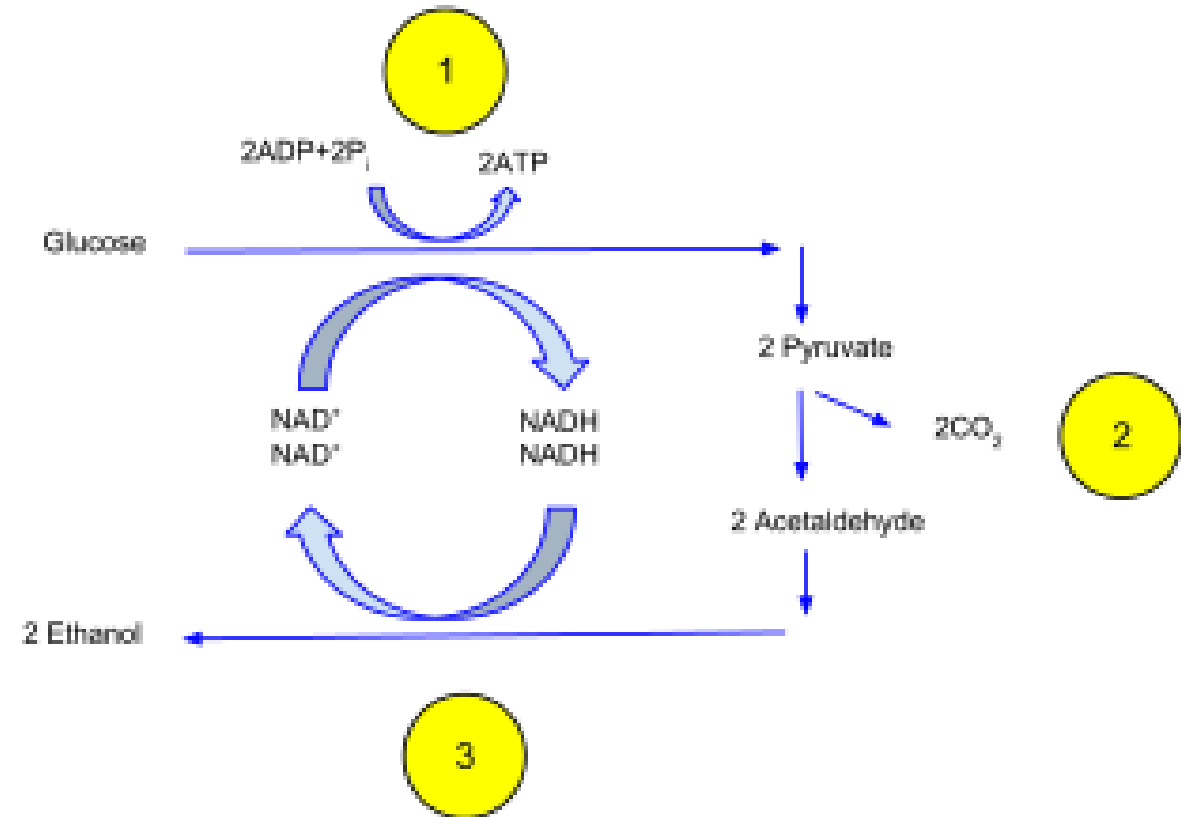
Yeast in Cider Fermentation

During traditional cider making, the yeast faces an increasingly hostile environment. During fermentation the medium rapidly becomes anaerobic and is increasingly laden with ethanol and other potentially inhibitory metabolites.

Yeast cells reproduce ($10^6 \rightarrow 10^7$) and disperse themselves throughout the fermenting juice, converting sugars to alcohols, carbon dioxide and various flavor compounds.

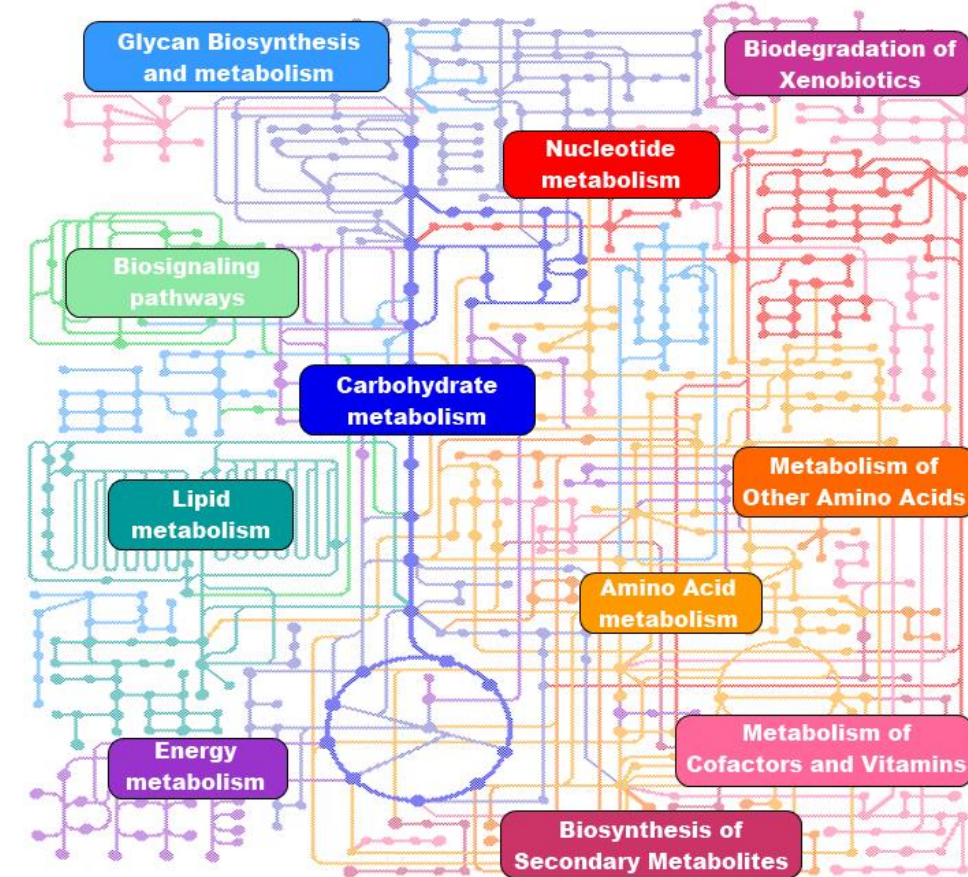


The cells absorb dissolved sugars, simple nitrogenous matter (amino acids, ammonium ions, and small peptides), vitamins, and ions through their plasma membrane. Subsequently, they employ a series of reactions known as metabolic pathways (glycolysis, biosynthesis of cellular constituents, etc.) and use these nutrient materials for growth and fermentation.

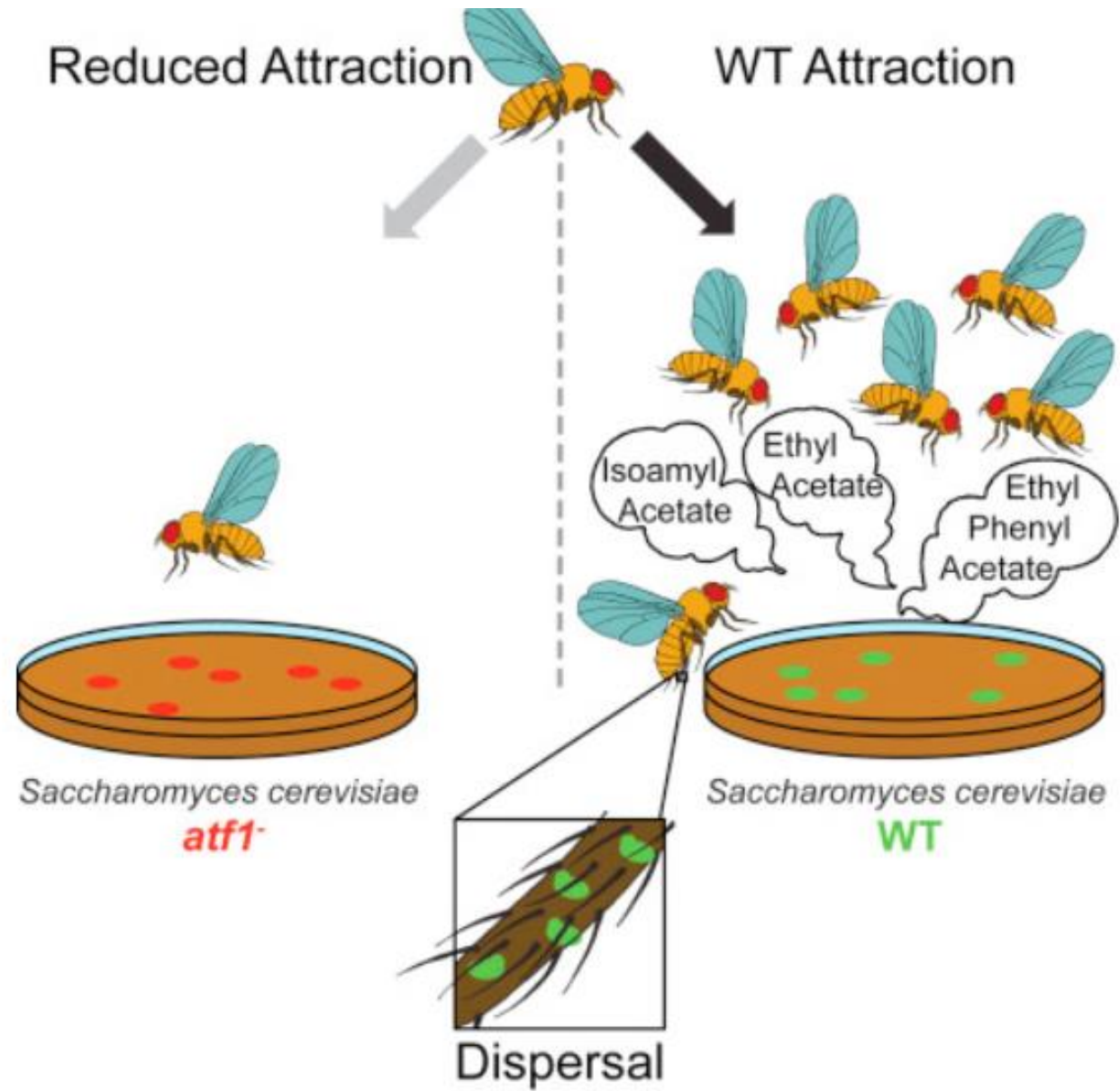


Why yeast cells produce these flavor-active molecules?

- Specific cellular building blocks, redox balancing and detoxification reactions
- Fundamental role in the lifestyle of yeast: signaling information to animal vectors, regulation of fungal growth and communication between yeast cells or colonies



(Richard *et al.* 1996; Bruce *et al.* 2005; Leroy *et al.* 2011; Davis *et al.* 2013)



[Christiaens](#) et al. 2014. The Fungal Aroma Gene ATF1 Promotes Dispersal of Yeast Cells through Insect Vectors

The aroma profile of fermented foods and beverages comprises hundreds of compounds

The flavour profiles of cider can principally be attributed to the biochemical activities during fermentation within the yeast cell

The proportional volatile fractions of cider : 49% alcohols, 36% esters, and 11% carbonyl compounds

The need to understand and control aroma compound synthesis is driven by the fact that these compounds play a key role in the sensorial quality of fermented alcoholic beverages

- Food fermentation is all about increasing the sensory quality for the consumer, and obtaining unique signature flavors that help to distinguish a product from others on the market

Hugenholtz, J. (2013) “Traditional biotechnology for new foods and beverages.”

- Consumers select products based on taste, preferring to pay more for a refined sensation, rather than less for quantity

Bisson, L.F. et al. (2002) “The present and future of the international wine industry.”

1. Involve in the biocontrol of moulds, which influences quality before harvest.

2. Perform alcoholic fermentation of must sugars and transform juice into cider; the *de novo* biosynthesis of the flavour and aroma compounds.

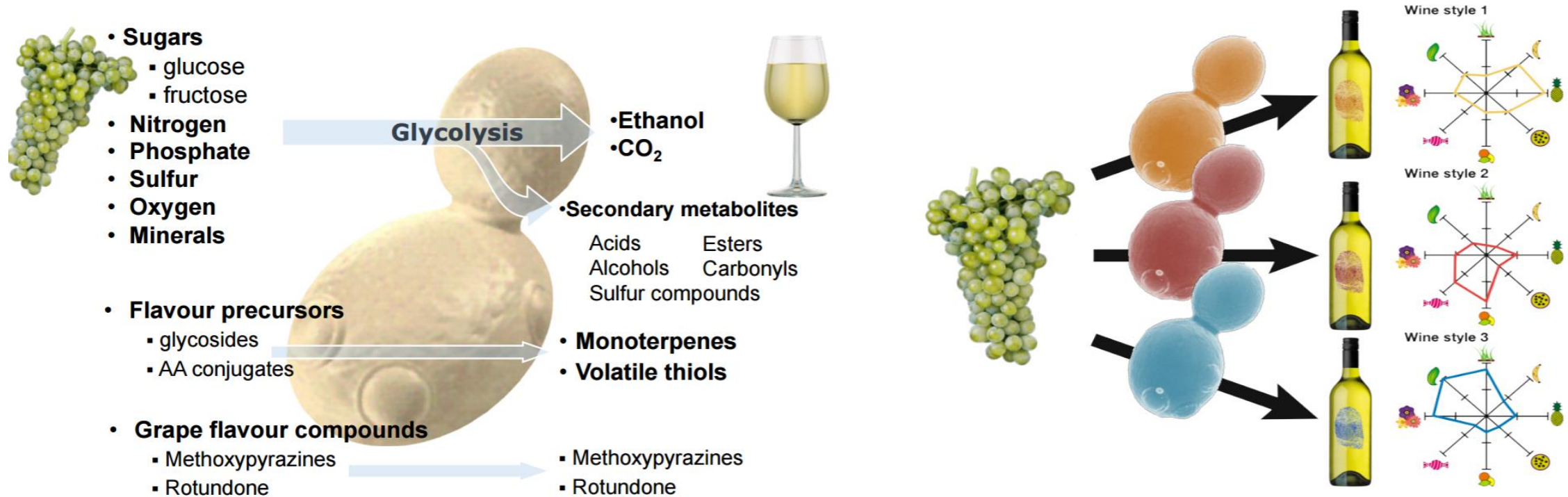
3. Enzymatic conversion of flavour neutral, grape components into odour-active compounds.

4. yeast autolysis products.

5. Absorption of juice components.

6. Spoilage during the storage period and even after packaging.

7. Influence growth of other spoilage microorganism, for example, lactic acid bacteria, acetic acid bacteria.



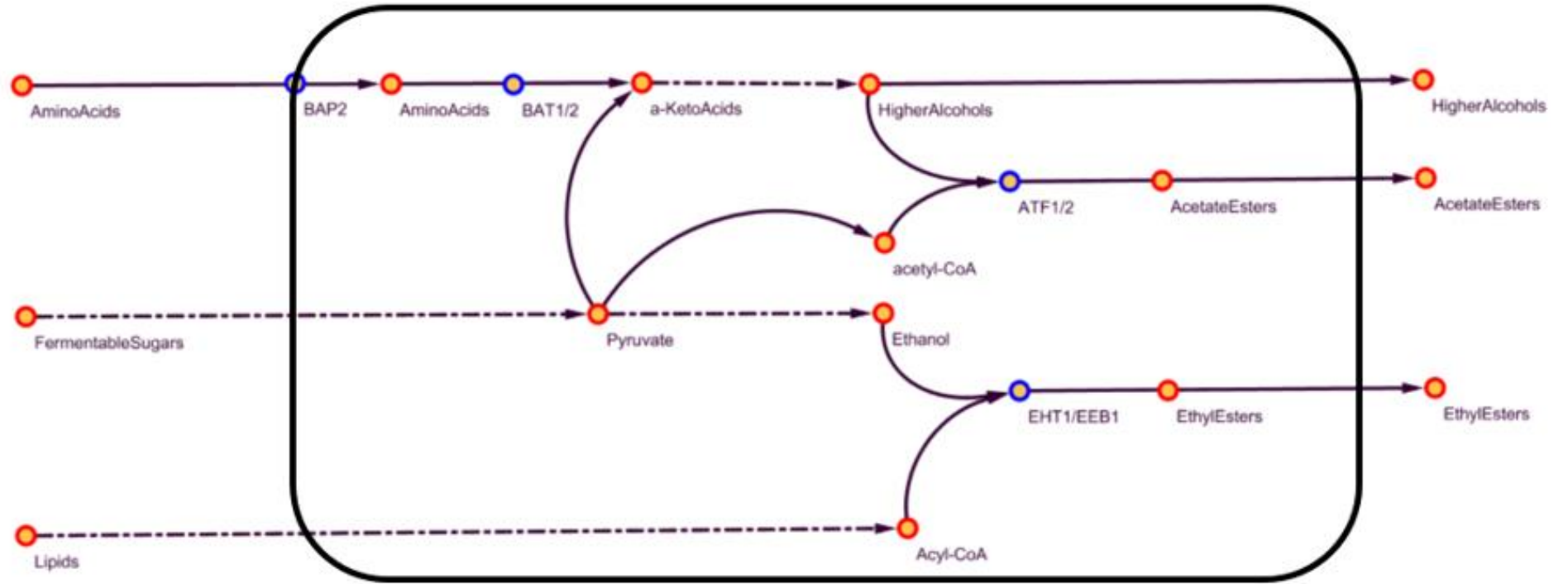
When presented with the appropriate nutrients, yeasts produce complex bouquets of aroma compounds including esters, higher alcohols, carbonyls, fatty acid derivatives and sulfur compounds. Moreover, while not directly synthesized by yeasts, volatile thiols and monoterpenes are sometimes released from odorless precursors by yeast-derived enzymes



1996 Release: Yeast Genome Sequenced

Our understanding of the fermentation process and the associated aroma production by yeast has increased exponentially over the last centuries, from the discovery of yeast cells in 1680, to the sequencing of the entire *Saccharomyces cerevisiae* genome just two decades ago

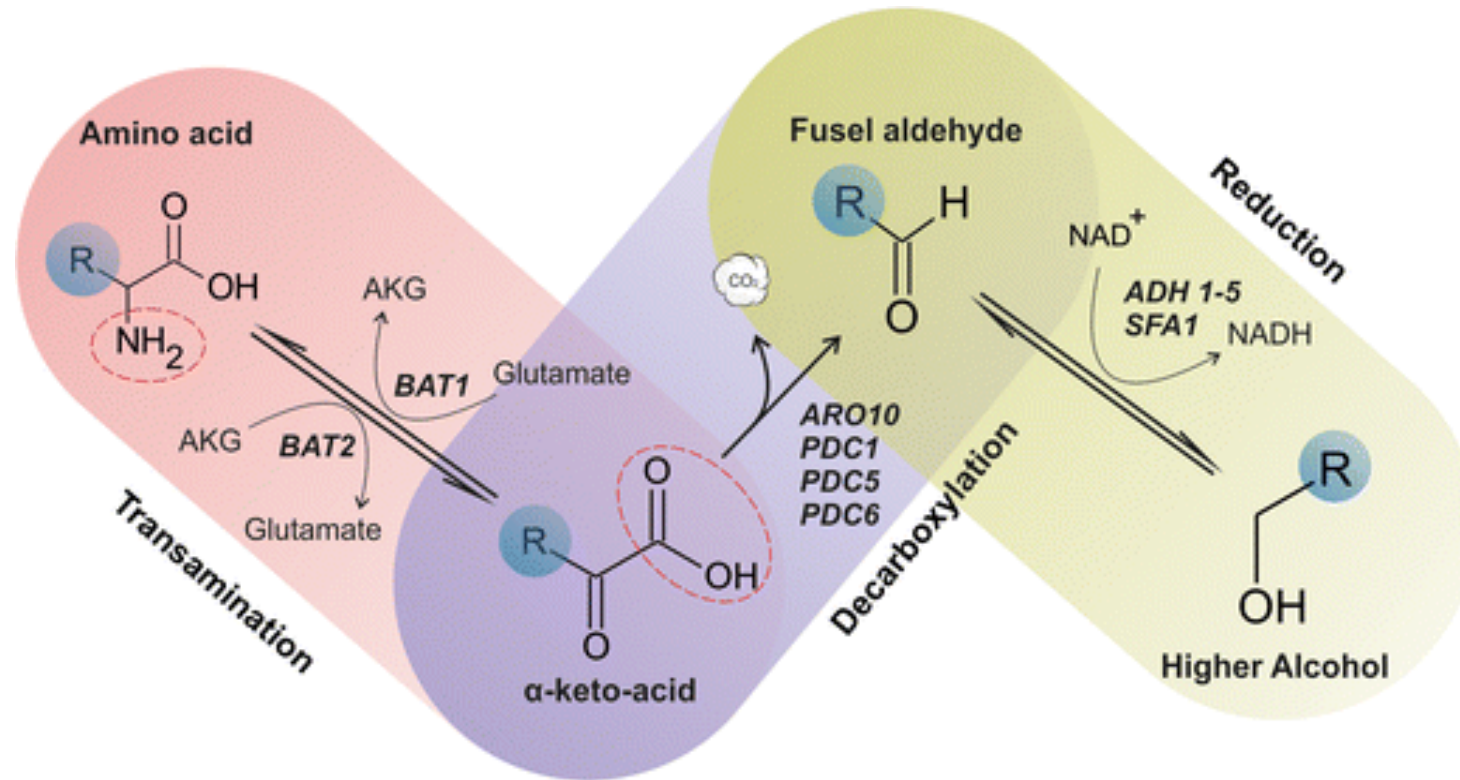
In-depth look at the phenotypic and genetic diversity of nearly 200 industrial yeasts profiled the of differences in aroma formation



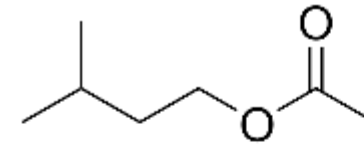
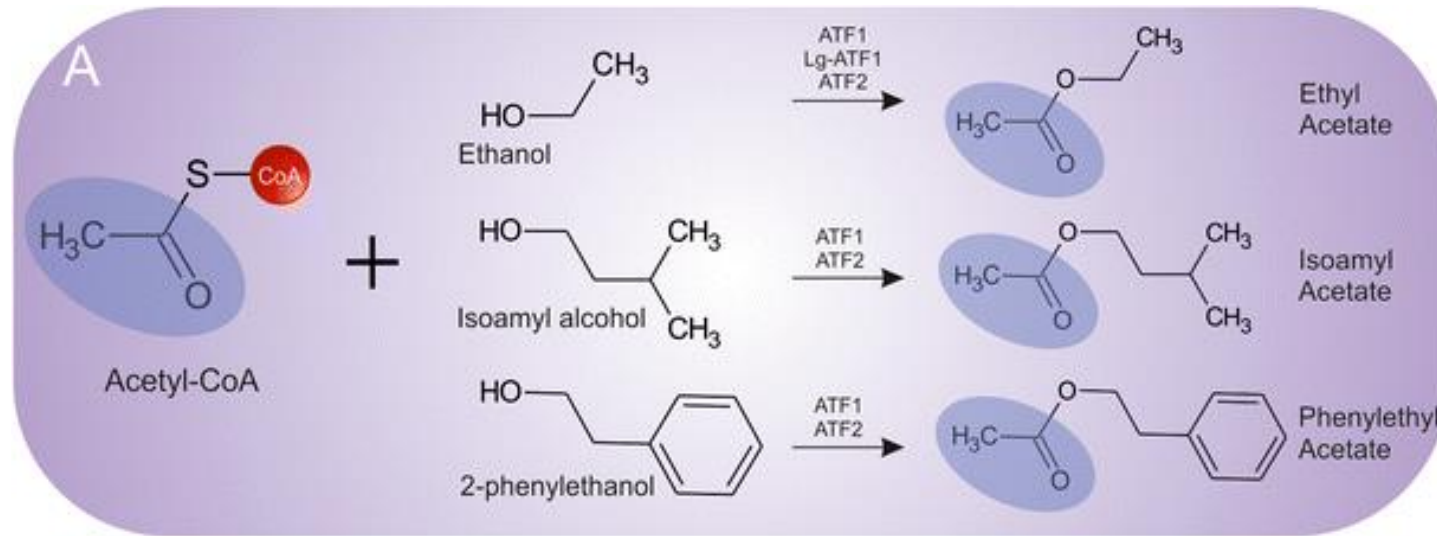
Gene	Enzyme	function
<i>BAP2</i>	Branched chain amino acid permease	Uptake of branched chain amino acids
<i>BAT1</i>	Mitochondrial branched chain amino acid aminotransferase	Branched chain amino acid transaminase activity
<i>BAT2</i>	Cytosolic branched chain amino acid aminotransferase	Amino acid catabolism and branched chain amino acid biosynthesis
<i>ATF1</i>	Alcohol acetyltransferase	Acetate ester production
<i>ATF2</i>	Alcohol acetyltransferase	Acetate ester production
<i>EEB1</i>	Acyl-coenzymeA/ethanol <i>O</i> -acyltransferase	Short-chain esterase activity
<i>EHT1</i>	Acyl-coenzymeA/ethanol <i>O</i> -acyltransferase	Short-chain esterase activity

(Procopio, Qian et al. 2011)

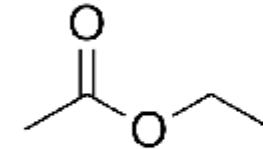
From AA to Higher Alcohols



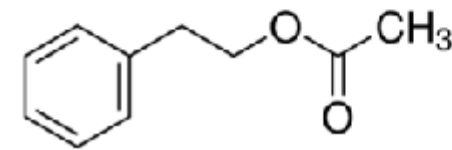
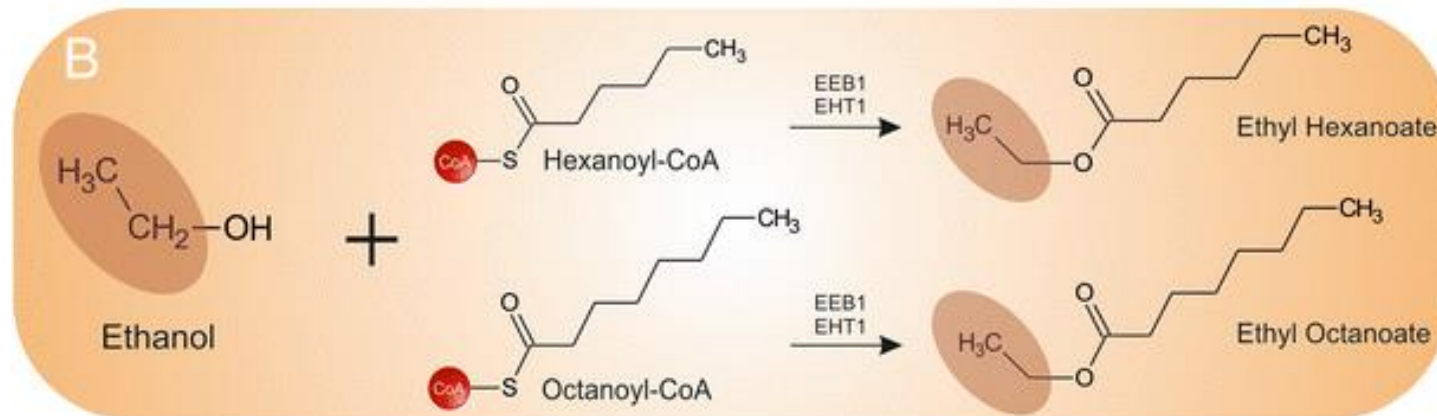
From AA and Lipids to Esters



Isoamyl acetate



Ethyl acetate



Phenylethyl acetate






























































































































Esters

Table of esters and their smells

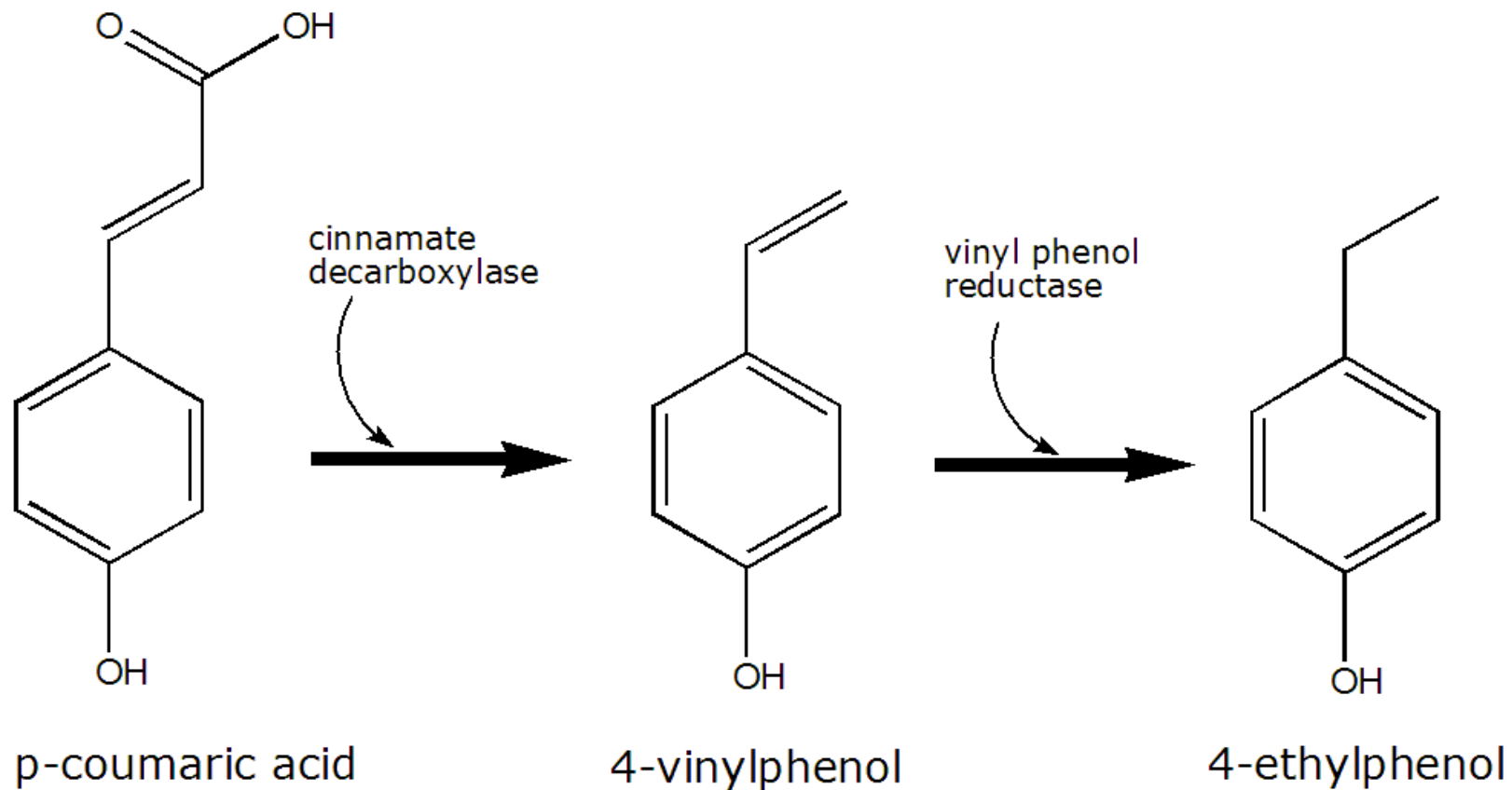
from the carboxylic acid (second word)

from the alcohol (first word)

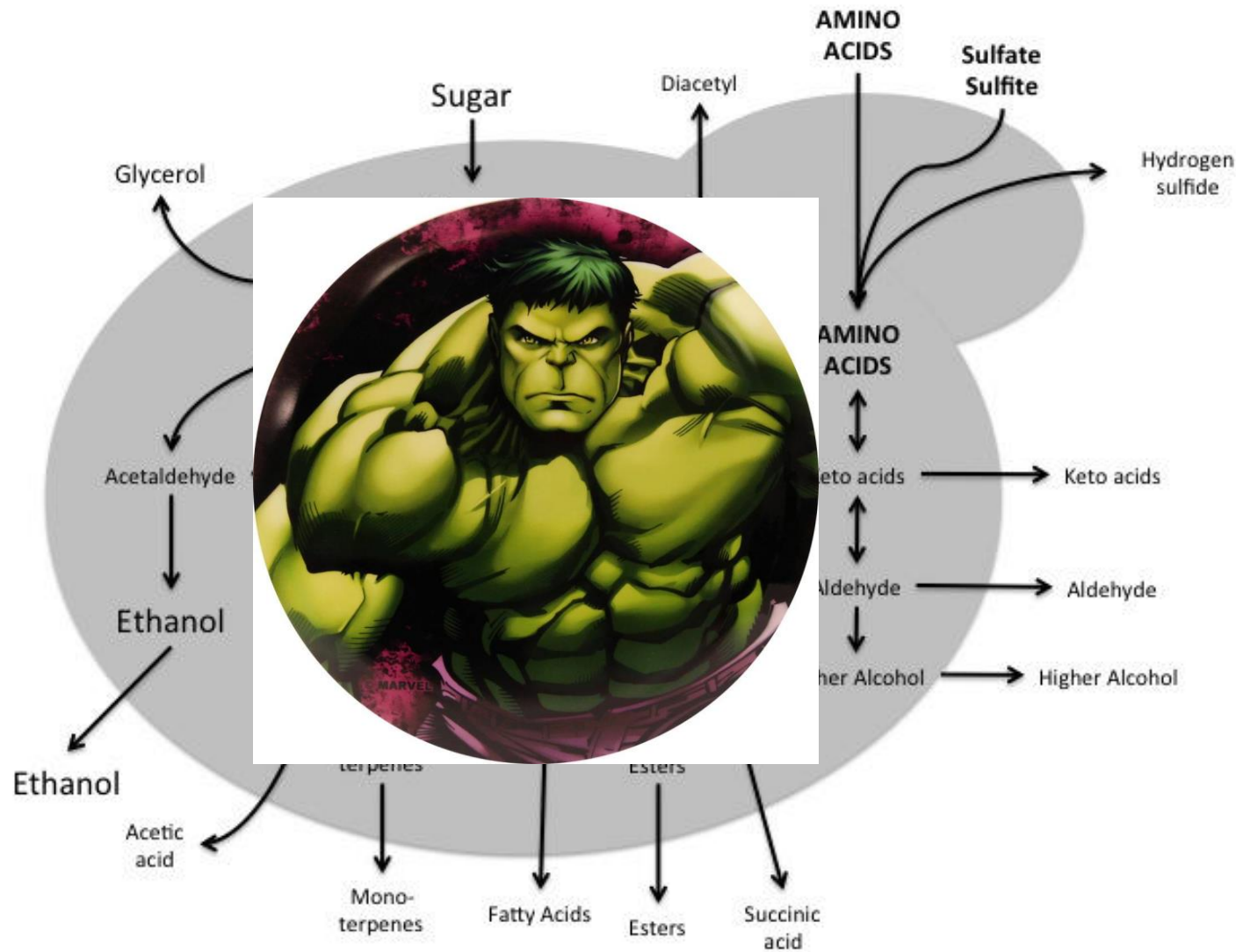
	methyl 1 carbon	ethyl 2 carbons	propyl 3 carbons	2-methyl propyl-	butyl 4 carbons	pentyl 5 carbons	hexyl 6 carbons	benzyl benzene ring	heptyl 7 carbons	octyl 8 carbons	nonyl 9 carbons
methanoate 1 carbon	ETHEREAL			ETHEREAL							?
ethanoate 2 carbons											
propanoate 3 carbons											?
2-methyl propanoate 4 carbons, branched		ETHEREAL									?
butanoate 4 carbons											?
pentanoate 5 carbons					ETHEREAL					?	?
hexanoate 6 carbons											
benzanoate benzene ring										?	
heptanoate 7 carbons						?					?
salicylate from salicylic acid								DIFFERENT PEOPLE PERCEIVE DIFFERENT AROMAS!	?		?
octanoate 8 carbons											
nonanoate 9 carbons										?	
cinnamate											?
decanoate 10 carbons							?	?	?	?	?

Phenols

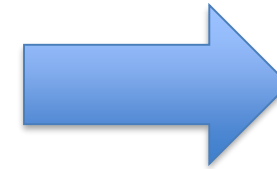
A *phenol* is an organic compound in which a hydroxyl group (-OH) is bonded to an aromatic hydrocarbon ring (also called a benzene ring).



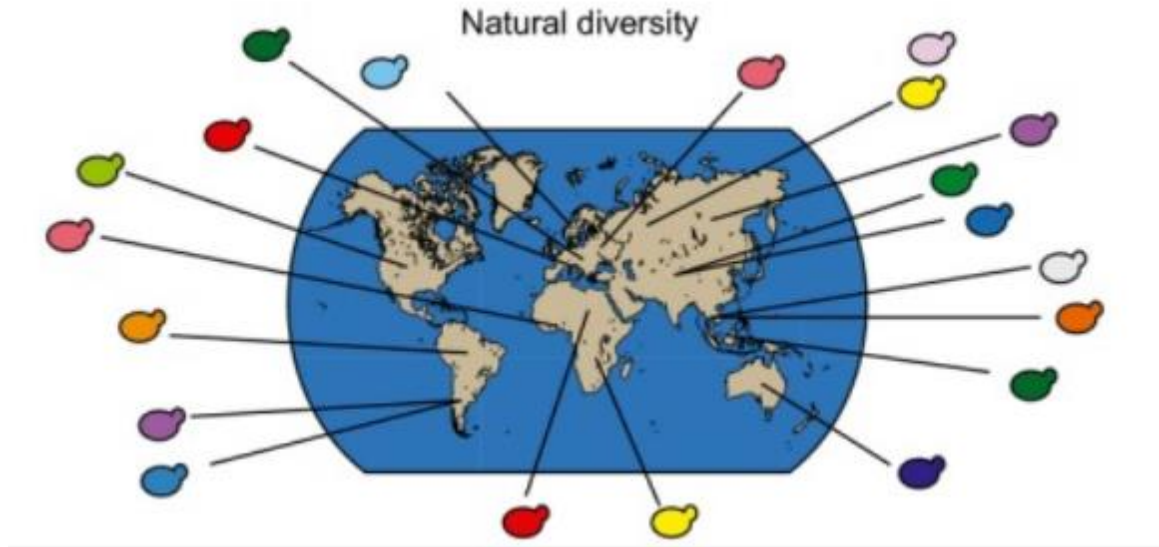
- Given its importance in product quality, much effort has been devoted to fine-tune flavor production by yeast in an industrial setting. Globally, two approaches can be applied to steer the yeast's physiology to alter aroma production: adjusting the fermentation environment or modifying the genotype of the production strain.



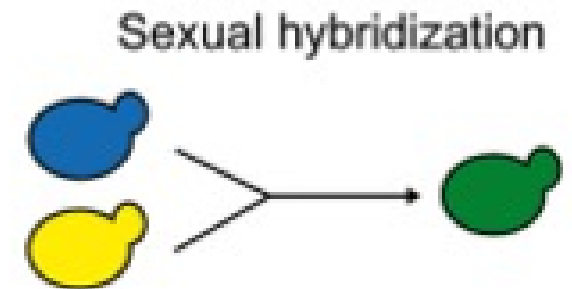
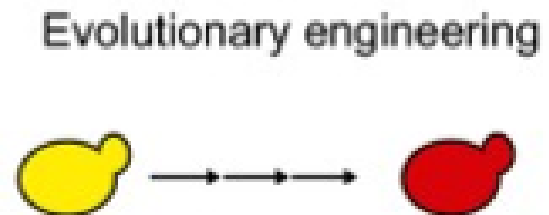
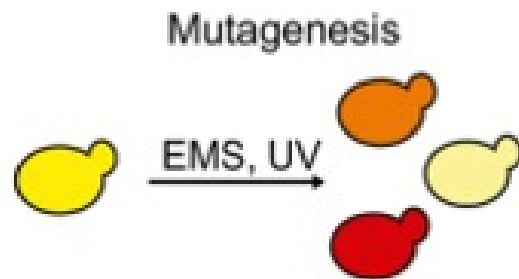
GMO?



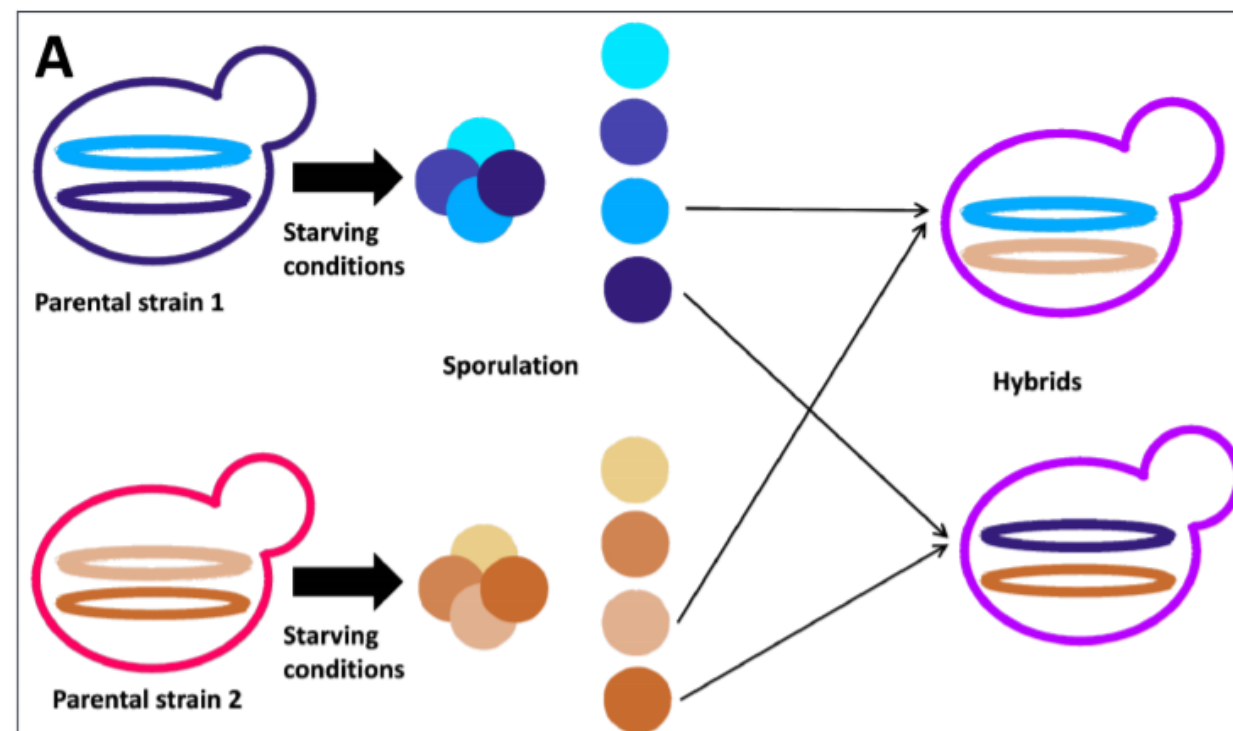
Not accepted
by consumers



Generation of artificial diversity



	<i>A</i>	<i>a</i>
<i>A</i>	<i>AA</i>	<i>Aa</i>
<i>a</i>	<i>Aa</i>	<i>aa</i>



Improved cider fermentation performance and quality with newly generated *Saccharomyces cerevisiae* × *Saccharomyces eubayanus* hybrids

[Frederico Magalhães](#),^{1,2} [Kristoffer Krogerus](#),^{1,2} [Virve Vidgren](#),¹ [Mari Sandell](#),³ and [Brian Gibson](#)¹


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Associated Data

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Abstract

Go to: 

Yeast cryotolerance may be advantageous for cider making, where low temperatures are usually employed. Here, we crossed the cryotolerant *S. eubayanus* with a *S. cerevisiae* wine strain and assessed the suitability of the hybrids for low-temperature cider fermentation. All strains fermented the juice to 5% ABV, but at different rates. Hybrid strains outperformed *S. cerevisiae*, which was sensitive to low temperatures. The

After crossing a wine strain with *S. eubayanus*, hybrid strains were expected to inherit the more pleasant aroma profile of the former and the reportedly high tolerance to low temperatures of the latter

Screening of cider yeasts for sparkling cider production (Champenoise method)

Belén Suárez Valles, Rosa Pando Bedriñana*, Ana Lastra Queipo, Juan José Mangas Alonso

Área de Tecnología de los Alimentos, Servicio Regional de Investigación y Desarrollo Agroalimentario (SERIDA), 33300 Villaviciosa, Asturias, Spain

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ABSTRACT

A total of 350 colonies isolated from a cider cellar in Asturias (Spain) were identified by rDNA ITS-RFLP restriction analysis. *Saccharomyces* spp. strains were characterized by mitochondrial DNA (mtDNA) restriction analysis. Fifty-four different *Saccharomyces* spp. strains were identified and tested to ascertain their capacity to carry out secondary fermentation of sparkling ciders. The screening of yeasts to determine their principal enological characteristics (tolerance to ethanol, production of volatile acidity and hydrogen sulphide) was accomplished by means of rapid, non-expensive assays (plate agar). As a result, 13 (24%) of the 54 initial *Saccharomyces* spp. yeast strains were eliminated. The technological

10 *S. cerevisiae* strains were found as true flocculants and were able to grow in ethanolic medium and in the presence of 200mg/l of sulphite



New Fermol Yeast strains:

- ✓✓ Fermol Elegance: does not produce sulfur compounds
- ✓✓ Fermol Glutaferm -1: allows to have very high levels of glutathione in the fermenting must and wine

NOTE TO THE EDITOR

Evolution-based strategy to generate non-genetically modified organisms *Saccharomyces cerevisiae* strains impaired in sulfate assimilation pathway

L. De Vero, L. Solieri and P. Giudici

Department of Agricultural and Food Sciences (DipSAA), University of Modena and Reggio Emilia, Reggio Emilia, Italy

Keywords

evolutionary strategy, sulfate assimilation, sulfite and hydrogen sulfide production, wine yeast strains.

Correspondence

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doi:10.1111/l.1472-765X.2011.03140.x

Abstract

Aims: An evolution-based strategy was designed to screen novel yeast strain impaired in sulfate assimilation. Specifically, molybdate and chromate resistance was used as selectable phenotype to select sulfate permease-deficient variants that unable to produce sulfites and hydrogen sulfide (H₂S).

Methods and Results: Four *Saccharomyces cerevisiae* parent strains were induced to sporulate. After tetrad digestion, spore suspensions were observed under the microscope to monitor the conjugation of gametes. Then, the cell suspension was inoculated in tubes containing YPD medium supplemented with ammonium molybdate or potassium chromate. Forty-four resistant strains were obtained and then tested in microvinifications. Three strains with a low sulfite production (SO₂ <10 mg l⁻¹) and with an impaired H₂S production in grape must without added sulfites were selected.



RESEARCH ARTICLE

Evolved *Saccharomyces cerevisiae* wine strains with enhanced glutathione production obtained by an evolution-based strategy

Francesco Mezzetti, Luciana De Vero & Paolo Giudici

Department of Life Sciences, University of Modena and Reggio Emilia, Reggio Emilia, Italy

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Editor: Isak Pretorius

Keywords

Saccharomyces cerevisiae; evolutionary strategies; glutathione; molybdate resistance; wine strain.

Abstract

In winemaking, the application of glutathione (GSH) has been the subject of ever-growing interest because of its important role in limiting must and wine oxidation and in protecting various aromatic compounds. Glutathione concentration in wine is highly variable, involving as it does several factors from must, through alcoholic fermentation, to yeast strain activity. Consequently, the development of new wine yeast strains able to improve flavor stability is in great demand. To generate evolved *Saccharomyces cerevisiae* strains with enhanced GSH production, we have applied an evolution-based strategy that combines the sexual recombination of spores with the application of molybdate, which is toxic for the cells at high concentration, as specific selective pressure. Eight molybdate-resistant strains were selected and further screened for GSH production in synthetic grape must and in microvinification assay. By this nongenetically modified strategy, we obtained two evolved strains, Mo21T2-5 and Mo21T2-12, both able to enhance GSH content in wine with an increase of 100% and 36%, respectively, compared with the parental strain 21T2, and 120% and 50% compared with initial GSH content in the must.

Fermol 2



Bayanus yeast suitable for the fermentation of cider, fruit wines and grape must.

It produces fruity nuances and carries over a regular fermentation with high alcohol tolerance and no H₂S production.

Cold & SO₂ tolerant and with short lag phase, it guarantees quick dominance over the wild yeast.

Parameters

- Temperature range
- Nitrogen Requirement
- Fermentation performance
- SO₂ tolerance
- Aroma productions
- Flocculation

Fermentation Facility:

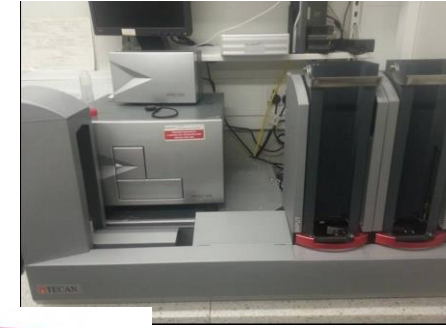
TEE-BOT high throughput small scale fermentation robot

- This machine is able to assist monitoring of the fermentation progress by autonomously collecting and cold-storing samples for further analysis.
- The Tee-Bot is a highly efficient tool for users who need to run fermentation experiments. 96 flasks of ~100 mL volume can be simultaneously monitored while maintaining temperature and agitation at user defined levels.
- The Tee-Bot has been specifically designed for wine fermentations, but it can be easily adapted for other fermentation media



Fermentation Facility:

- Platemaster, Gilson
- Tecan 200 spectrophotometer + stacker
- Guava® easyCyte HT Sampling Flow Cytometer, Millipore
- ChemWell® 2910 Automated EIA and Chemistry Analyzer



Its technology has been custom built with a high degree of automation for process reliability, cost-effectiveness and ultimately more robust research outcomes

Analytical Chemistry facility:

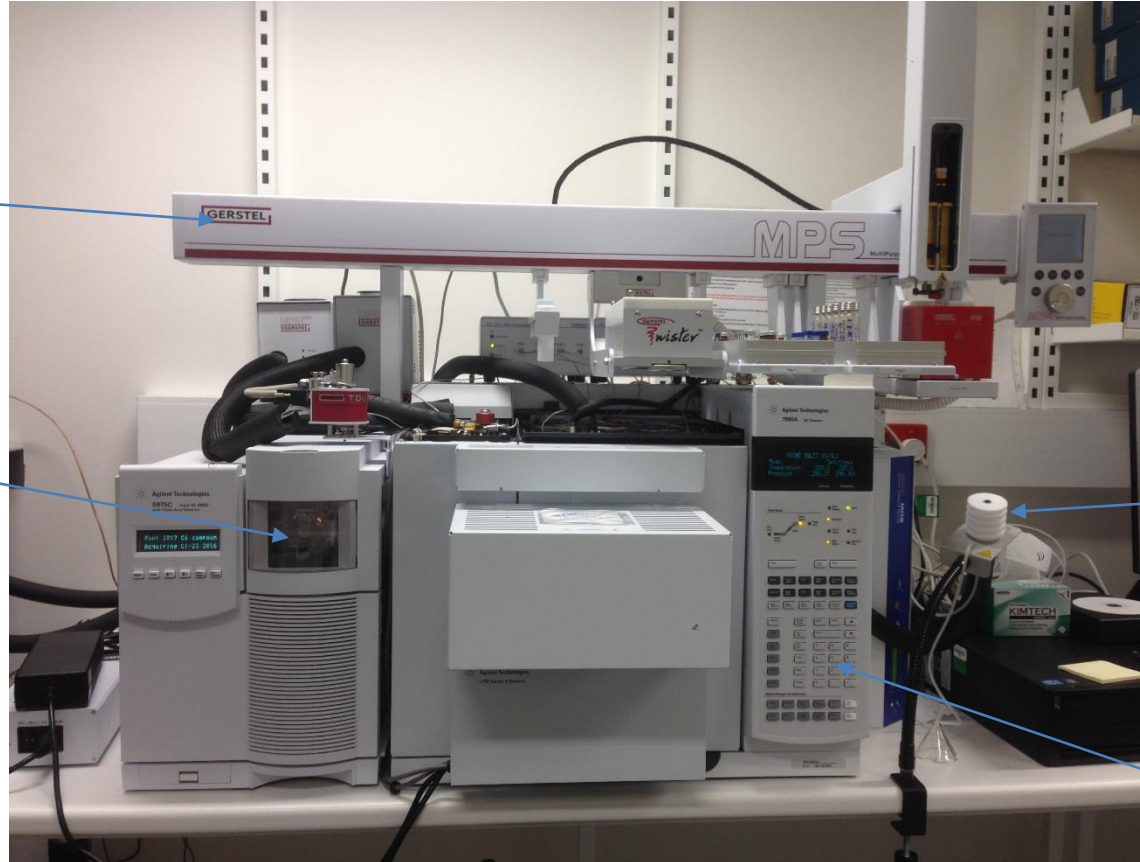
Agilent 7890A GC coupled to 5976C MSD detector

Gerstel MPS
Autosampler

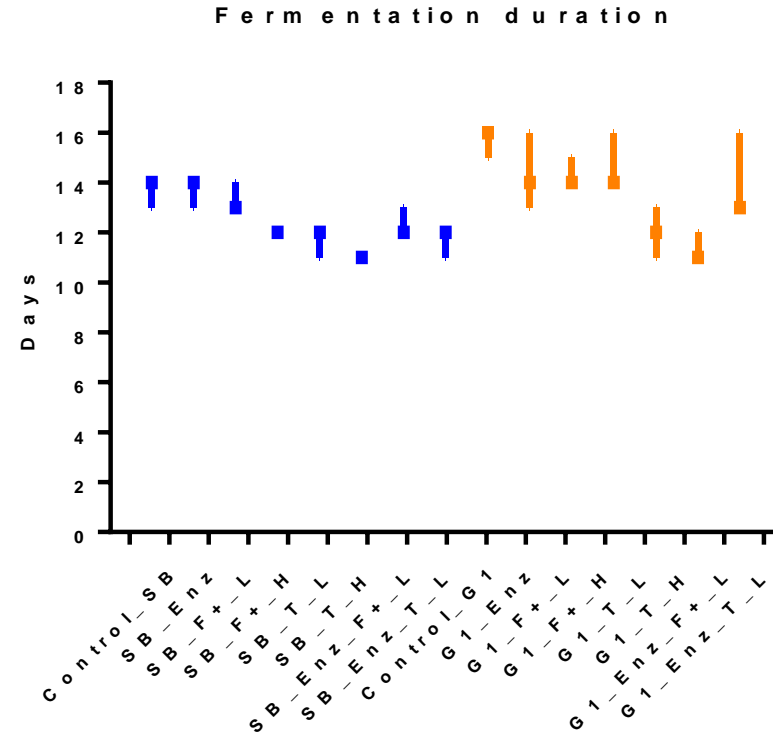
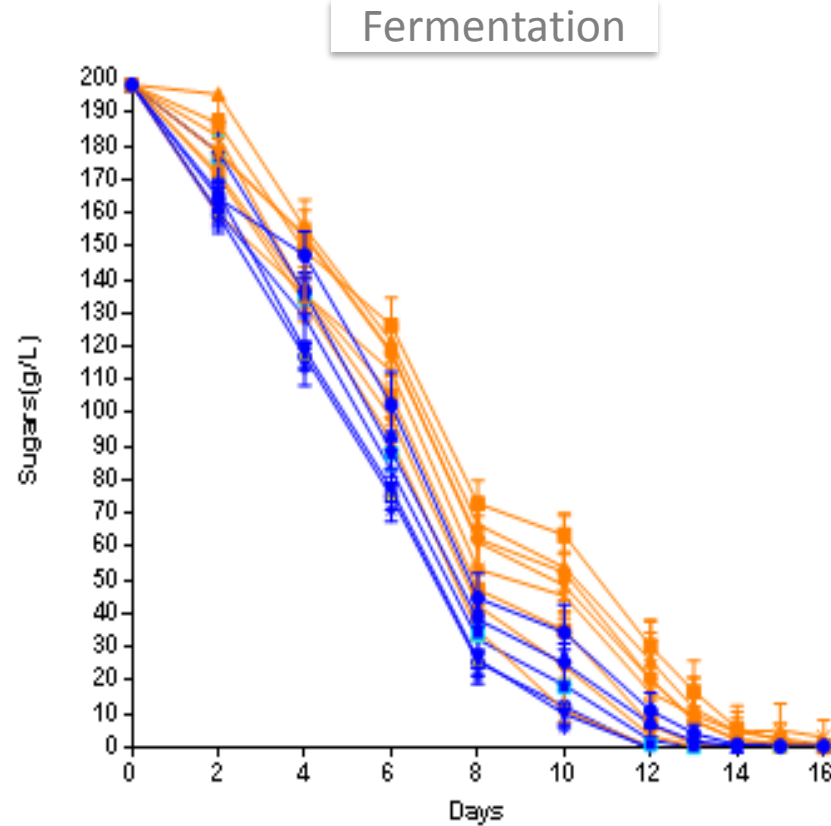
MS detector

ODO port

Gas
chromatograph



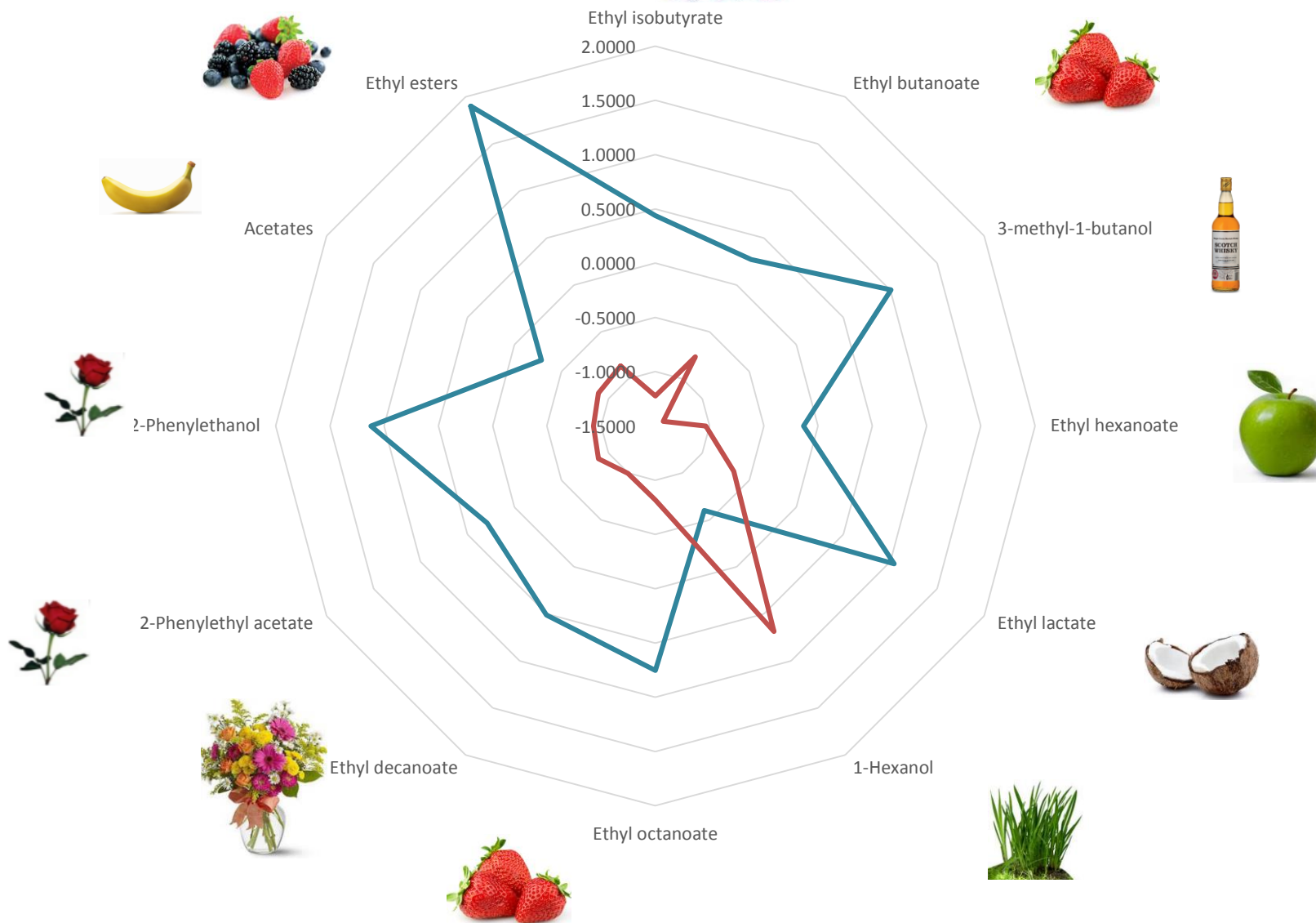
Fermentation performance



Fermol® Sauvignon	SB
GlutafermONE	G1
control	Control_G1
enzyme 5 g/hl	Enz
FermoPlus 250 mg/L	F+_L
FermoPlus 400 mg/L	F+_H
Tropical 250 mg/L	T_L
Tropical 400 mg/L	T_H
enzyme 5 mg/hl + Ferm 250 mg/L	Enz_F+_L
enzyme 5mg/hl + Tropical 250 mg/L	Enz_T_L

Esters and alcohols

— SB - Control — G1 - Control



Increasing demand for innovative products, alternative flavours, and low-alcohol beers has stimulated research into the potential benefits of alternative yeasts; in particular, non-Saccharomyces species have been isolated and characterized for the development of specialty beers.

M. pulcherrima (*LEVULIA PULCHERRIMA*) : 3% ethanol, extracellular enzymes, bioprotection, less acetic acid

L. thermotolerans (*LEVULIA ALCOLMENO*) : 7%, different esters production, lactic acid production

T. delbrueckii (*LEVULIA TORULA*) : 9% , different esters production, extracellular enzymes, mannoprotein, less acetic acid

SCREENING OF ENZYMATIC ACTIVITIES IN NON-SACCHAROMYCES CIDER YEASTS

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ABSTRACT

The activities of polygalacturonase, pectin lyase, β -glucosidase, β -xylosidase and protease were determined using solid media in 420 wild non-*Saccharomyces* cider strains identified by internal transcribed spacer-restriction fragment length polymorphism. The identified species corresponded to *Hanseniaspora valbyensis*, *Hanseniaspora uvarum*, *Hanseniaspora osmophila*, *Metschnikowia pulcherrima*, *Candida parapsilosis* and *Pichia guilliermondii*. The most common activity exhibited was that of β -glucosidase (33%), with all the analyzed species having some strains able to develop this activity. Strains of *M. pulcherrima* showed the greatest capacity to produce β -glucosidase and protease. β -xylosidase was detected in 17 yeast strains

The results from the screening of enzymatic activities in non-*Saccharomyces* cider yeasts suggest their potential to improve the aroma and flavor of cider used as natural inocula

How to assess yeast for apple juice fermentation?



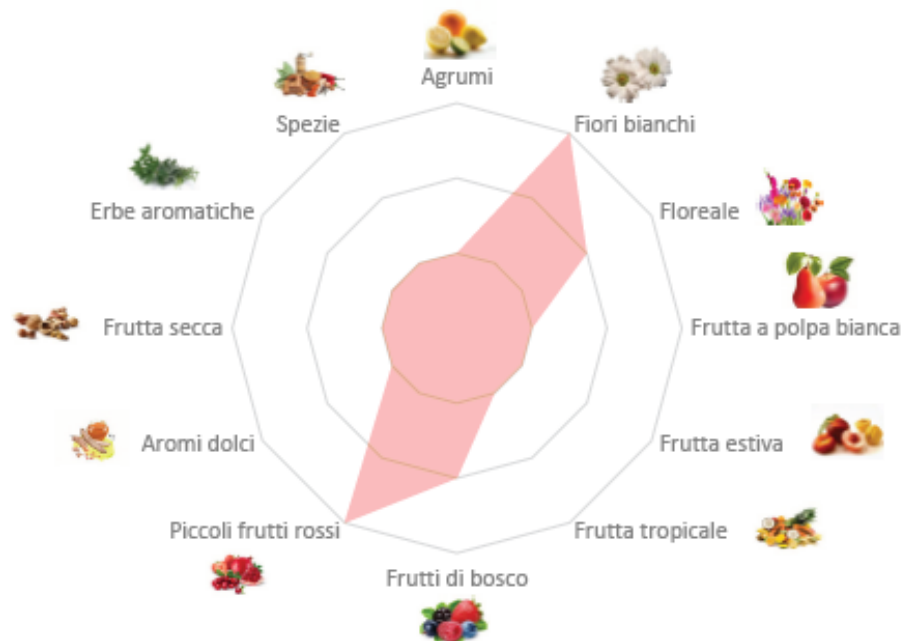
CIDER > PRODUCTS > YEASTS > ZYMASIL® CEREVISIAE

ZYMASIL® Bayanus
ZYMASIL® Special Cider

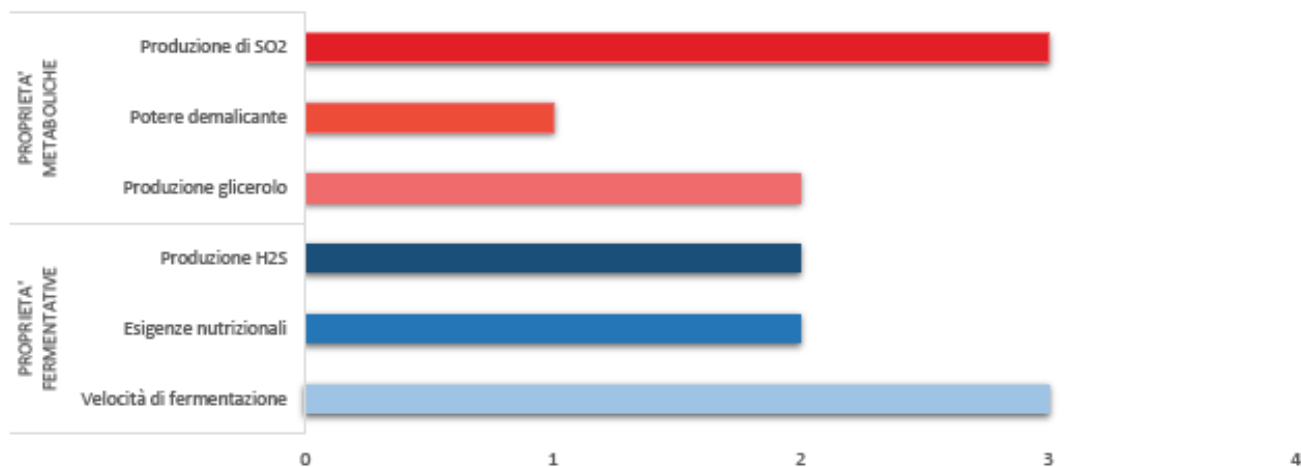
ZYMASIL® Cerevisiae

ZYMASIL® Cider

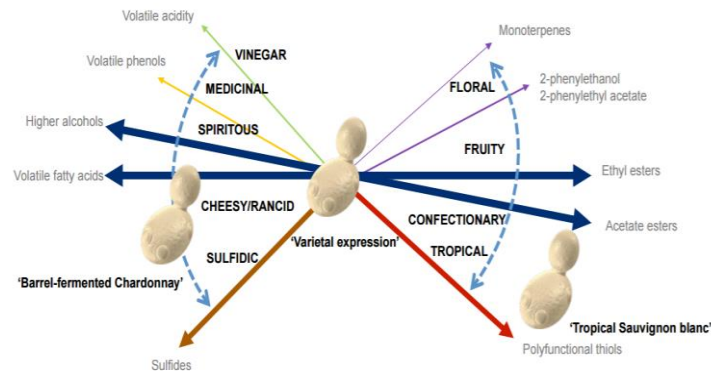
DESCRITTORI ORGANOLETTICI



PROPRIETÀ METABOLICHE E ORGANOLETTICHE



How to assess yeast for apple juice fermentation?




Apple Contents

- An apple contains:
 - 80% water
(varies with irrigation practices and weather conditions)
 - 10% carbohydrate
 - Sugars (mostly fructose, with some glucose—**100% fermentable**)
 - Fiber/cellulose – removed by pressing
 - 4% vitamins/minerals
 - 6% of:
 - Organic acids (primarily malic acid)
 - Pectin – **pectinase highly recommended**
 - Polyphenols – flavonoids and, to a varying degree, tannins
 - **Very small amounts of proteins (added yeast nutrition is often needed!)**

	Wort	Grape	Apple
Sugars (g/L)			
Fructose	3.1	83.4	82.1
Glucose	116.8	80.4	30.3
Sucrose	4.6	0	7.7
Maltose	3.7	0	0
Amino acids (mg/L)			
Aspartic acid	80	35	154
Glutamic acid	130	60	72
Serine	83	43	29
Histidine	56	39	4
Glycine	44	9	2
Threonine	86	52	10
Arginine	142	610	6
Alanine	132	75	16
Tyrosine	125	16	3
Methionine	45	3	3
Valine	156	26	10
Phenylalanine	155	16	5
Isoleucine	78	12	4
Leucine	187	25	6
Lysine	99	16	2
Minerals (mg/L)			
Calcium	36.4	147.3	27.9
Magnesium	106.3	90.2	37.7
Sodium	17.9	36	29.6
Potassium	785	1138	1184
Zinc	0.4	0.84	1.04
Copper	0.08	1.12	0.1
Iron	0.22	2.62	0.12
Aluminium	0	3.34	0.01
Manganese	0.08	1.16	0.31
Phosphorus	528.2	143	74
Silica	21	18.3	0.4
Chloride	292	31.7	11.7
Sulphate	47.2	204	47.9
Nitrate	0.6	11.2	1.5
Free oxalic acid	26.9	28.7	4.8
Phosphate	1299.3	352.5	163.9

Ctrl-Ferm a patented, unique and innovative system for the detection of **H₂S** and **CO₂** during alcoholic fermentation (AF). Ctrl-Ferm is easy to mount and simple to use: once positioned the detector tube on the top of the fermenter, the instrument aspires and measures the gases, uploading the data real-time to a website, accessible from the winemaker phone or personal computer. Ctrl-ferm is available in two version, for the measurement of 1 fermentation or up to 5 tanks simultaneously.

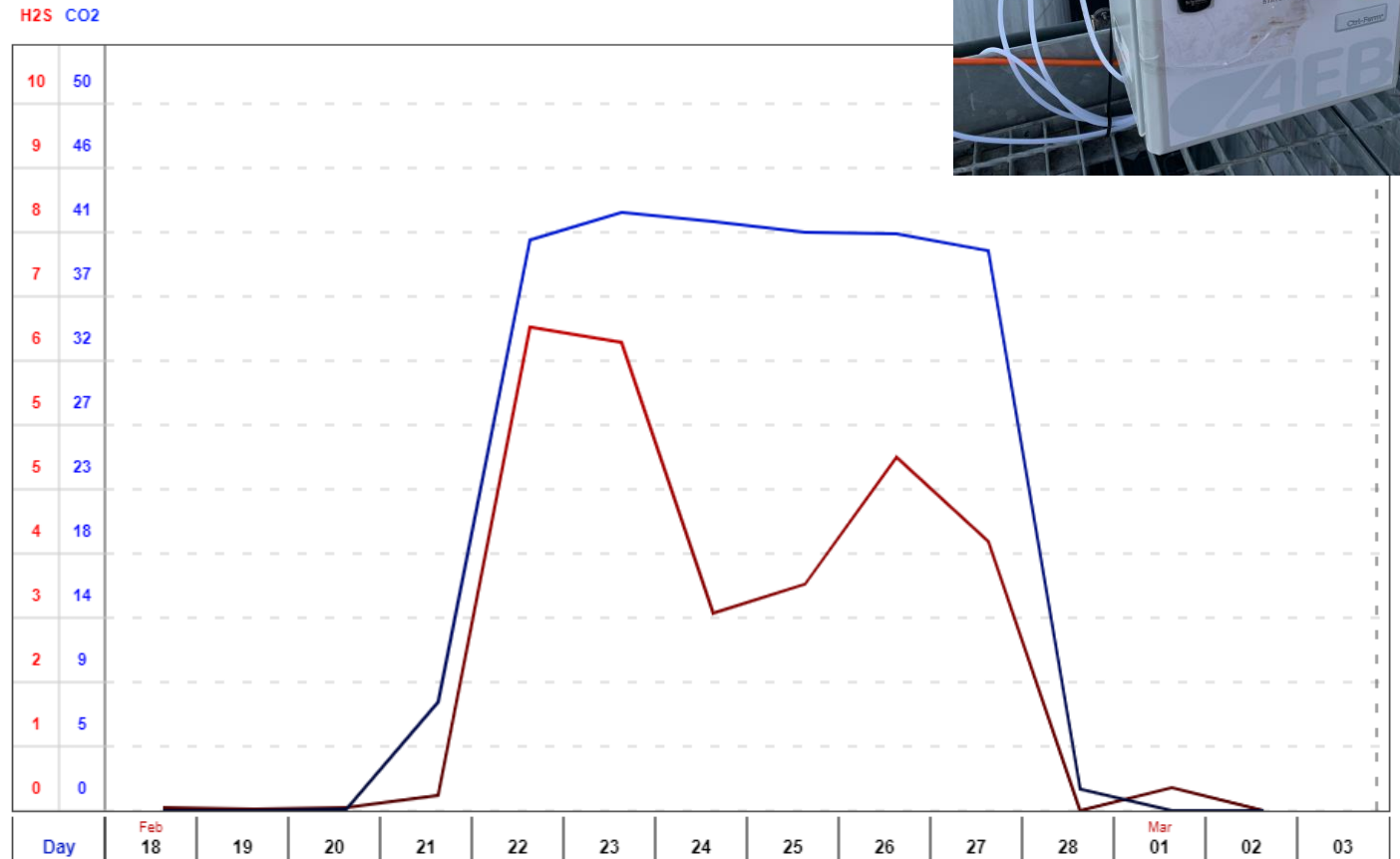
Channel: Channel B ▼ T441 - Pulsair 

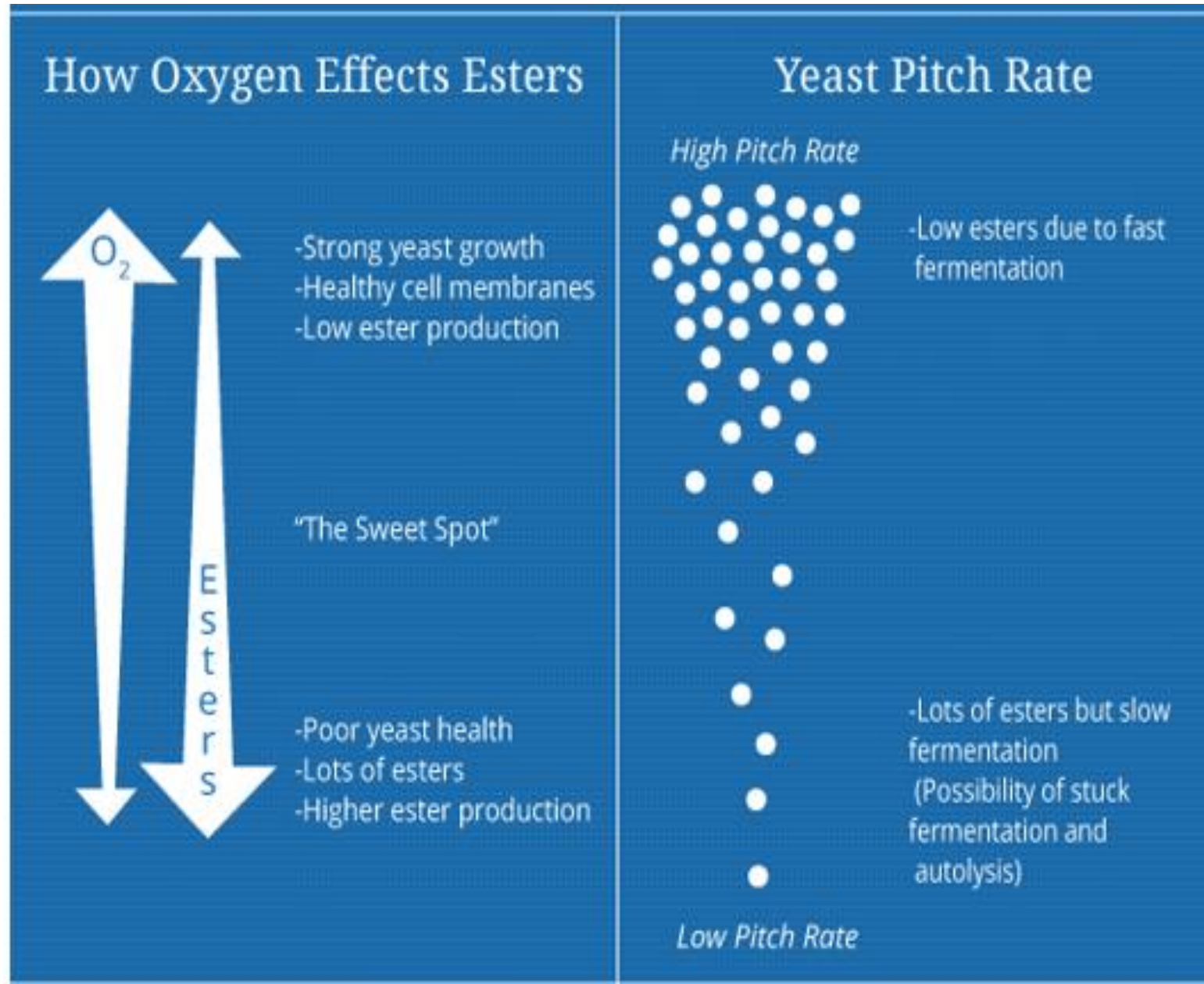
Yeast (g/hl): Update

View: ☒ H₂S ☒ CO₂

Range type: Real (normalized) ▼

AEB Ctrl-Ferm





Temperature

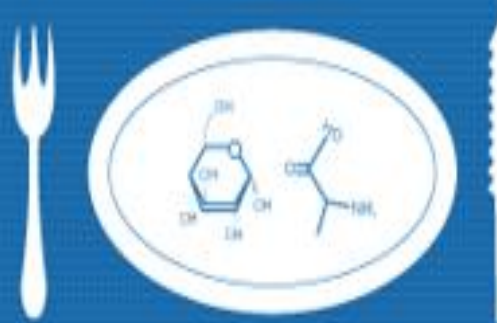


- High temperature results in fruity esters

- Slightly lower temperature makes less esters but more phenols

- Lower temperature still produces very low esters and phenols (Ideal for lagers)

Sugar and FANS



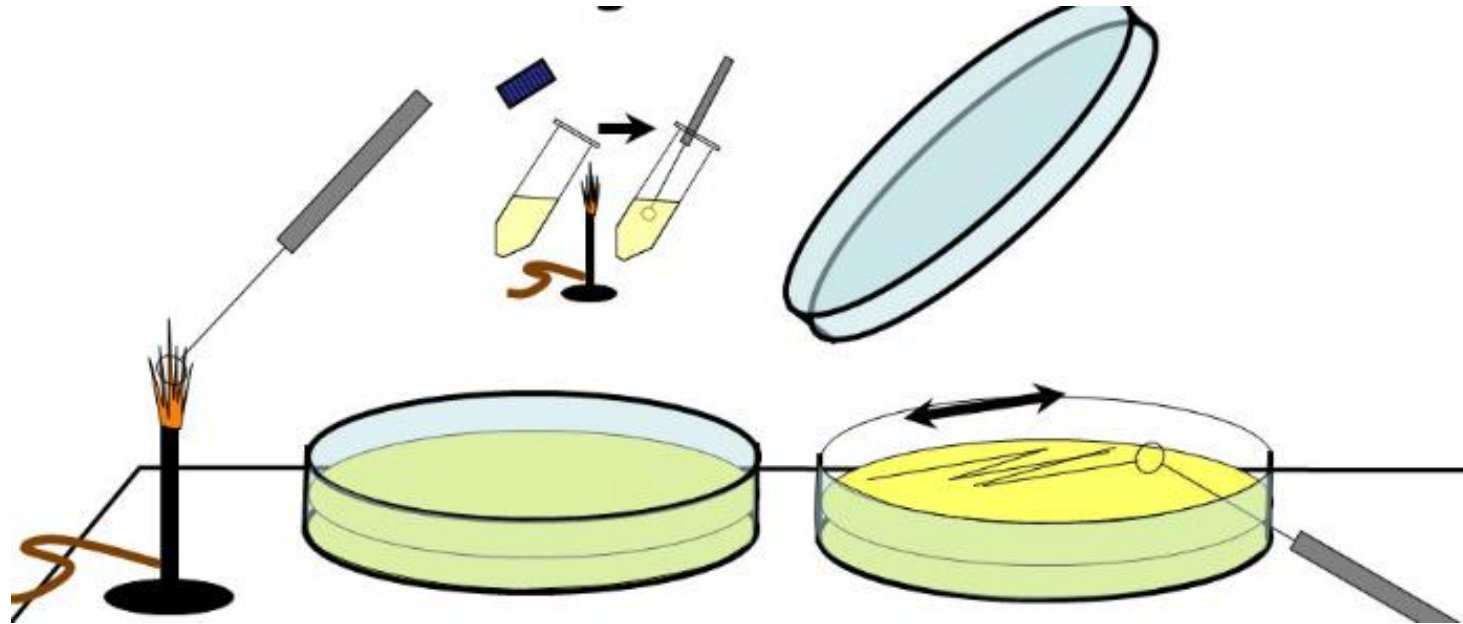
Sugars and FANS are yeast food

More sugar = More esters
Too much sugar = Acetaldehyde

Free Amino Nitrogens (FANS)
More FAN = Better yeast health

How to isolate yeasts from various fruit?

- Collected samples should be diced and place in 9 ml of YPD (Yeast extract 1%, Bacteria peptone 2%, Glucose 2%)



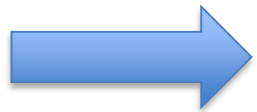
Smelling or 'sniffing' approach

To make the solid media, 20 g/L agar was added and dissolved into pre-heated apple juice at 80°C, then poured into Petri dishes. All non-conventional strains were streaked onto the agar plates and incubated at room temperature for 2–7 days.

The aim of this screening was to identify diverse aroma compounds typically found in fermented beverages as desired compounds or off-flavours, or simply strains characterised by a strong and novel aroma profile. Plates were 'sniffed' directly and classified as: 'pleasant aroma', 'unpleasant aroma' or 'no growth'. Additionally, 'pleasant aroma' was ranked as 'intermediate' or 'strong' depending on the level of perception. 'Unpleasant aroma' was classified as 'phenolic' or 'acetic' as both are common undesirable compounds produced by non-conventional yeasts.

Laboratory-Scale

- APPLE JUICE
- 1.5 L fermentation
- 3 Replicates
- Temperature controlled
- 5×10^6 cells/ml inoculum
- Sample daily
- Taste



Species Identification (PCR/ITS REGION IDENTIFICATION)



Glycerol Stock



FEDERICO@AEBUSA.COM

IC-Gene

IC-Gene brings the power of molecular biology in the vineyard and in the cellar. It allows a rapid detection of spoilage microorganisms (i.e. *Brettanomyces*, *Botrytis*). The analysis is based on a new PCR reaction, with improved sensitivity and specificity that reduce time of the assay and false outcome. Thanks to its easy execution and real time results, this method can be done ON SITE, directly from the matrix (wine, berries, waters, etc.). Low cost and the easy reproducibility allow to manage the flow of the winery in complete safety. Ten different samples can be processed simultaneously, and the time required is less than 2 hours.

We recommend IC-gene for:

- (i) Identify unnoticed *Botrytis* contaminated must. If recognized, you can avoid negative organoleptic effects.
- (ii) Test wine samples, rinsing water and wood surfaces to keep your winery free from *Brettanomyces*.

(NEW) Detection of harmful bacteria in storage water.

