

Hefen – Stoffwechsel und Förderung der Aromatik

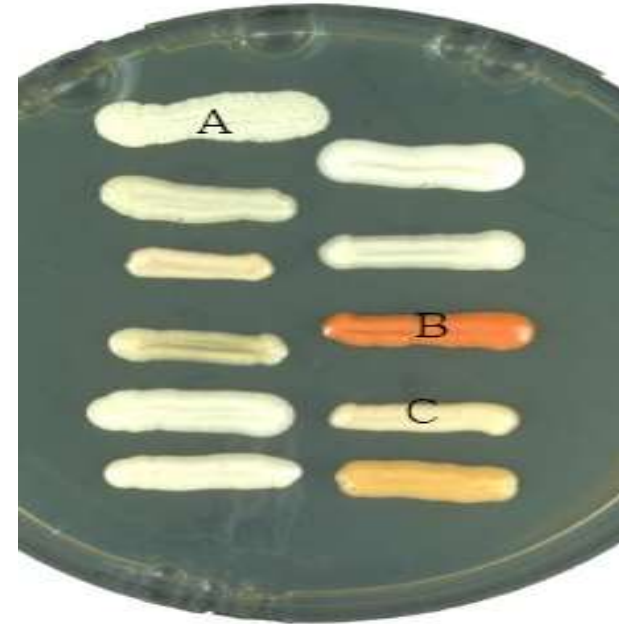
Andreas Bühlmann
Agroscope

Hefepopulation auf reifen Trauben (%)

***Hanseniaspora uvarum* (51 – 89%) (C)**
***Rhodotorula* (0 – 26%) (B)**

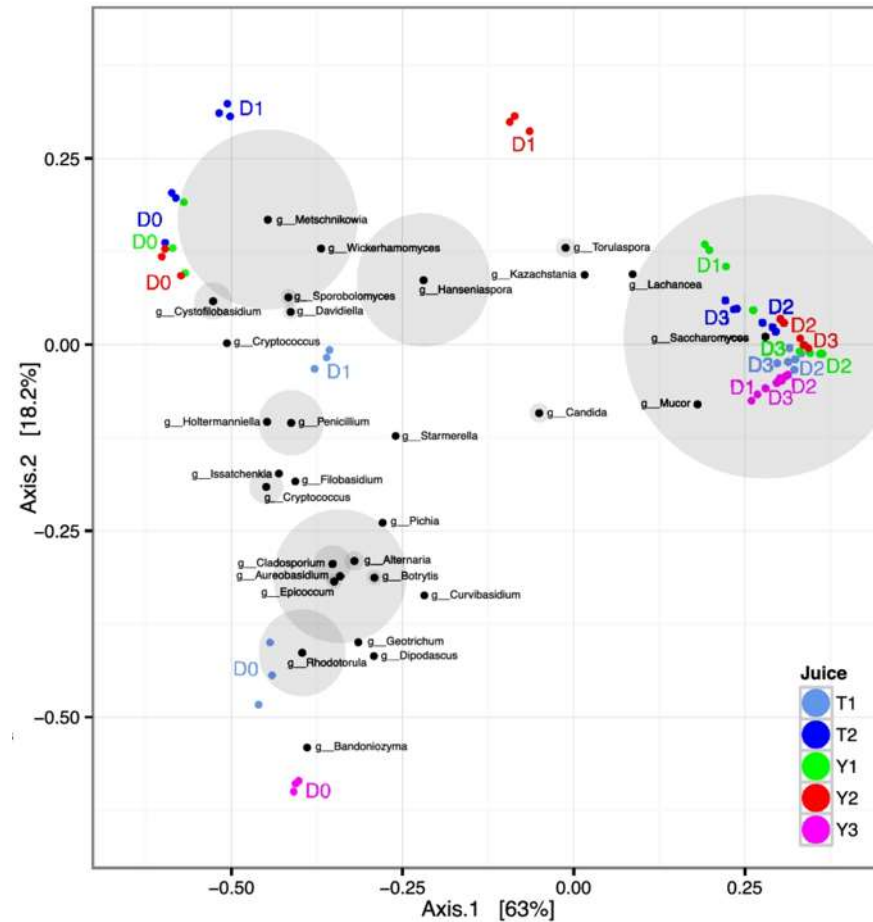
***Candida glabrata* (4 – 7%)**
***Zygosaccharomyces bailii* (1 – 4%)**
***Saccharomyces cerevisiae* (0.3 – 3%)**
***Metschnikowia pulcherima* (0.5 – 3%) (A)**

***Debaryomyces* (0.6 – 2%)**
***Candida zeylanoides* (1 – 2%)**
***Pichia kluveri* (0.4 – 1%)**
***Candida stellata* (0.5 – 0.9%)**
***Brettanomyces bruxellensis*, *Hyphopichia butonii*, *Kluyveromyces*,
Williopsis sat. *Hyphopichia butonii*, *Cryptococcus*, *Lipomyces* (0 – 0.4)**



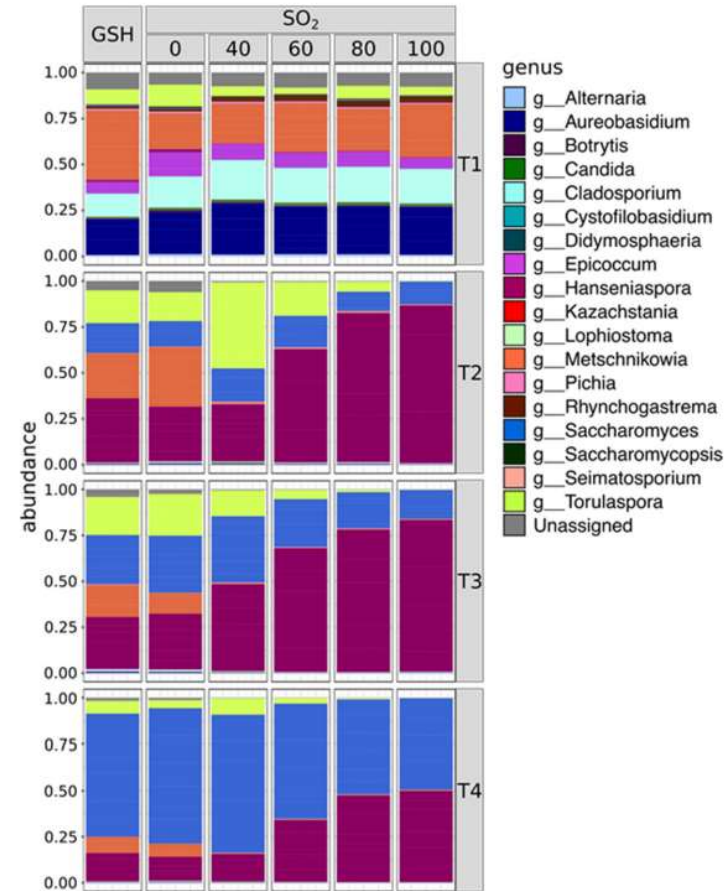
Hefe Spontane Gärung

- Alles mögliche bei Beginn
- Nach 3 Tagen nur noch *Saccharomyces cerevisiae*



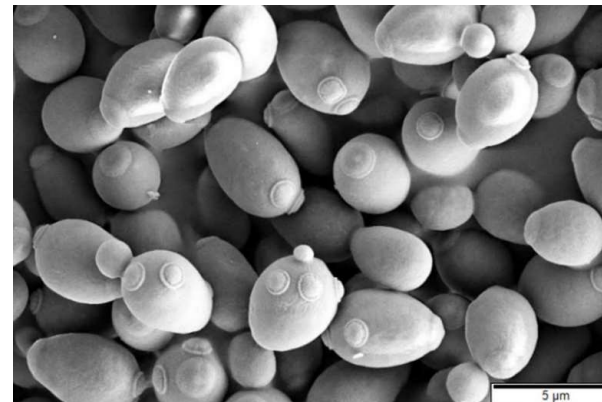
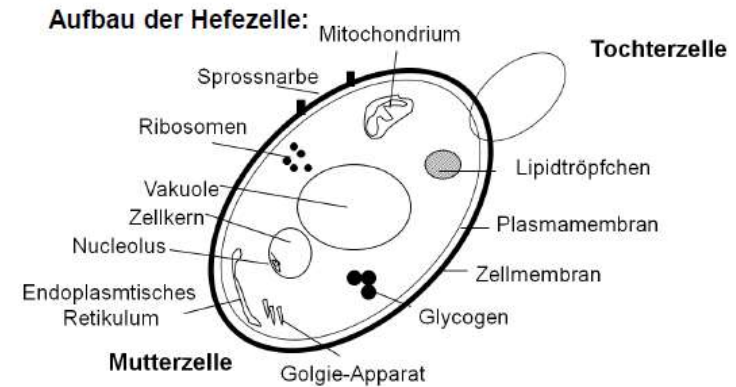
Spontane Gärung - Effekt SO2

- SO2 reduziert diversität im Most
- Nach 1-2 Tagen nur noch 2 Genera
- Hanseniaspora und Saccharomyces

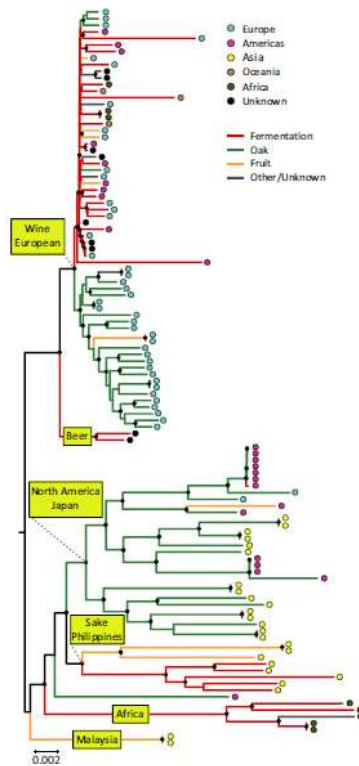


Saccharomyces cerevisiae

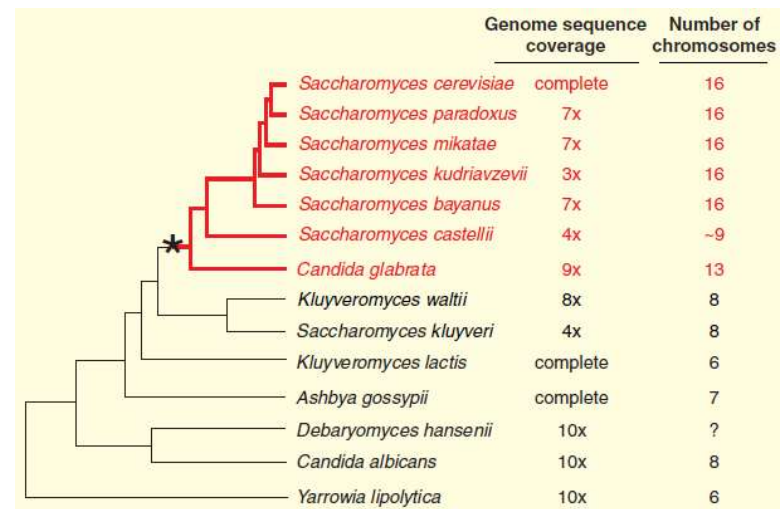
- Diploider Ascomycota
- 5-10µm
- Meistens asexuelle Vermehrung
- Lebt auf Holz >> Frucht
- Modellorganismus der Biologie
- 12MB genome, 16 chromosomes
- «Einfache» Genetik



S.cerevisiae Herkunft Biologie und Geographie

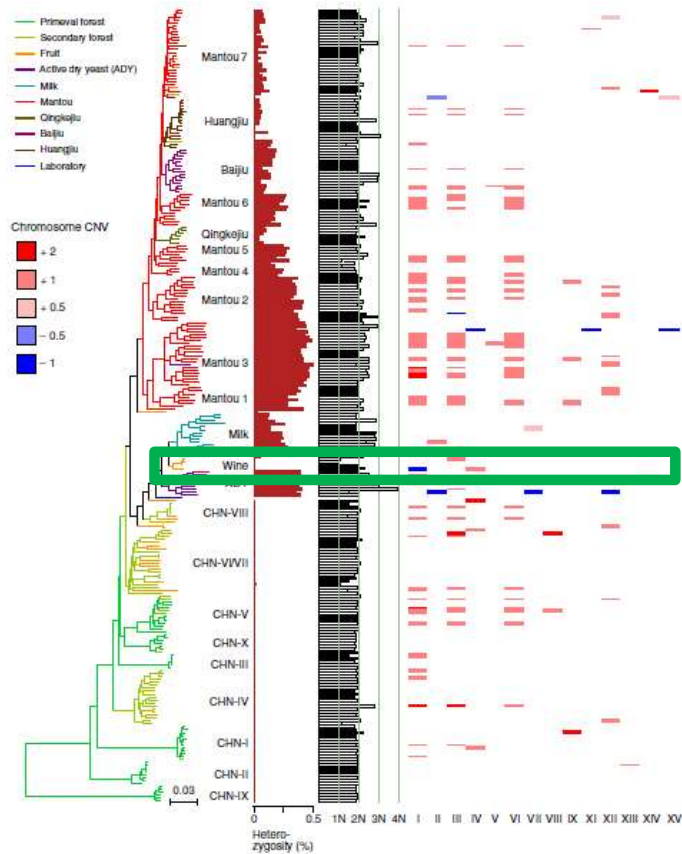


Whole genome SNP tree

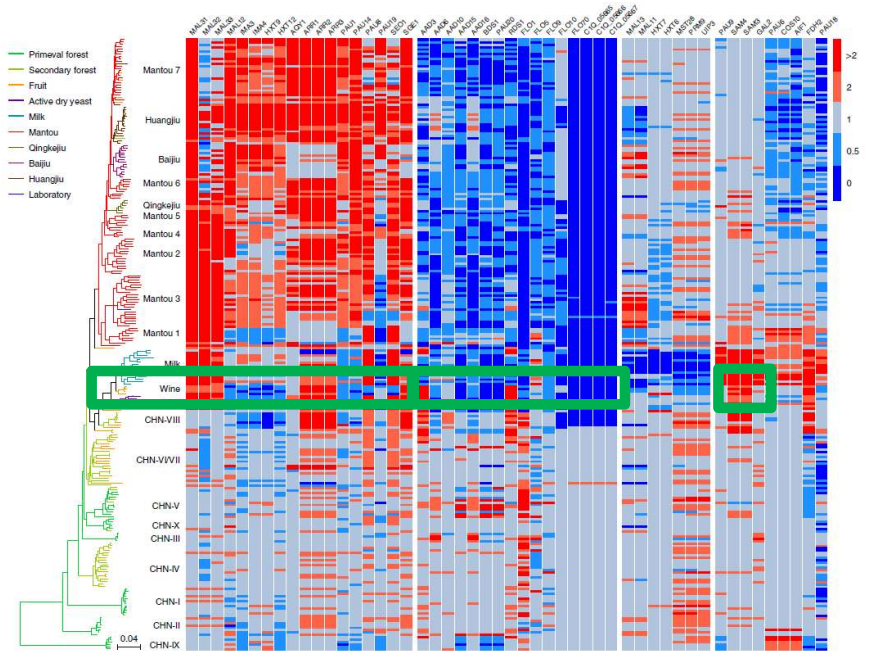


PDR5 gene tree

S.cerevisiae domestication



Chromosome CNV



Gene CNV

Reinzuchthefen

- Wildhefen in Spontangärungen können Fehler verursachen
- Unsaubere Gärung öffnet Nische für Bakterien (Acetobacter, Lactobacillus, Pediococcus)
- Lösung – mit bekannten Hefen animpfen, erlaubt «Design»
- Hefeselektion
- Entwickeln neuer Funktionen

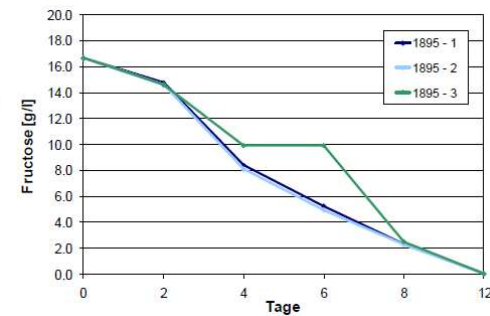
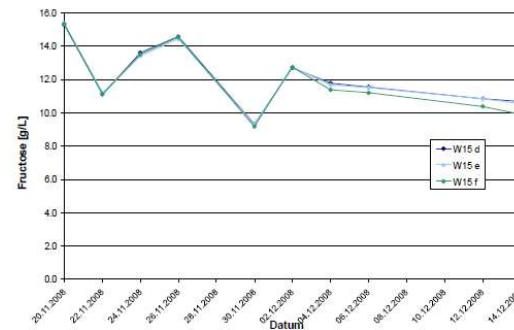


Hefeselektion



Fähigkeiten

- Fructophil – Gärstockungen 1895c
- Aroma (Esterproduktion)
- Alkoholtoleranz – Champagner
- Kältetoleranz – Weisswein
- Reduktion von H_2S and SO_2
- Farbstabilität



Entwicklung - Evolutionär

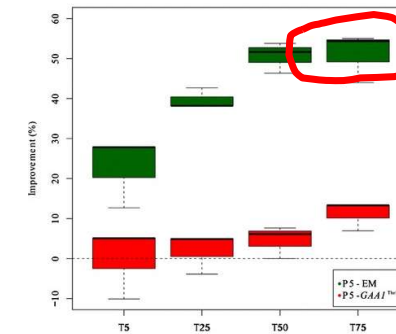
Kälteadaptation

- 27 bekannte Stämme
- 200 Passagen bei 12°C
- In weissem Most

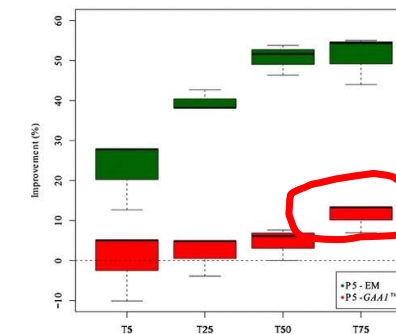
Genome Sequenierung

Chr	Coordinate	Ref.	Mut.	Change	Het/Hom	Gene	Molecular function
IV	119953	G	A	S547F	het	<i>UPD2</i>	ubiquitin-ubiquitin ligase activity
IV	974831	C	T	E318K	het	<i>YAP6</i>	sequence-specific DNA binding RNA polymerase II transcription factor activity
IX	108277	C	T	G1654D	het	<i>HIO3</i>	component of the RAM signaling network (molecular_function unknown)
V	143758	C	T	H623Y	het	<i>MTI</i>	transcriptional regulator of pseudohyphal growth (molecular_function unknown)
XII	316429	C	T	T108I	het	<i>STB1</i>	contributes to GPI-anchor transamidase activity
XIII	257816	G	A	P201S	het	<i>GIS4</i>	CAAX box containing protein of unknown function
XIV	128913	C	T	D58N	hom	<i>SEC2</i>	guanyl-nucleotide exchange factor activity
XV	848746	C	T	T90I	het	<i>HUA2</i>	cytoplasmic protein of unknown function

Fermentation performance



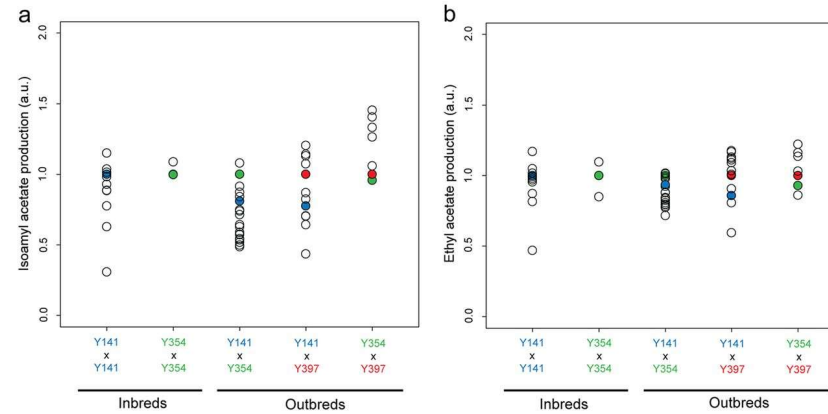
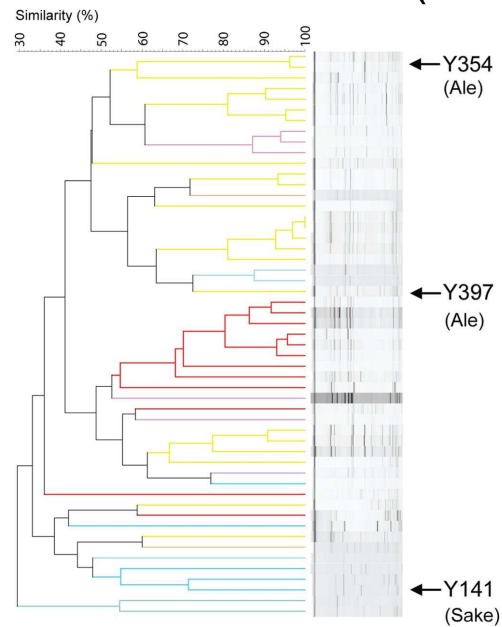
Fermentation performance



Entwicklung - Züchtung

Züchtung für Aroma (viel Isoamylacetate wenig Ethylacetate)

- 3 *S. cerevisiae* ausgewählt und gekreuzt
- 46 nachkommen (inbreeding, outbreeding)

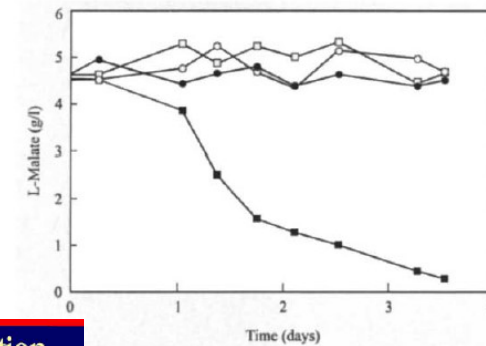


Entwicklung - GMOs

Malolactic fermentation strain ML01 Registered in US, CA, Moldova

Table 1. Constructs used to engineer malate degrading pathways in *S. cerevisiae* YPH259 (ref. 23).

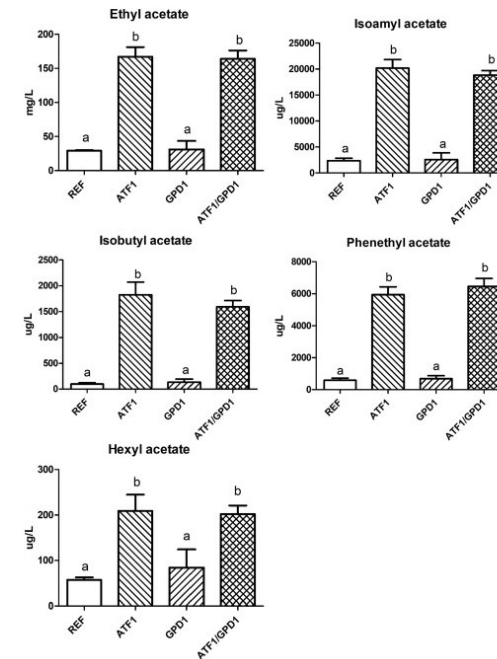
Construct	Description	Recombinant strain
pHVX1	Shuttle vector YEplac181 (ref. 24), containing the <i>ADH1₂-ADH1</i> , expression cassette	YADH
pHVX2	Shuttle vector YEplac181, containing the <i>PGK1₂-PGK1</i> , expression cassette	YPGK
pHV1	pHVX1 with <i>mae1</i> ORF (<i>ADH1₂-mae1-ADH1</i>)	YADH- <i>mae1</i>
pHV2	pHVX1 with <i>mae2</i> ORF (<i>ADH1₂-mae2-ADH1</i>)	YADH- <i>mae2</i>
pHV3	pHVX2 with <i>mae1</i> ORF (<i>PGK1₂-mae1-PGK1</i>)	YPGK- <i>mae1</i>
pHV4	pHVX2 with <i>mae2</i> ORF (<i>PGK1₂-mae2-PGK1</i>)	YPGK- <i>mae2</i>
pHV5	YEplac181-based vector containing the <i>ADH1₂-mae1-ADH1</i> , : <i>PGK1₂-mae2-PGK1</i> , expression system	ME1
pHV6	YEplac181-based vector containing the <i>ADH1₂-mae2-ADH1</i> , : <i>PGK1₂-mae1-PGK1</i> , expression system	ME2
pMDMALO ³	Multicopy episomal plasmid containing the <i>mleS</i> ORF inserted between the <i>PGK1</i> promoter and terminator sequences, as well as the <i>URA3</i> marker gene.	YPGK- <i>mleS</i>



Agency Response Letter GRAS Notice No. GRN 000120

Entwicklung - (nonGMOs) CRISPR CAS

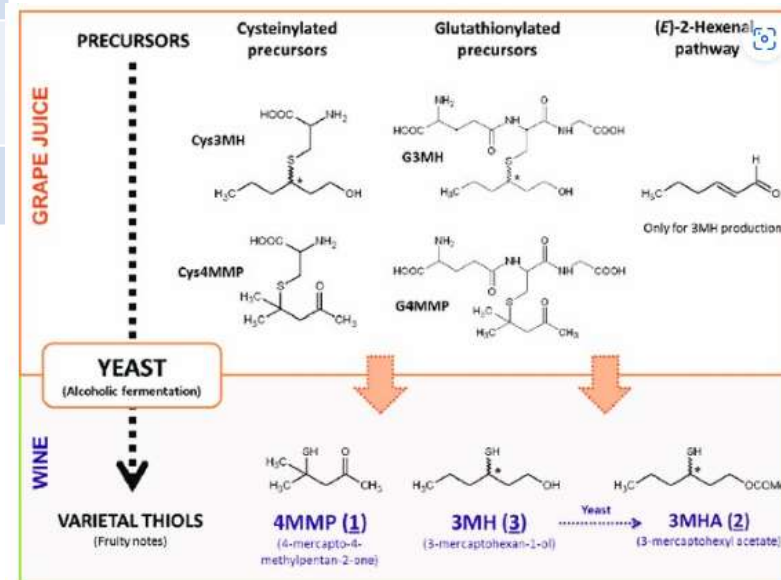
- Harnstoff Reduktion
 - Reduziert bildung von Ethylcarbammat
 - Karzinogen
- Isoamylacetat (Banane)
- Glycerolproduktion (Kältetoleranz)
- Akzeptanz am Markt?



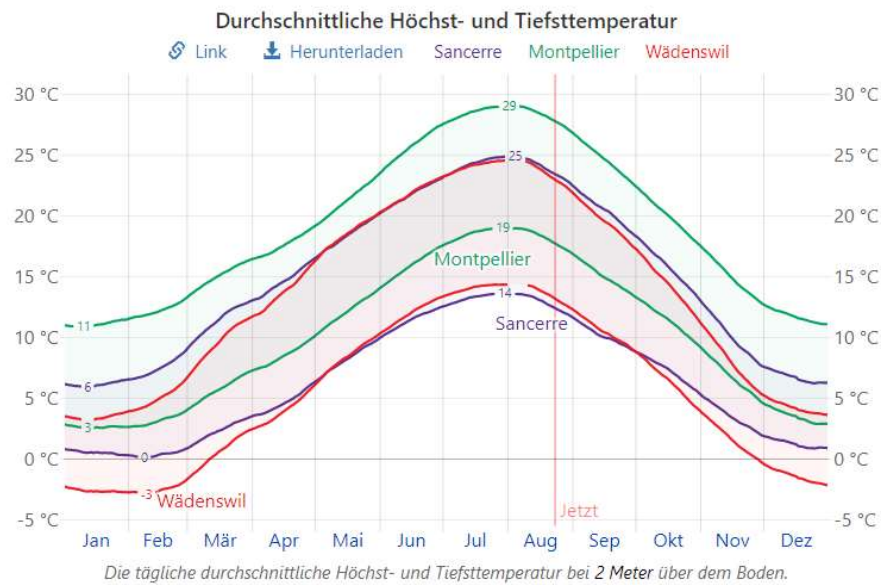
Sauvignon Blanc - Aroma

Thiolverbindung	Aromabeschreibung	Wahrnehmungsschwelle (ng/l)	[c] in NZ Sauvignon Blanc (ng/ul)
4-Mercapton-4-methyl-2-pentanon (4MMP)	Buchsbaum, Ginster, Cassis, Tomatenlaub, Katzenurin	0.8	2- 50
3-Mercaptohexanolacetat (3MHA)	Grapefruit, Passionsfrucht, Stachelbeere, Buchsbaum, Maracuja	4	5- 2'500
3-Mercaptohexanol (3MH)	Grapefruit, Passionsfrucht, Stachelbeere	60	100- 20'000

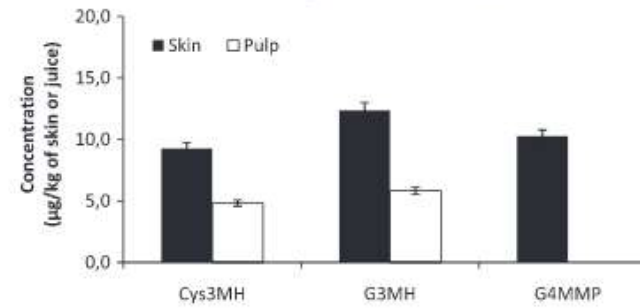
- Vorstufen in Traube
- Freisetzung durch Enzyme in Hefe, Speichel



Sauvignon Blanc Aroma im Anbau - Klima



B. Distribution of thiol precursors in Sauvignon Blanc from Sancerre



C. Distribution of thiol precursors in Sauvignon Blanc from Montpellier

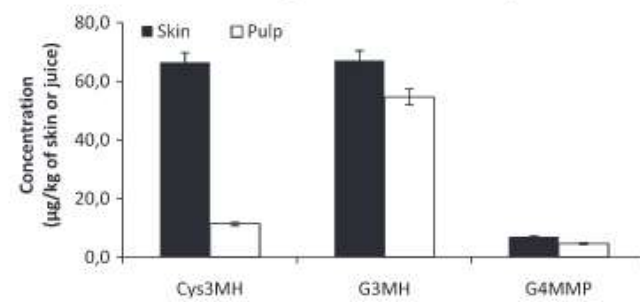
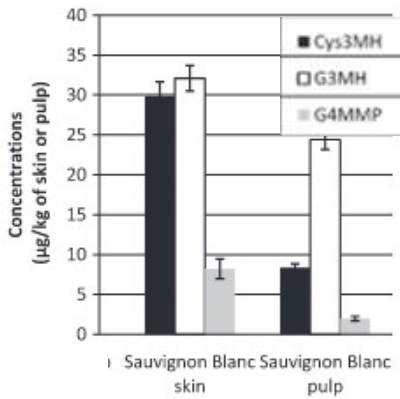
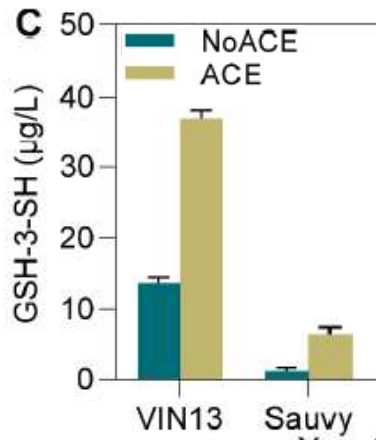


Fig. 2. Influence of vineyard locations on distribution of thiol precursors in Sauvignon Blanc from Tours (A), Sancerre (B) and Montpellier (B).

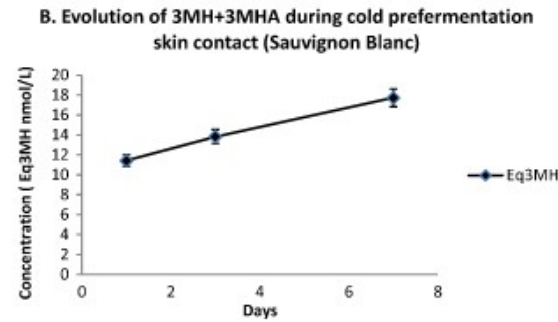
Sauvignon Blanc Aroma - Keller



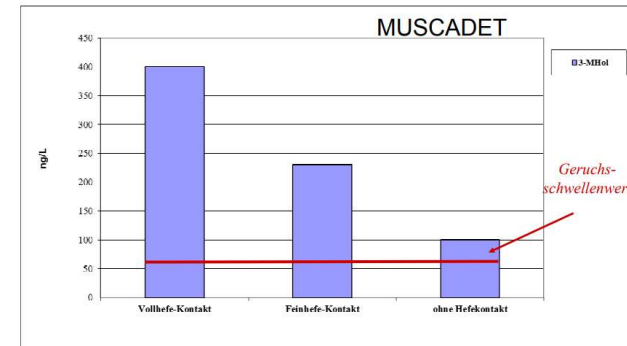
Verteilung in Beere



ACE

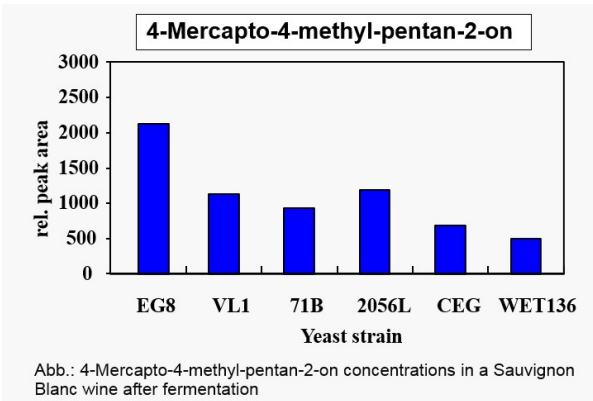


Kaltstandzeit

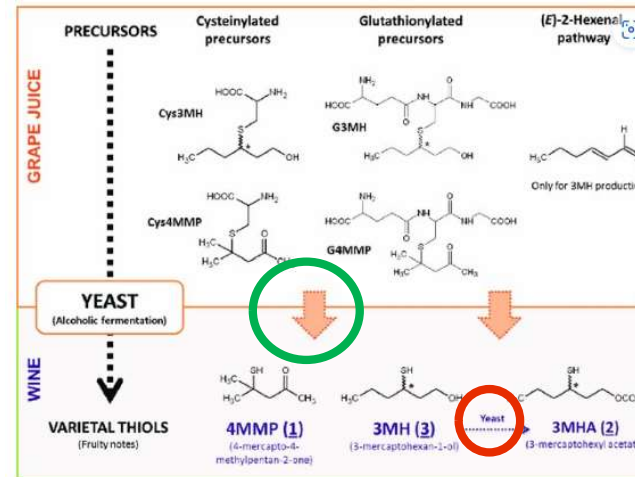
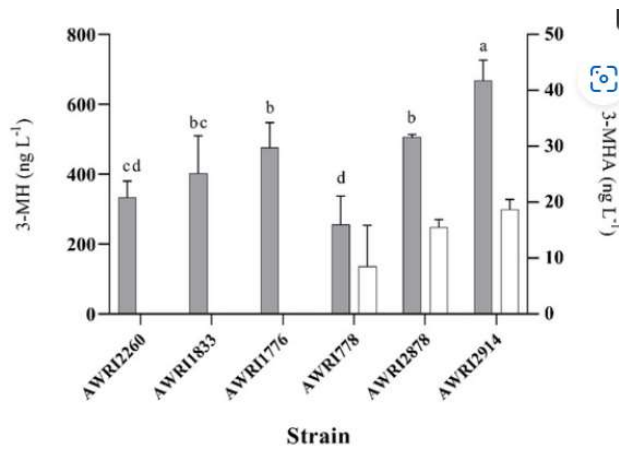
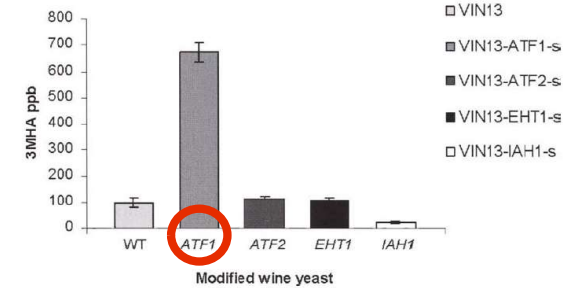


Hefekontakt

Sauvignon Blanc Aroma - Hefen

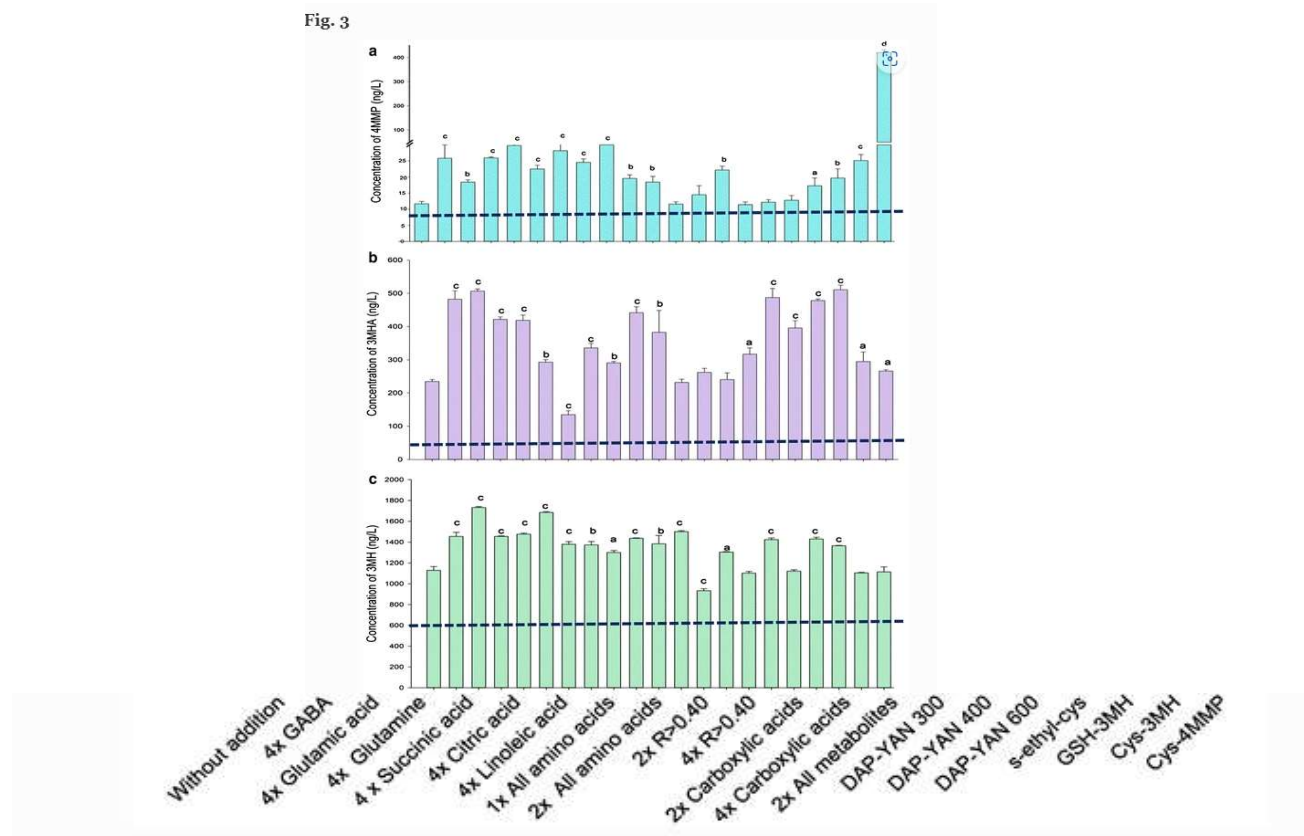


Strain	3MH (ng/liter) ^a	P ^b
VIN 13	1,084 ± 66	
VIN 13 STR3	1,362 ± 84	0.014



Sauvignon Blanc Aroma - Nährstoffe

Fig. 3



Sauvignon Blanc Aroma >> Thiole

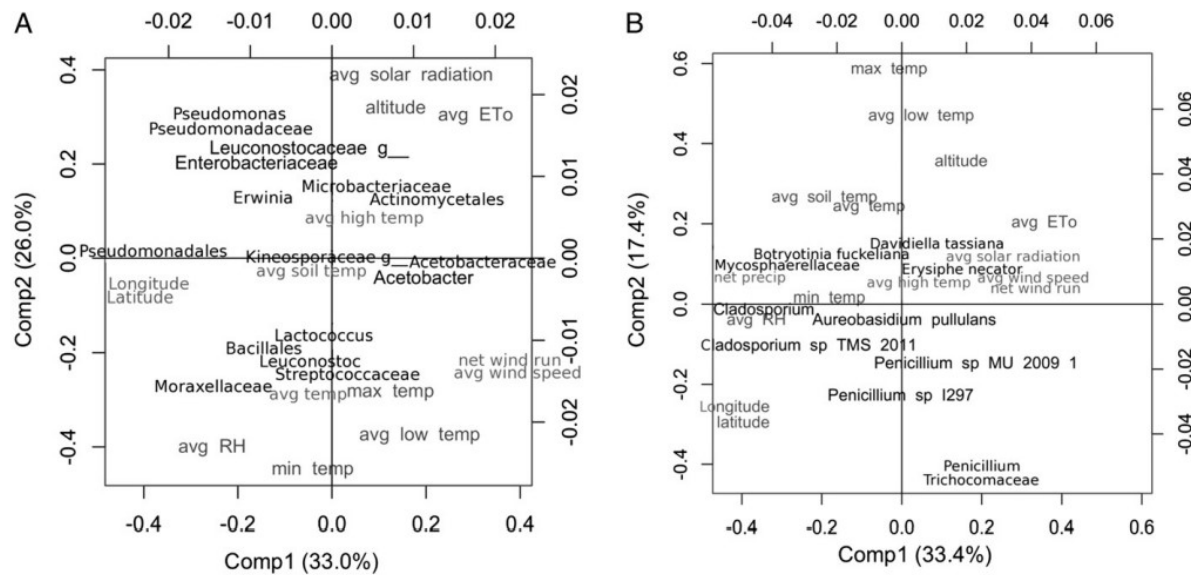
Compound class	Compound	Aroma	Odour threshold	C in Control Wine ug/L	OAV in Control Wine	OAV in
Acetate esters	Isoamyl acetate	Banana	30	3,175.57	105.85	175.18–22.50
Ethyl esters	Ethyl hexanoate	Pineapple, apple peel, fruity	45	737.62	52.69	70.50–23.83
Ethyl esters	Ethyl butanoate	Fruity, apple	20	463.89	23.19	31.97–7.12
Fatty acids	Decanoic acid	Fatty acid, dry, woody	1,000	20,187.46	20.19	21.36–8.24
Fatty acids	Isovaleric acid	Sweet, cheese	34	460.23	13.54	18.02–3.82
Fatty acids	Hexanoic acid	Sweet, cheese, spicy	420	5,094.66	12.13	15.48–7.85
Norisoprenoids	β -Ionone	Floral	0.07	0.47	6.65	6.74–5.53
Alcohols	Isoamyl alcohol	Whisky, malt, burnt	30,000	112,701.73	3.76	6.55–2.92
Fatty acids	Octanoic acid	Fatty acid, dry, dairy	220	680	3.09	3.09

Compound class	Compound	Aroma	Odour threshold	C in Control Wine ug/L	OAV in Control Wine	OAV in
C6 Alcohols /aldehyde	Cis-3-hexen-1-ol	Green, cut grass	400	910.42	2.27	2.74–1.57
C6 Alcohols /aldehyde	Hexanol	Resin, flower, green, cut grass	1,100	2,499.60	2.25	3.91–1.42
Acetate esters	β -Phenylethyl acetate	Flowery, fruity, olive	250	511.13	2.03	3.05–0.60
Alcohols	Methionol	Cooked cabbage	1,000	1,936.85	1.94	8.52–0.46
Ethyl esters	Ethyl decanoate	Fruity, floral	200	318.42	1.59	2.36–0.65
Norisoprenoids	β -Damascenone	Rose	0.05	0.08	1.53	2.60–0.47
Ethyl esters	Ethyl octanoate	Sweet, ripe banana, pear	600	731.47	1.26	2.30–0.63
Ethyl esters	Ethyl isovalerate	Fruity, cheese	3	3.25	1.08	3.30–0.75

The art of not doing anything - but doing everything right

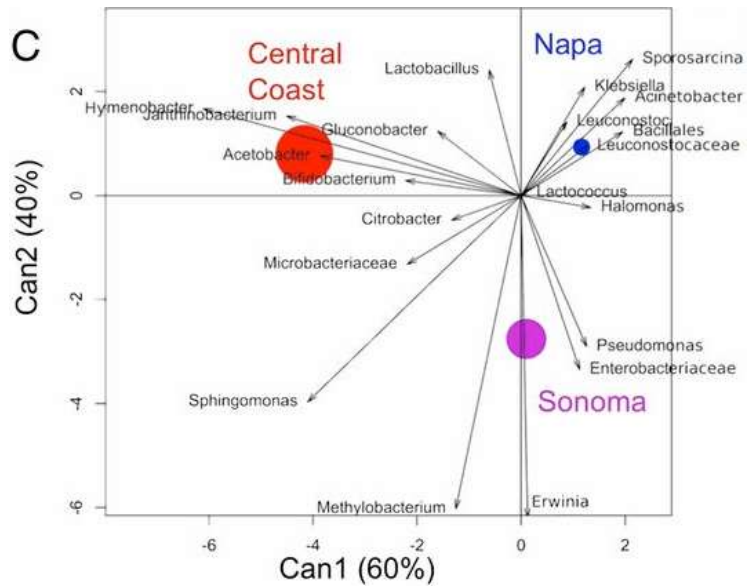
Terroir – abiotic factors

Orange wine, Natural wine, PetNat, Kvevri
Terroir?



The art of not doing anything - but doing everything right

Terroir- geography



Sauvignon Blanc Aroma

- Werkzeug ist vorhanden
- Entscheidung beim Produzent
- Mehrwert von Mischkulturen, Spontangärungen - TBD

