Hefetagung 2021

HBLA und Bundesamt Klosterneuburg
Wein- und Obstbau

Das Gärungs-Ökosystem und seine optimisierte Nutzung

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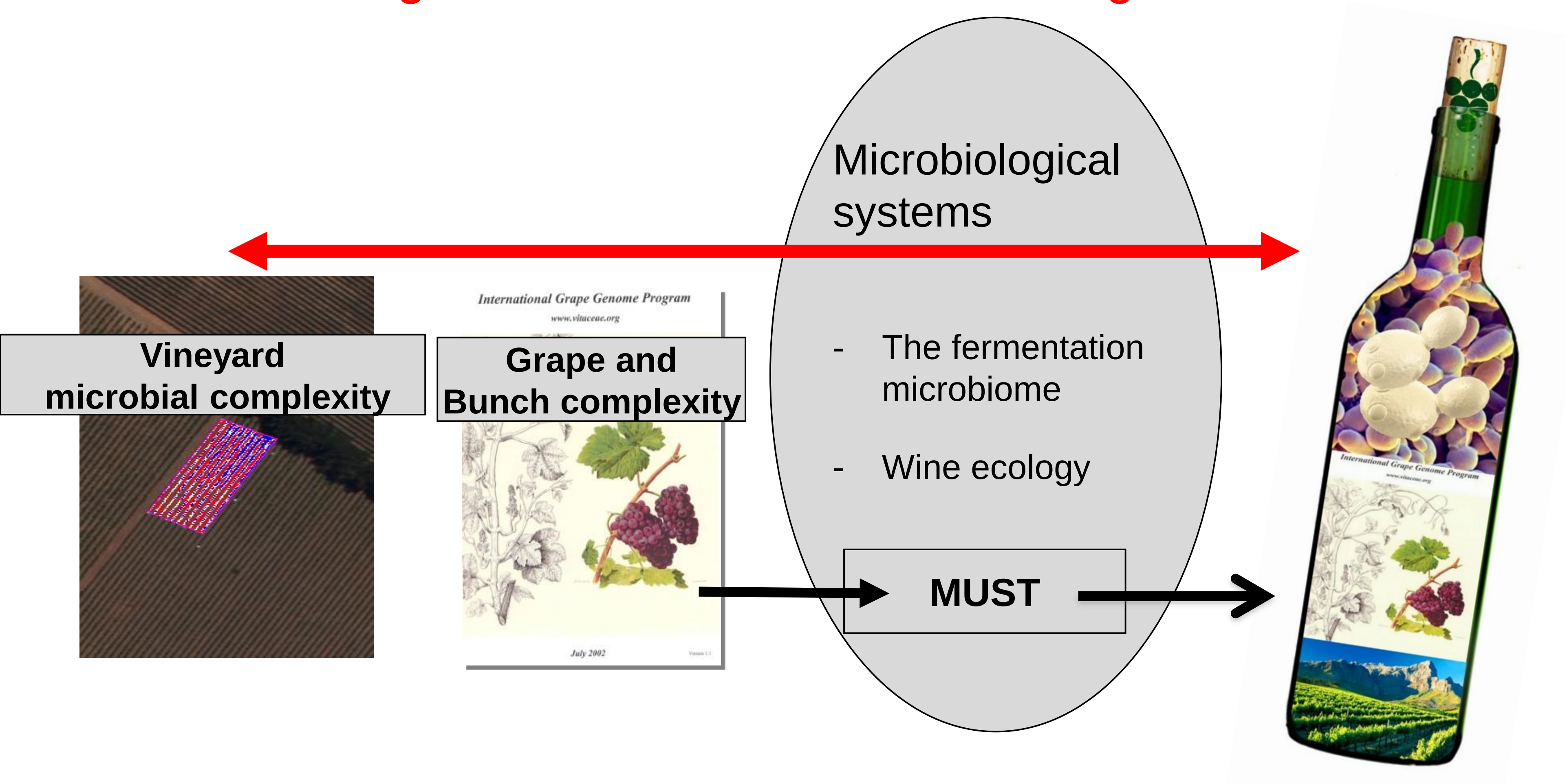
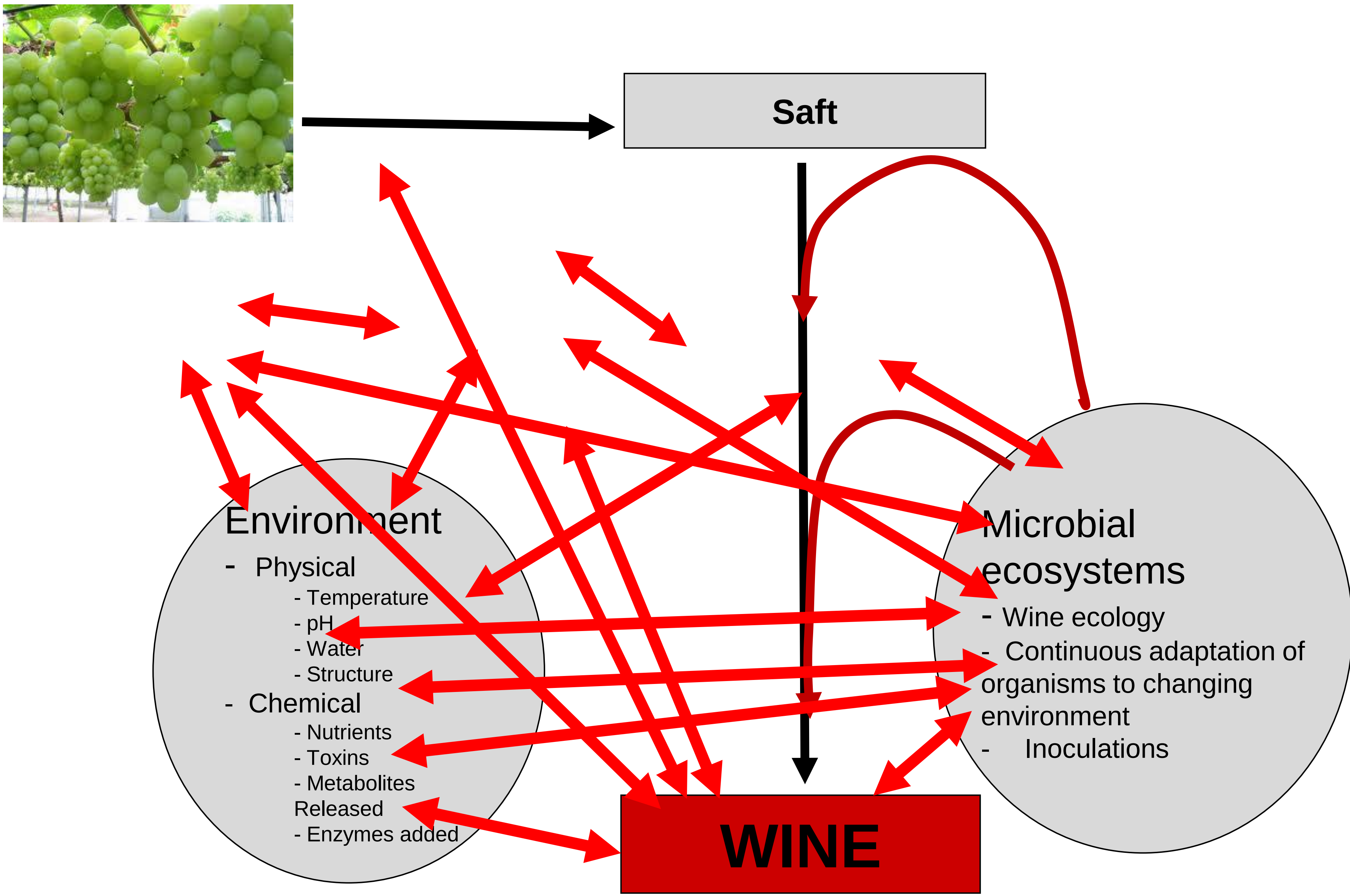
UNIVERSITEIT STELLENBOSCH UNIVERSITY

Die Wein-Landschaft aus Sicht der Mikroben:

Weinberg/Traube/Wein mikrobische Őkosysteme
oder:
Őkologische Nischen so weit das Auge reicht



“Die Gärung”


Saft
Environment
- Physical
- Temperature
- pH
- Water
- Structure
- Chemical
- Nutrients
- Toxins
- Metabolites Released
- Enzymes added
Microbial ecosystems
- Wine ecology
- Continuous adaptation of organisms to changing environment
- Inoculations
WINE

Optimisierte Nutzung des Gärungsökosystems

Warum?

- Allgemein wachsendes Verständnis der Rolle der mikrobiologischen Bio-diversität (im Menschen, aber auch im Weinberg und im Keller)
    - Auch bei Animpfung mit Zuchthefe…
- Anekdotische Evidenz: “Spontane” Gärungen werden (wieder?) mehr genutzt
    - Aber auch Zunahme an Berichten über Probleme
        - Off-flavours
        - Stuck/sluggish fermentation
- Koennen wir diese Probleme in den Griff bekommen?
    - Verstehen wir das Őkosystem?
    - Können wir das System kontrollieren oder vorhersagen?

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Wie wir den Gärungsprozess heute kontrollieren

- Temperature, oxygenation and other cellar practices
- Inoculation with yeast and bacterial strains
  - *S. cerevisiae* and some non-*Saccharomyces* yeast as co-inoculants
    - *Lachancea thermotolerans,Torulaspora delbrueckii, Candida zemplinina, Metschnikowia pulcherrima*
  - Lactic acid bacteria
    - *Oenococcus oeni, Lactobacillus plantarum*
- Chemical or enzymatic agents
  - SO_2
  - Pectinases, cellulases, proteases, lysozyme
  - Yeast nutrients (Ammonium, complex survival factors etc)

Impacts on costs, health and organoleptic properties - generic application

Können wir das besser machen?

Scientific Understanding of Ecosystem Function

- Study species individually
- Study species and their interactions

Modeling Ecosystem

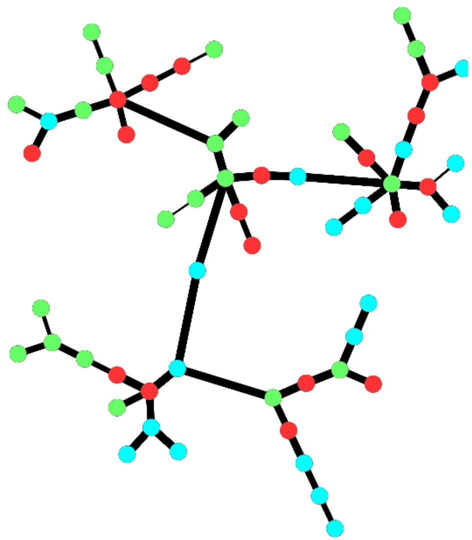
- “Engineering” approach
- Treat ecosystem as “Blackbox”

Können wir das besser machen?

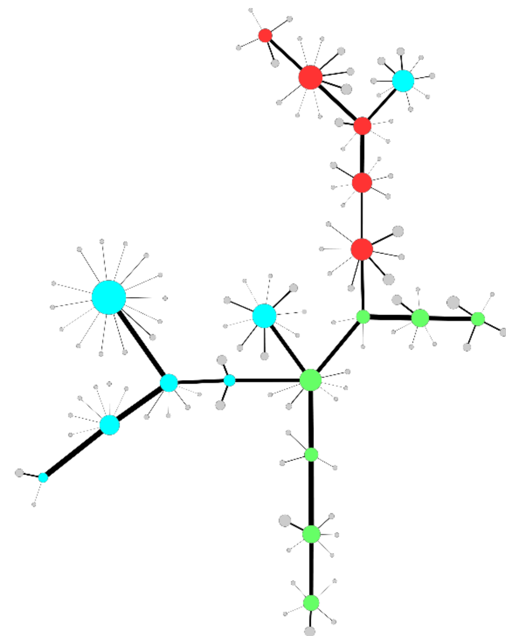
Understanding ecosystem function

- Mapping (Kartographieren) the microbial ecosystem in grapes and wine
- Understanding:
  - Ecological interactions between species
    - Molecular mechanisms?
    - Use of model consortia
  - Evolving species in response to biotic selection pressures
- Integrating data to describe and understand the ecosystem “bottom-up”

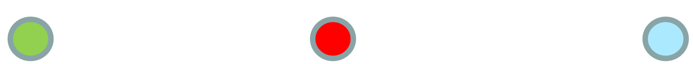
Kartographie der Heterogenität:
Die mikrobische Landkarte der Weinberge



Cultivable yeast



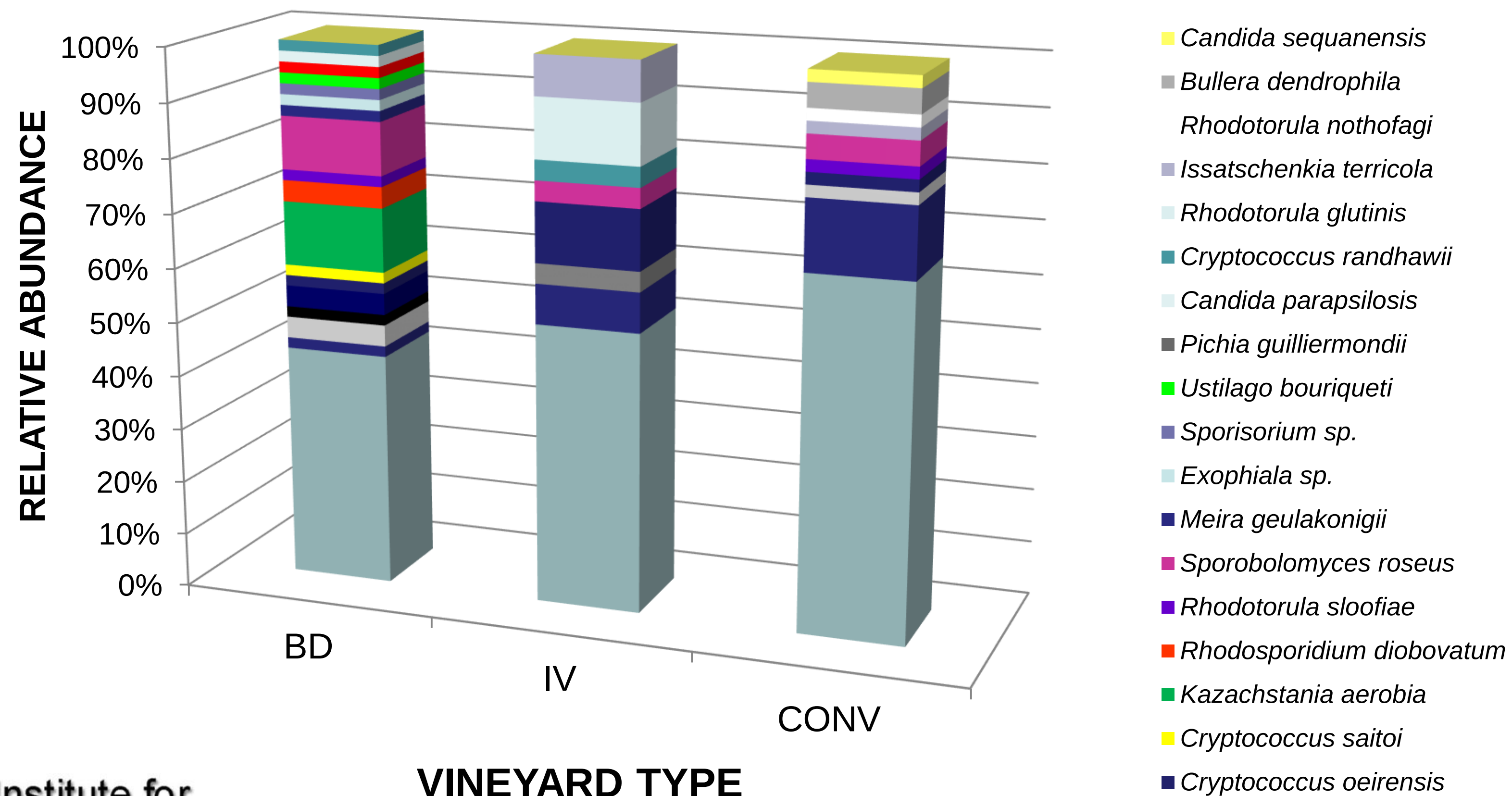
DNA Sequence based



“Biodynamic” vs “integrated” vs “conventional”.
Each node represents one sample

Die Kartographie der Trauben…

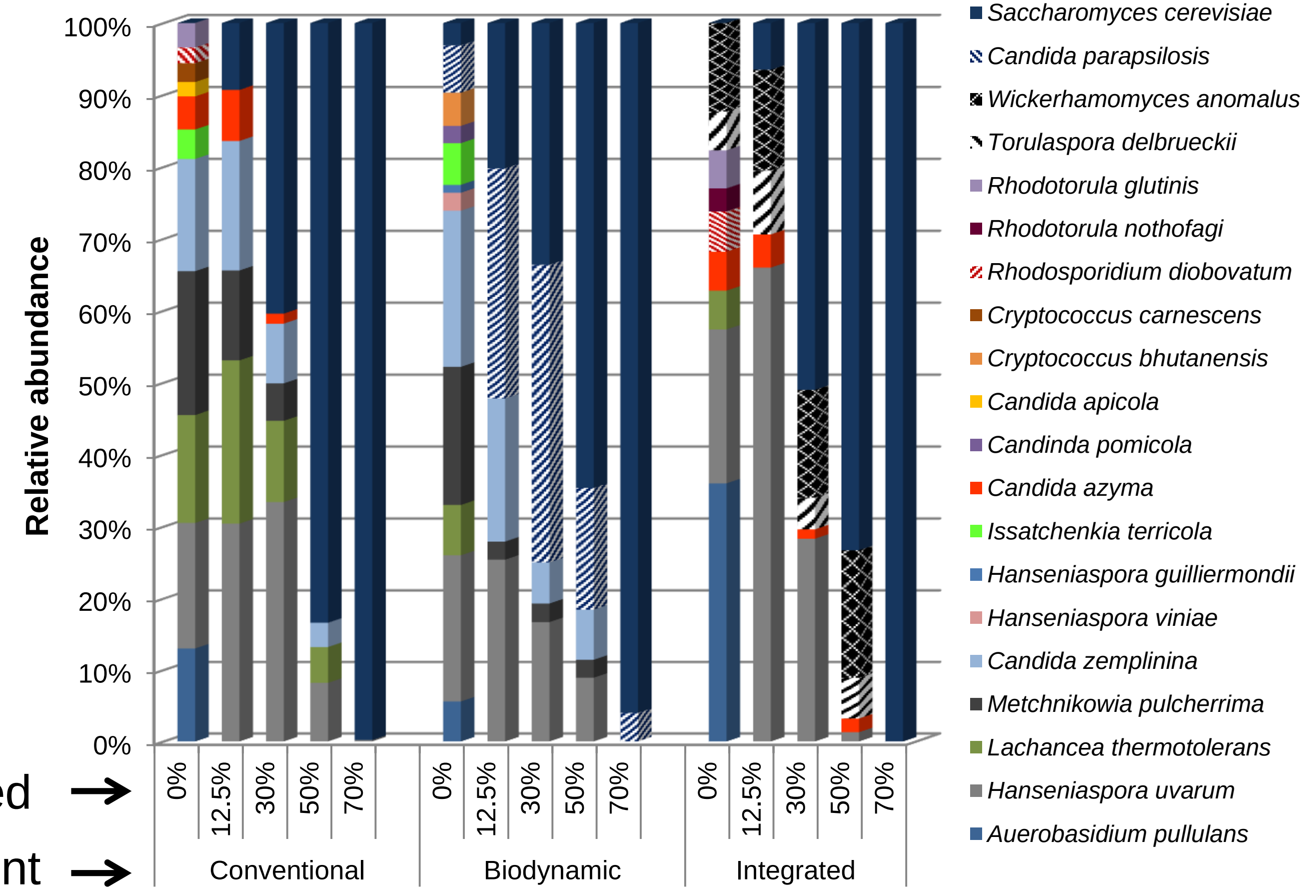
"Biodynamic" vs "integrated" vs "conventional"



Institute for Wine Biotechnology
Stellenbosch University

(Setati et al. PlosOne 2012)

…und die Kartographie der spontanen Gärung



% sugar consumed →

Vineyard treatment →

Unpredictable ecosystem dynamics

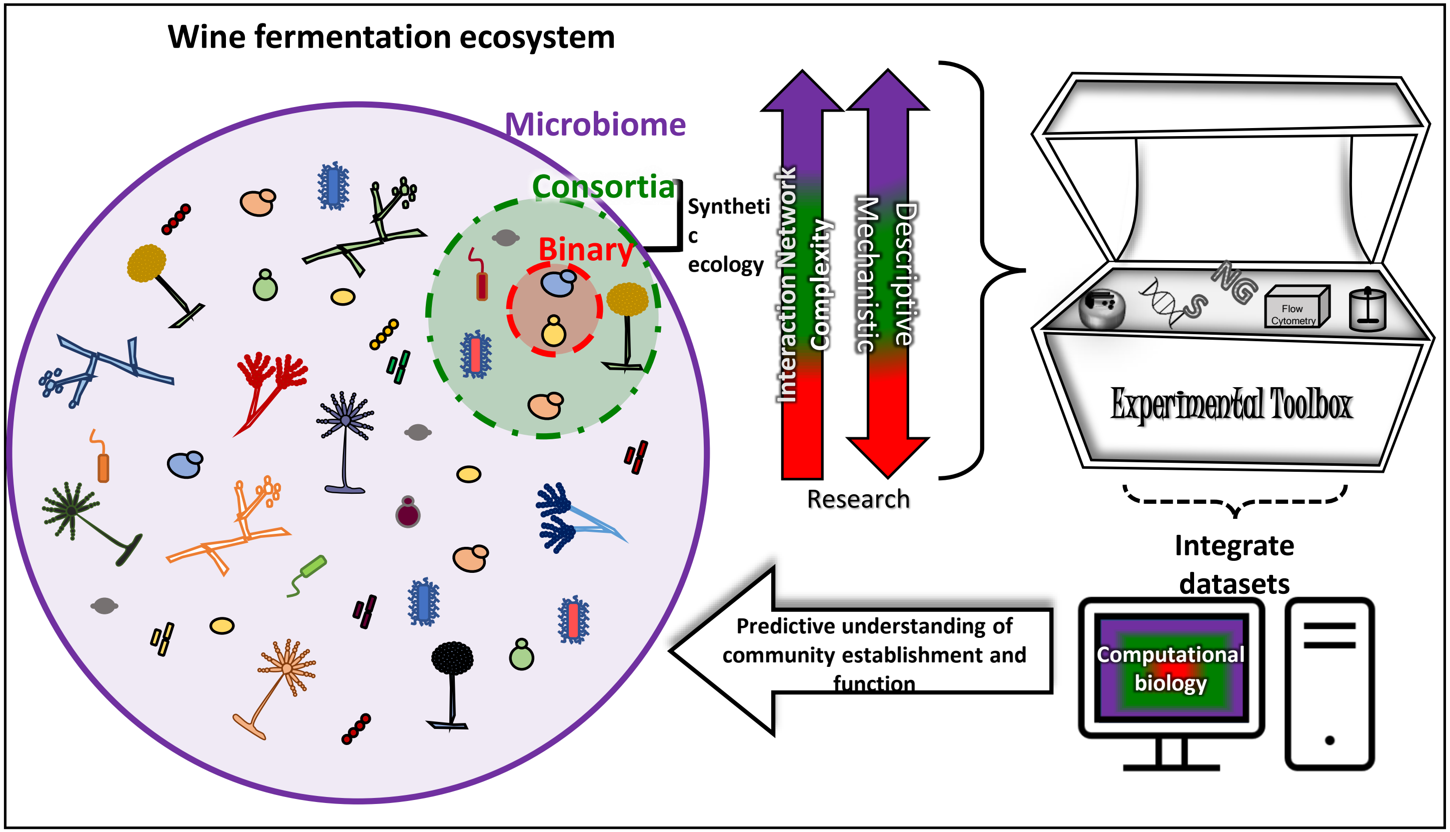
Setati et al. 2015 *Frontiers in Microbiology* 6:1358.

Viele “gute” Hefen, aber einige sind nicht erwünscht…

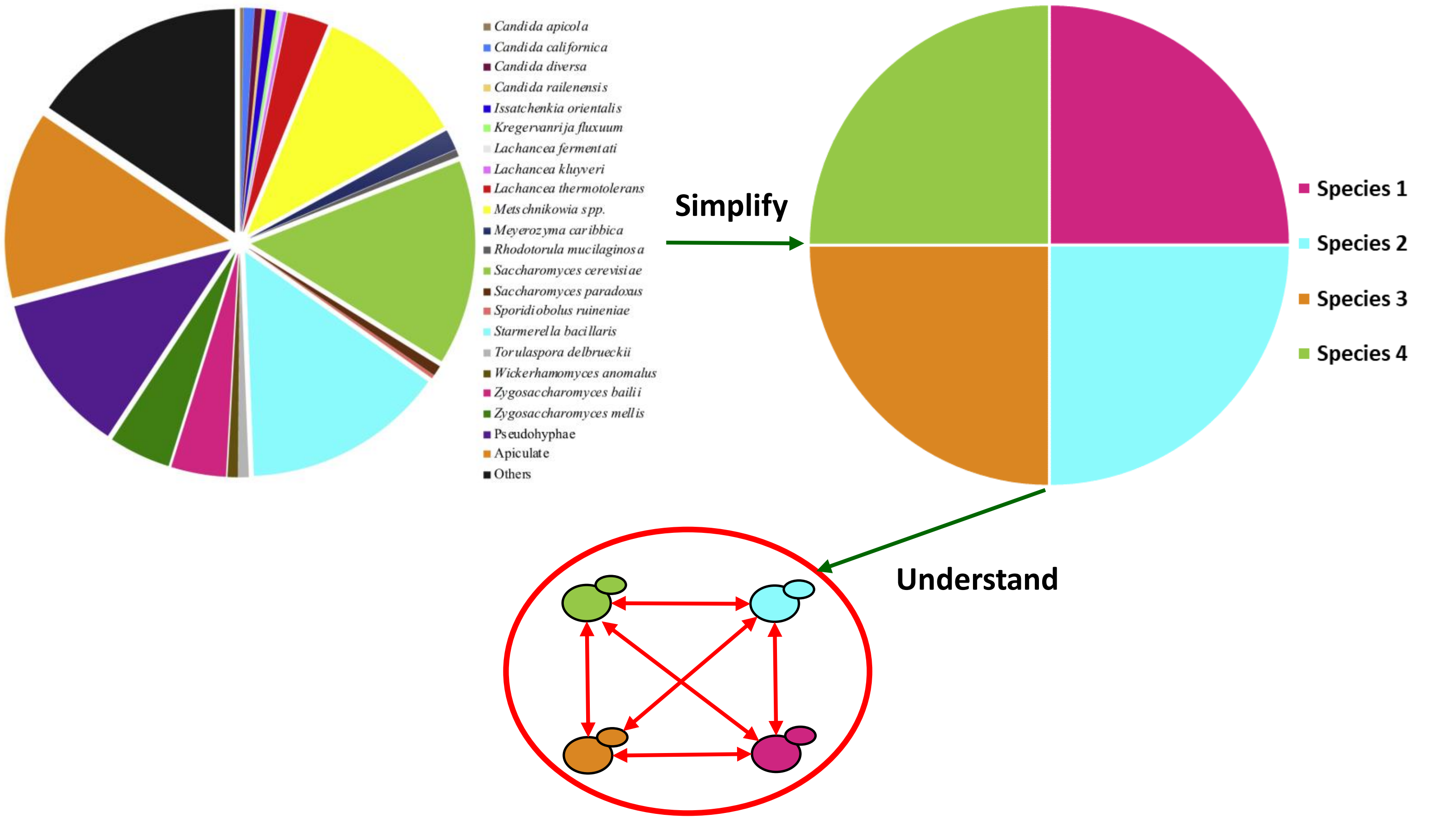
Yeast spp.	ITS SIZE	Characteristic	Yeast spp.	ITS SIZE	Characteristic
M. pulcherrima	374	Persist until final stage of fermentation Reduction in Alcohol Level Reduction in Total Acidity	*L. thermotolerans*	*678*	Persist until final stage of fermentation Reduction in Acetic Acid Increase Glycerol level
I. terricola	*411*	Persist until final stage of fermentation	*C. parapsilosis*	*520*	Persist until final stage of fermentation
C. zemplinina	*359*	Persist until final stage of fermentation Reduction in Alcohol Leve Increase glycerol level Decrease Acetaldehyde and Acetic Acid level	*W. anomalus*	*617*	Persist until final stage of fermentation Improve wine aroma Reduction in Alcohol level Utilize Malic Acid
H. uvarum	*751*	Persist until final stage of fermentation Strong reduction in Alcohol and Ethyl acetate level	*S. cerevisiae*	*843*	Strong fermentative yeasts

Wie kann man das Wein-Ökosystem studieren?

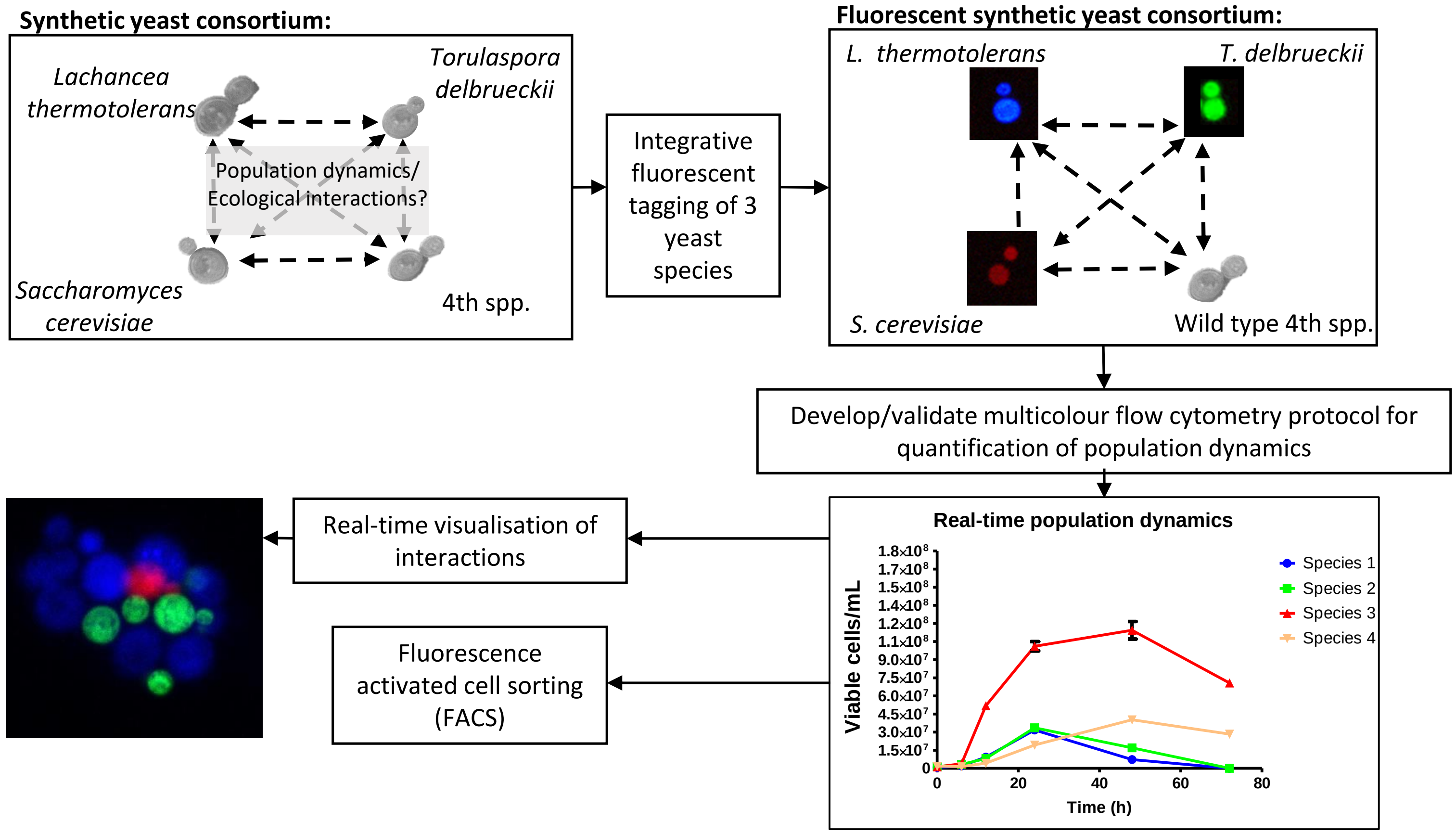
The ecology of wine fermentation: a model for the study of complex microbial ecosystems



Reducing complexity to increase understanding

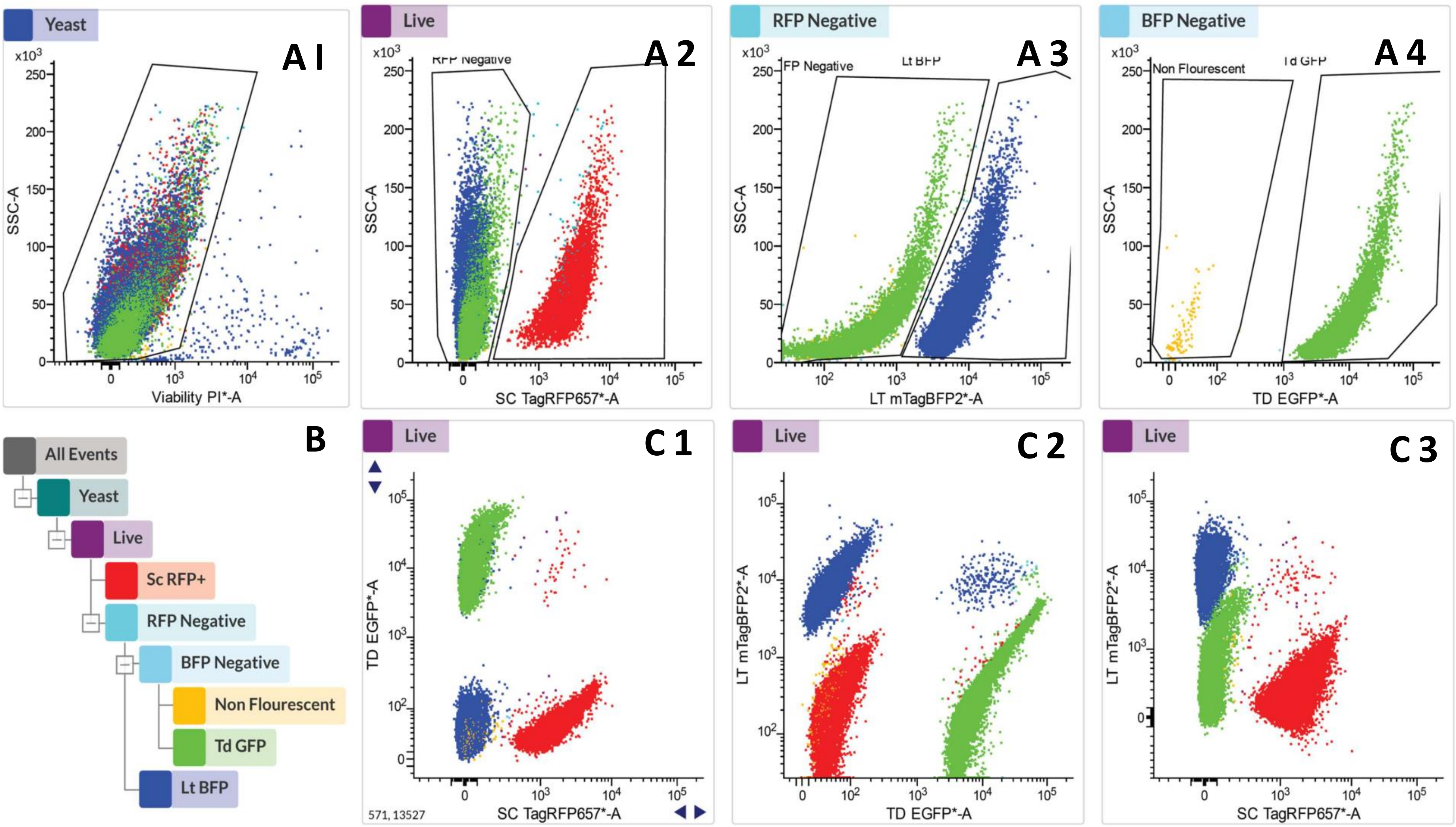


A new way to study population dynamics



Conacher et al. 2020

High throughput monitoring – change conditions, change species



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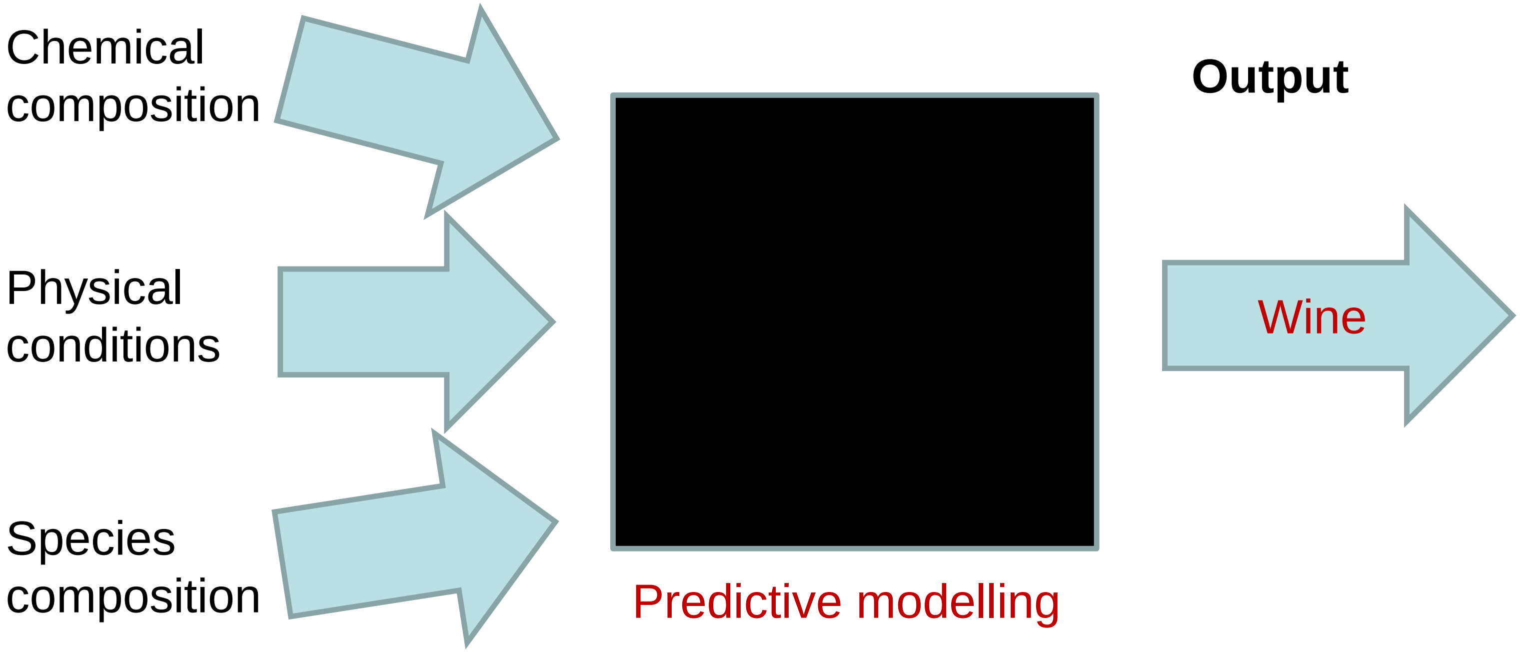
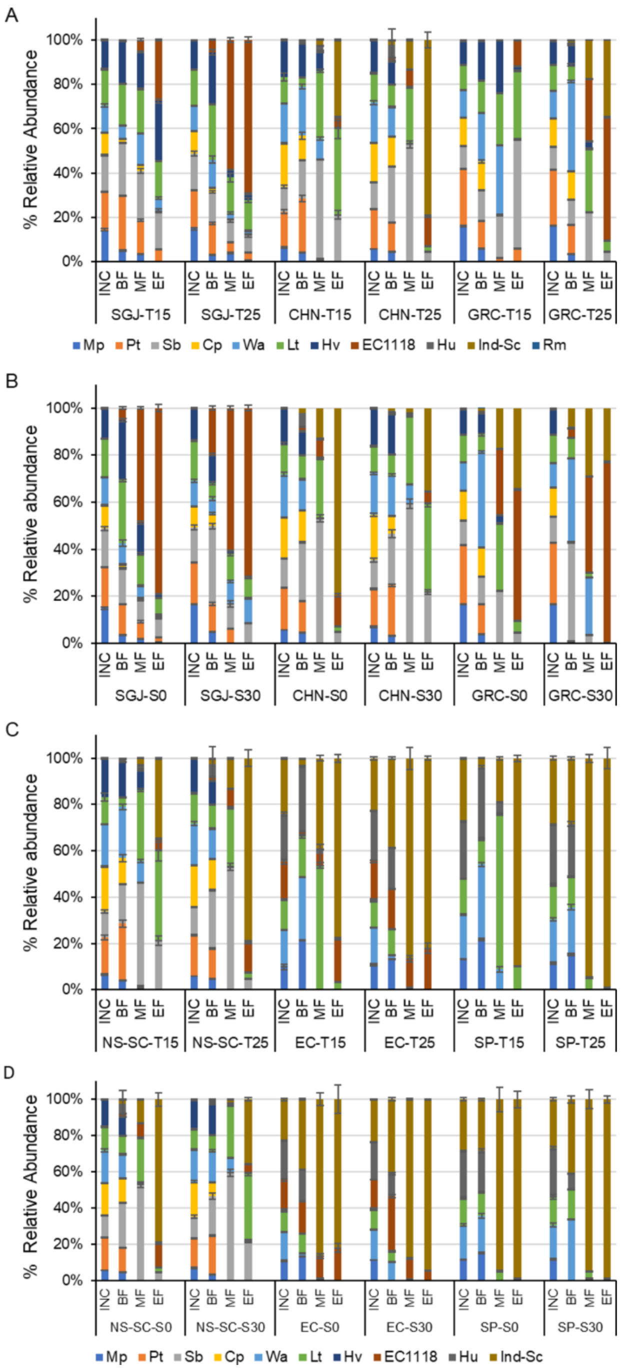
Enabling the black box approach

Input parameters



The big picture

Ecological interactions are a primary driver of population dynamics in wine ecosystems



Bagheri et al. 2020, Sci. Rep.

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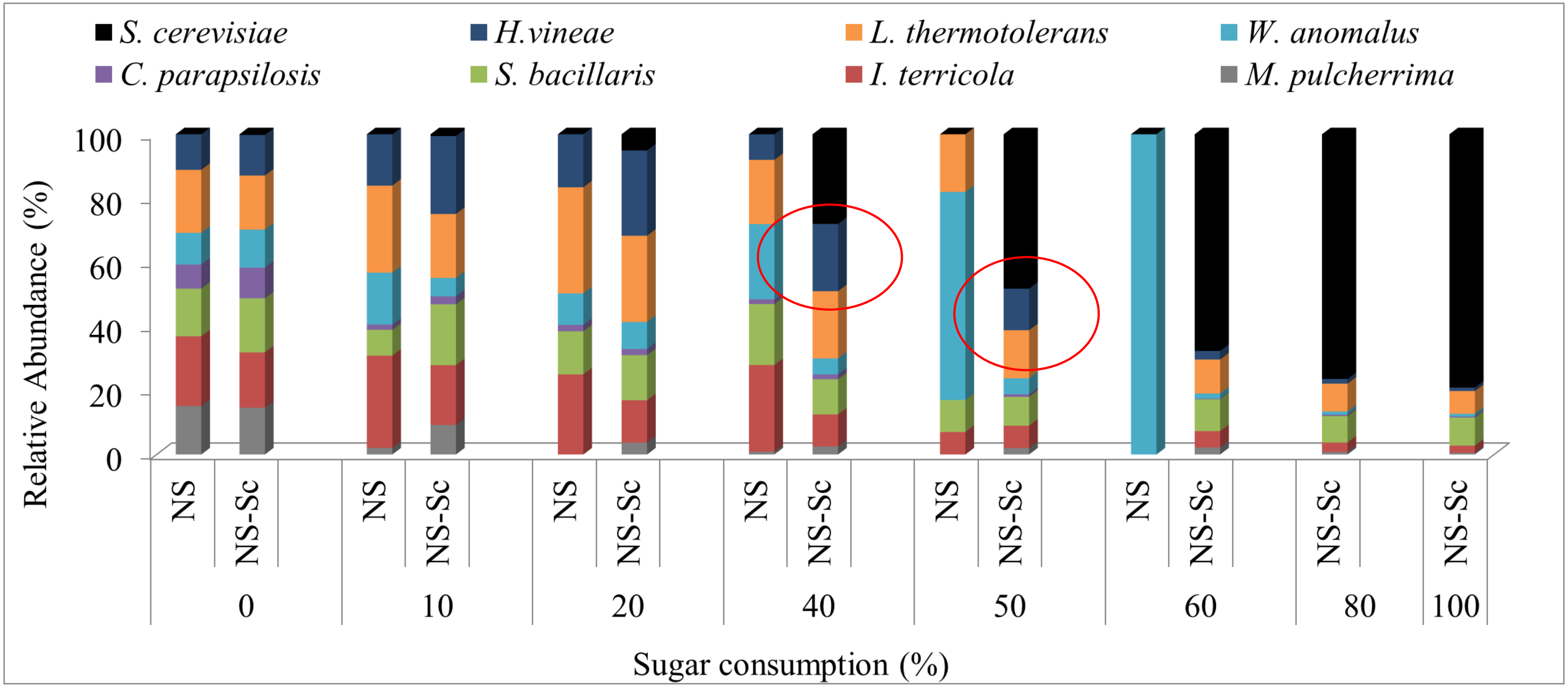
Oder in mehr Detail:

Inoculation of *S. cerevisiae* differentially impacts other species

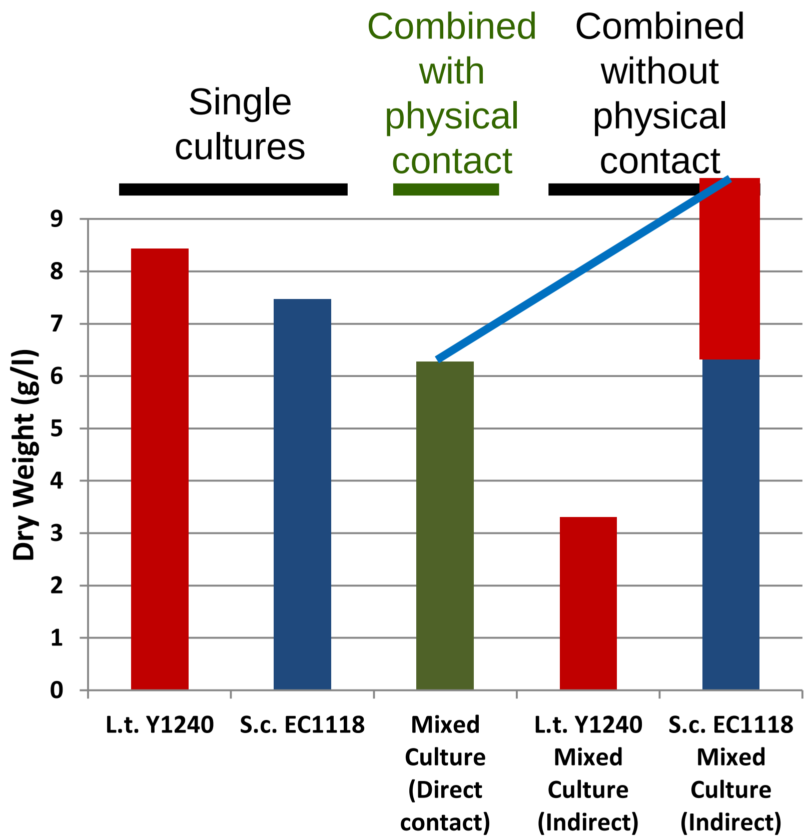


Bagheri et al. 2018, AMAB

Und die Mechanismen?

Ein Beispiel:
Direkter physischer Kontakt zwischen Zellen und Populationsdynamik

Example of *L. thermotolerans* and *S. cerevisiae*

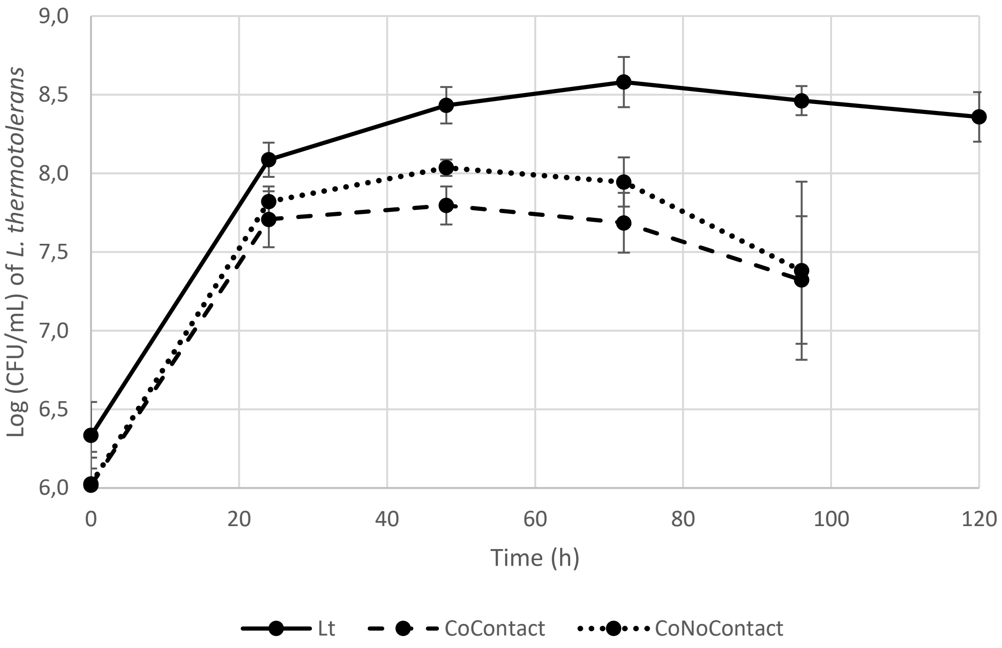


Double compartment bioreactor:
- Metabolic interactions maintained
- Physical interactions are either blocked (Mixed culture – indirect) or maintained (Mixed culture – direct)

Luyt et al. MSc thesis 2015

Direkter physischer Kontakt zwischen Zellen und Populationsdynamik

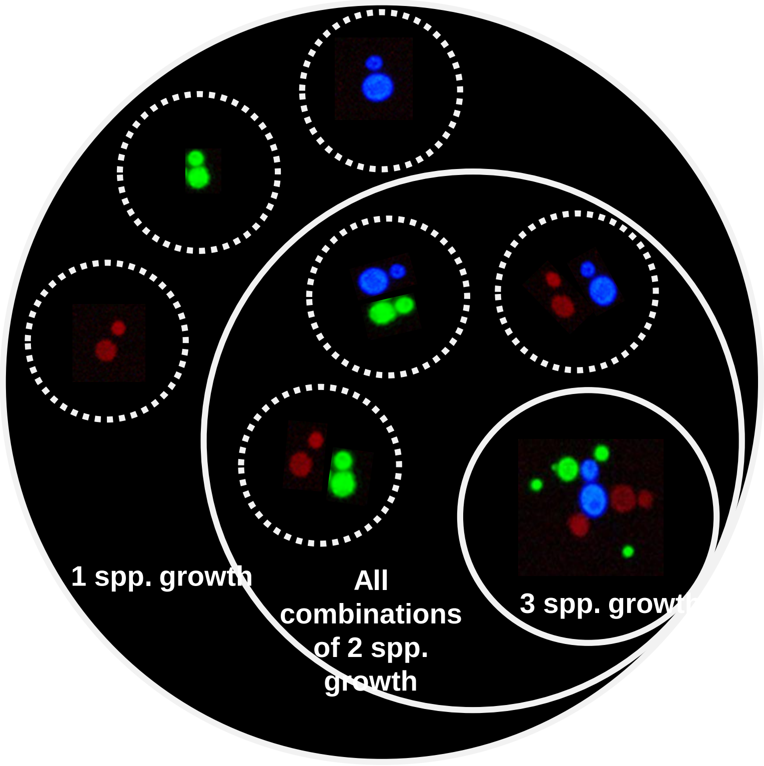
Example of *L. thermotolerans* and *S. cerevisiae*



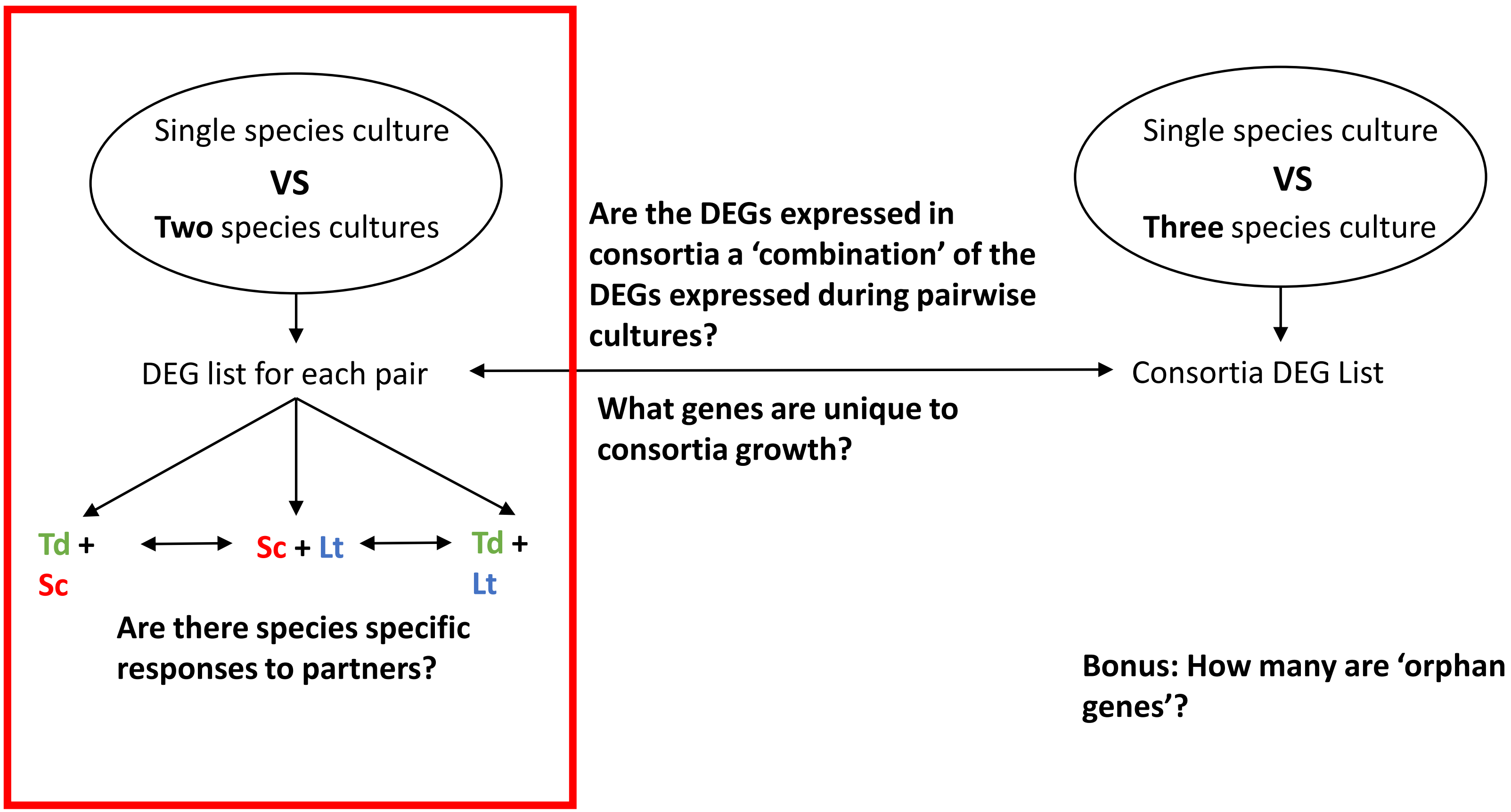
Und dann Multi-species omics….

RNASeq:

Identifying the transcriptomic signature of binary versus higher order interactions in a tri-species synthetic yeast consortium

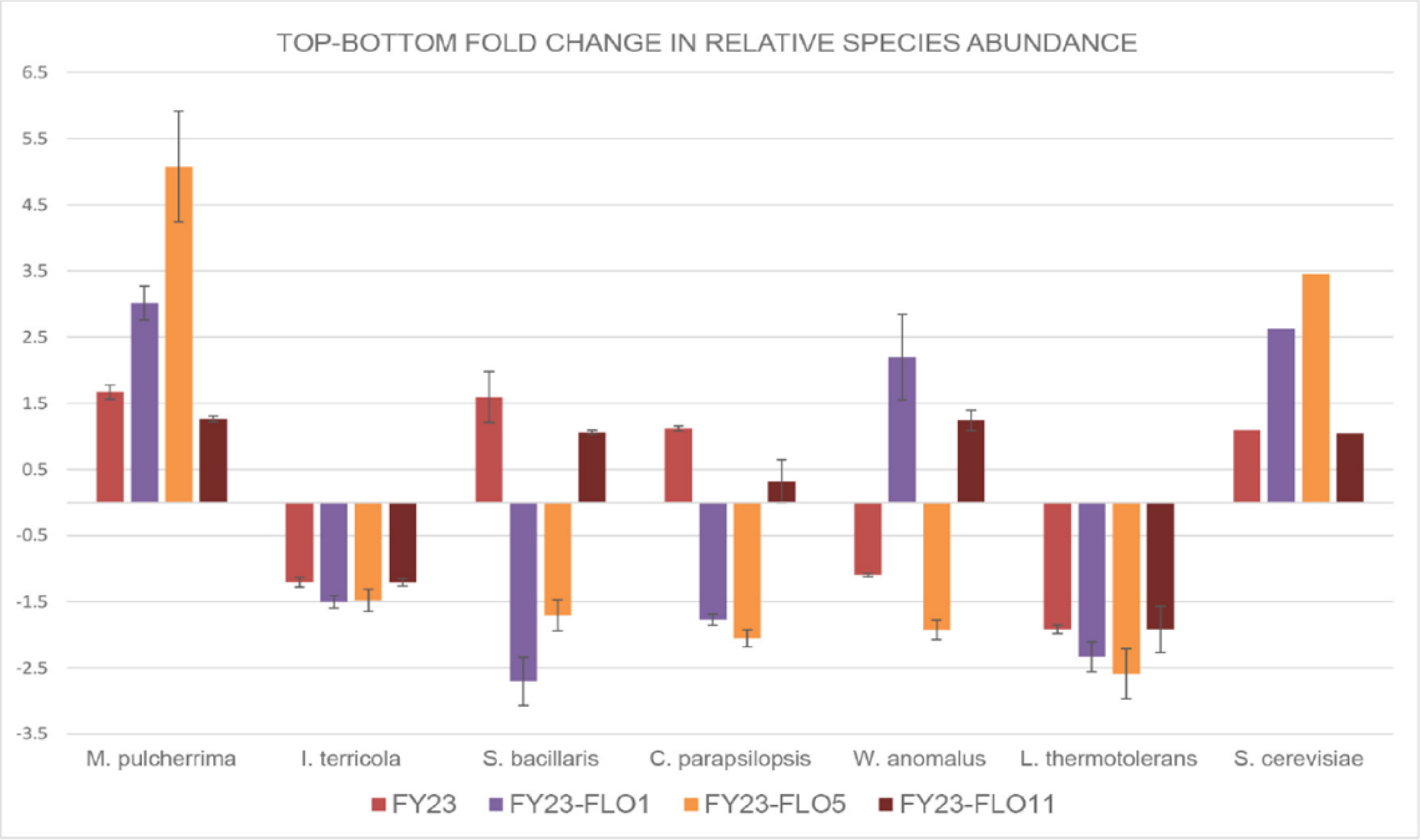


RNASeq Data Interpretation Framework

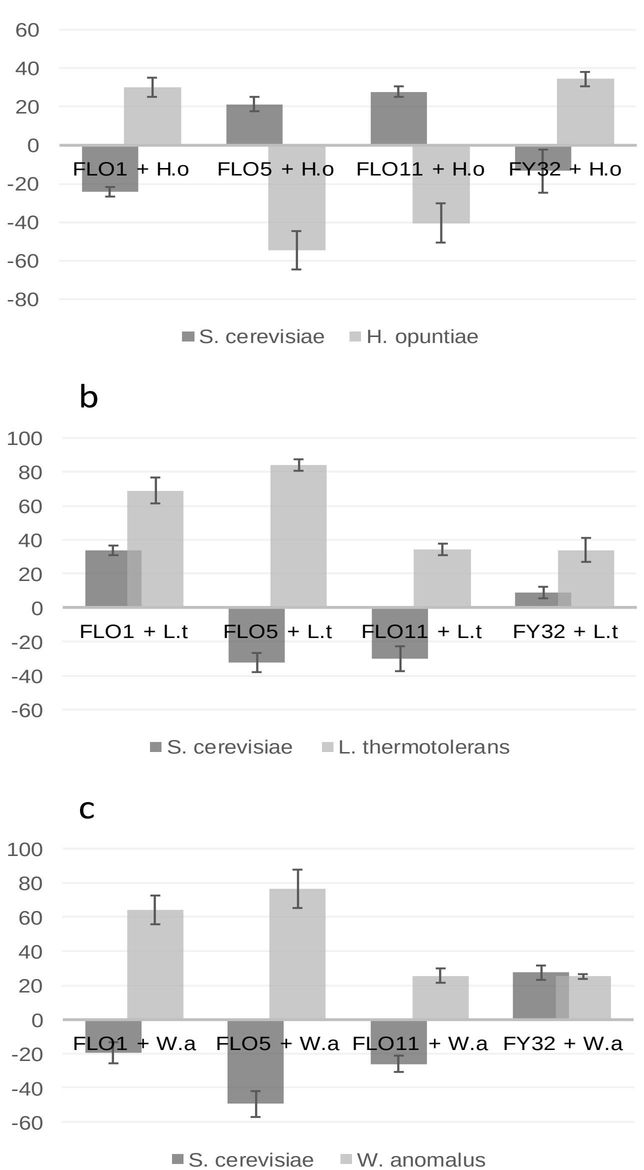


FLO Gene kontrollieren Interspezies Interaktionen

Individual *FLO* genes *FLO1*, *FLO5* and *FLO11* under the control of inducible promoters



…und beeinflussen das Ueberleben in der Gruppe



Percentage increase (or decrease) in survival after 24hr of co-culture of two species grown under flocculating conditions when compared to non-flocculating conditions.

And gene expression (RNAseq):

L.termotolerans: ↑ *FLO1* ortholog

S. cerevisiae: ↓ *FLO5*

Rossouw et al. mSphere 2018

Was bedeutet das für die Zukunft?

- Prediction tools for fermentation
  - Conditions
    - Chemical composition
    - Temp/pH/O2
  - Species composition
- Full exploitation of natural microbiota
- Rational application of oenological tools
  - Inoculation?
  - Nutrients
  - Enzymes/oenological tools
- Precision oenology?

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Zurück zur Natur – durch Forschung

Back to nature – through science

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Acknowledgements

NRF

Winetech
Wine Industry Network of Expertise and Technology
Netwerk vir Kundigheid en Tegnologie vir die Wynbedryf

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Hefetagung Klosterneuburg 2021

Einfluss von Hefen auf Unterbindung von Proteintrübungen im Wein

Florian Bauer

mit Thulile Ndlovu und Tyrone Chuene

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Proteintrübung

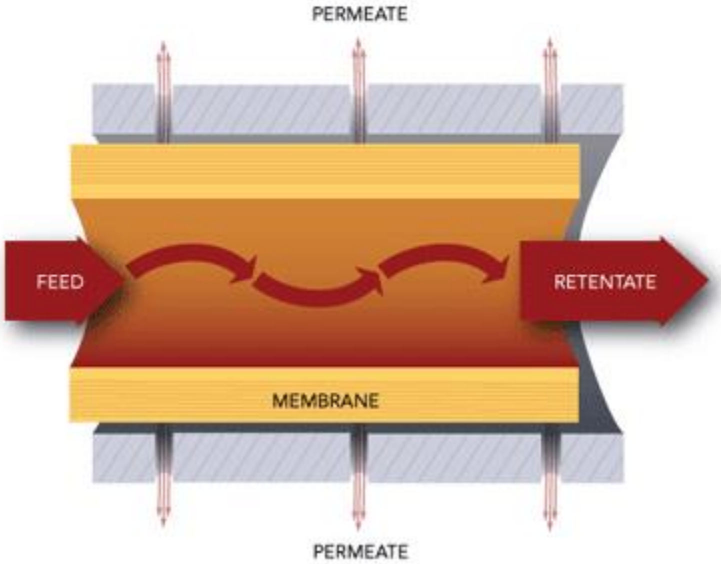
- Aggregate von Pathogenese-bezogenen Proteinen sind die Hauptursache der Trübung
  - “Thaumatin-like”
  - Chitinases

- Die Chitinasen haben den grössten Einfluss auf die Trübung

- Bentonite ist noch immer die bevorzugte Lösung, hat aber viele Nachteile

- Alternativen werden gesucht!

Clarifying agents used in industry



Und was ist mit Hefen?

Percentage decrease in haze formation relative to the control
Dead or alive

S. blanc	day 1		Chardonnay	day 1
Control	0		**Control**	0
Bent 0.5	62.80		**Bent 0.5**	69.52
Bent 1.5	94.57		**Bent 1.5**	91.63
ECIII8 dry	29.51		**ECIII8 dry**	-6.97
RO88 dry	26.13		**RO88 dry**	-18.92
ECIII8 live	30.23		**ECIII8 live**	12.94
RO88 live	50.10		**RO88 live**	19.92
ECIII8 dead	35.15		**ECIII8 dead**	-3.18
RO88 dead	33.71		**RO88 dead**	1.79

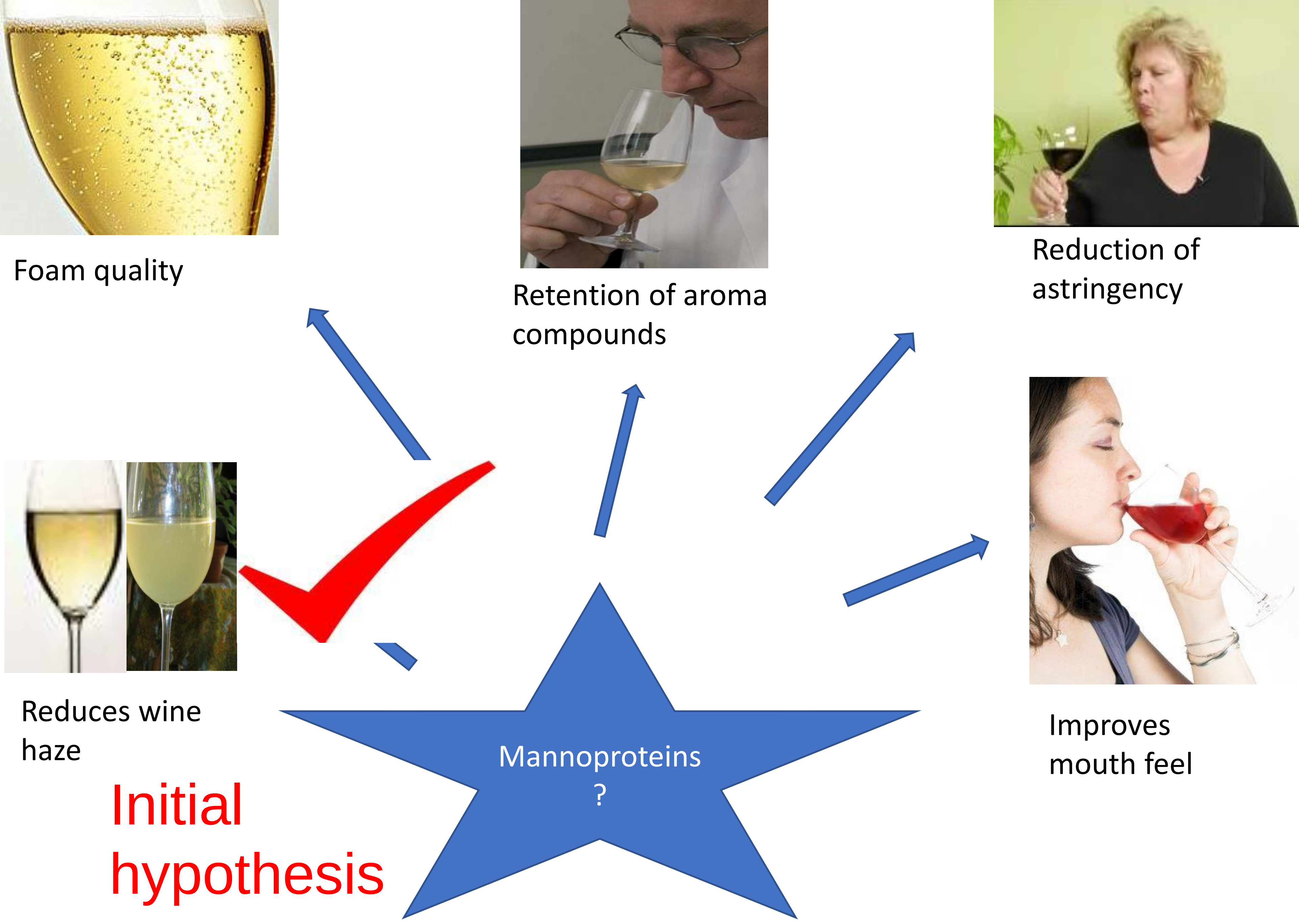
EC1118 wet live

S. Blanc - Control

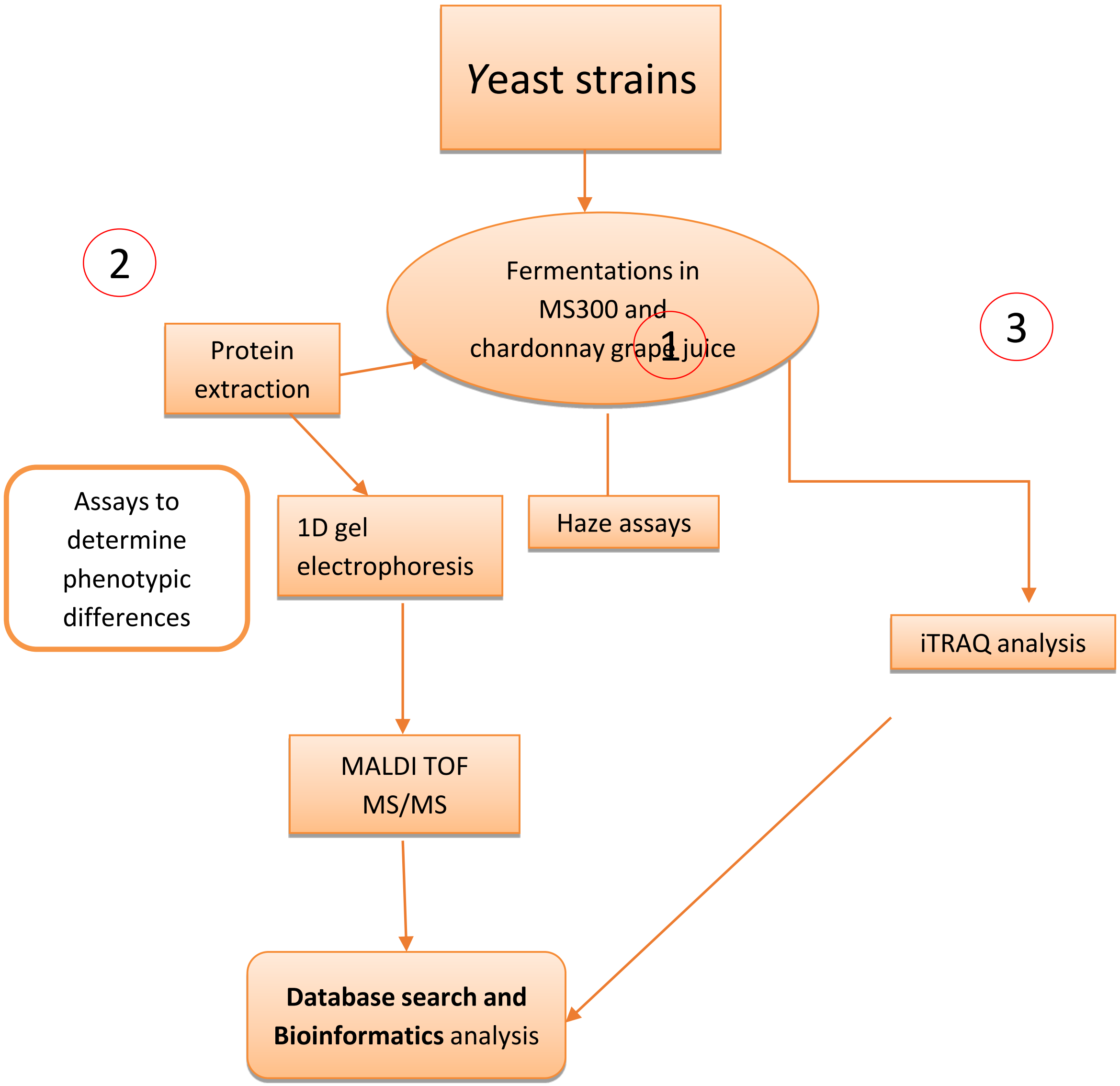
RO88 - wet live

Bentonite - 0.5g/l

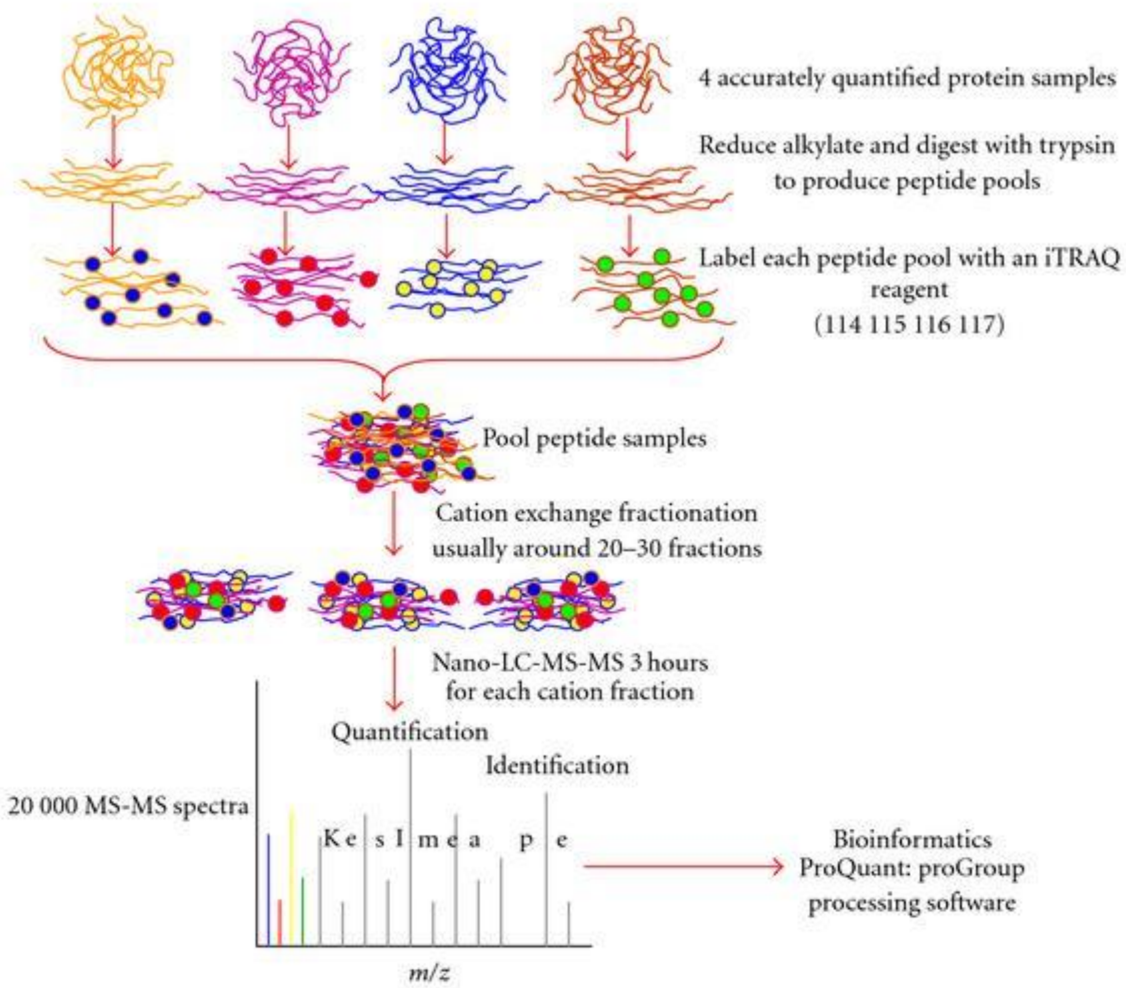
Some strains with high efficiency!


Foam quality
Retention of aroma compounds
Reduction of astringency
Reduces wine haze
Improves mouth feel
Mannoproteins ?
Initial hypothesis

Sind Unterschiede in Mannoprotein Sekretion verantworlich fuer diese Differenzen?



iTRAQ: A comparison between strains exoproteome secreted during fermentation



Secreted proteome (Secretome)

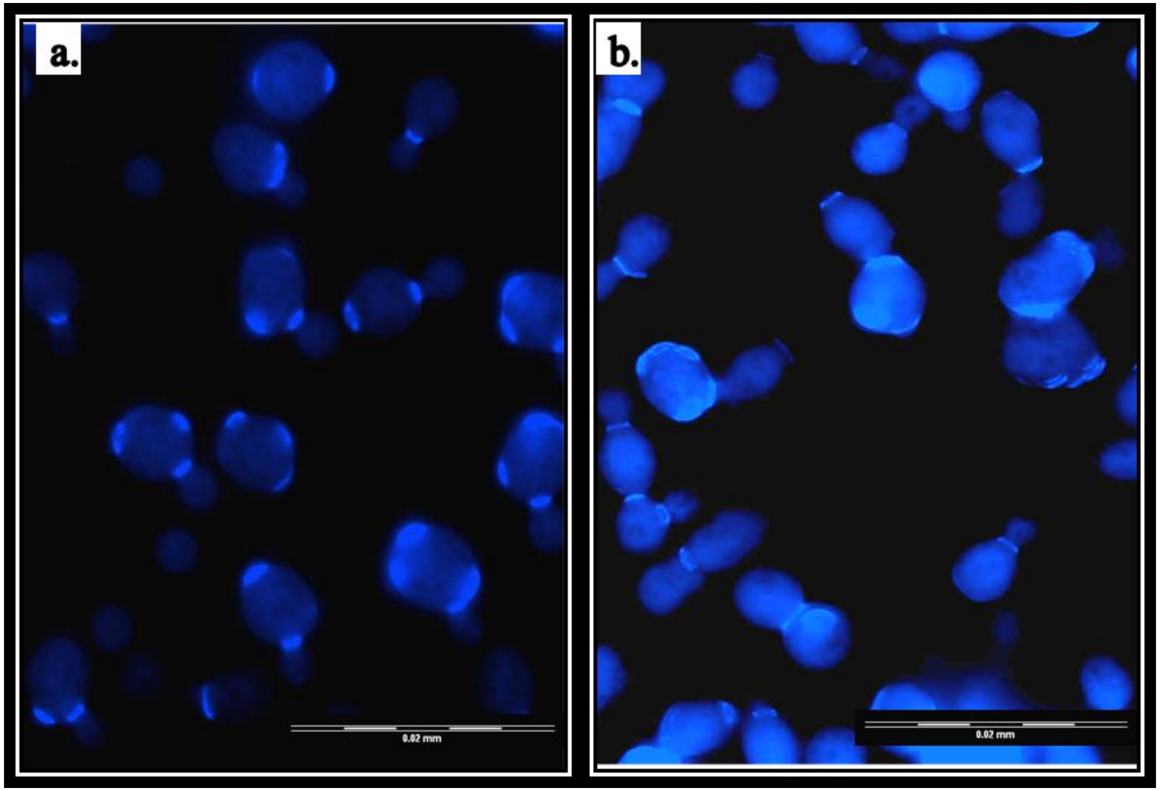
Protein name and gene name	Fold change
Daughter-specific expression-related protein, DSE4√	1.515
Phosphoglycerate mutase 1, GPM1	1.538
carboxyPEPtidase Y-deficient, PEP4√	1.587
Heat shock protein, HSP26	1.637
Glycosylphosphatidylinositol (GPI)-anchored cell wall endoglucanase, EGT2√	1.650
Glycophospholipid-anchored surface protein, GAS1√	1.675
Target of Sbf, TOS1	1.706
Phospholipase B, PLB3	1.715
Glyceraldehyde-3-phosphate dehydrogenase 3, TDH3	1.736
Exo-1,3-beta-glucanase, EXG2	1.776
Cell wall mannoprotein- Protoplasts-Secreted PST1/HPF2	1.818
60S ribosomal protein L17-B, RPL17B	1.835
Glutaredoxin-2, mitochondrial, GRX2	1.845

Protein name and gene name	Fold change
Glycolipid-anchored surface protein 3	1.965
YLR179C-LIKE PROTEIN	2.012
Similar to uniprot|P04838 Saccharomyces cerevisiae YLL039c UBI4 ubiquitin√	2.160
3-isopropylmalate dehydrogenase	2.208
Invertase-Sucrose fermentation, SUC2	2.681
EXo-1,3-beta-Glucanase, EXG1	3.247
ExtraCellular Mutant, ECM33√	3.322
ThioRedoXin, TRX1	3.344
Pathogen Related in Yeast, PRY3	3.584
Transaldolase, TAL1	4.608
ThioRedoXin, TRX2	4.739
Mitochondrial Matrix Factor, MMF1	4.878
YIL169C, HPF1'	11.111

Several proteins are produced at different levels by different strains, but overall differences are minor, and no clear trends.

Other factors?

Microscopy –
clear difference between strains with calcofluor white staining

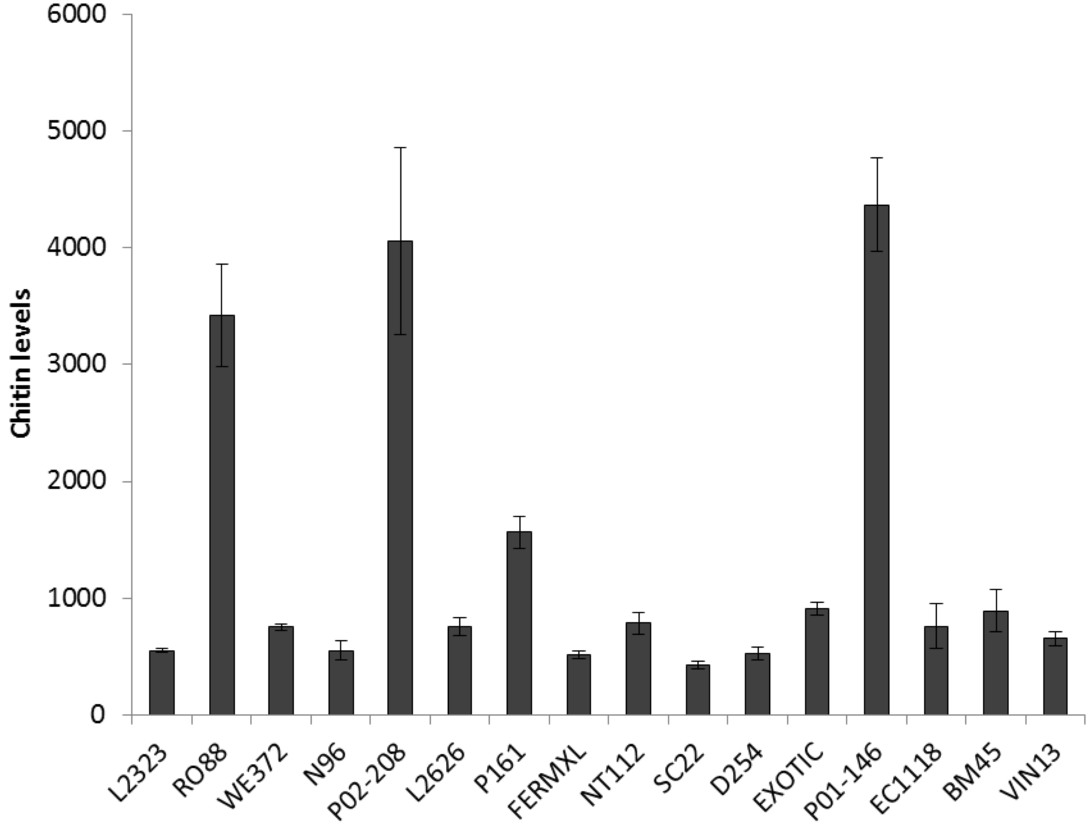


Could **cell wall chitin** be responsible for differences in haze protection?

Ndlovu et al. 2018. AEM

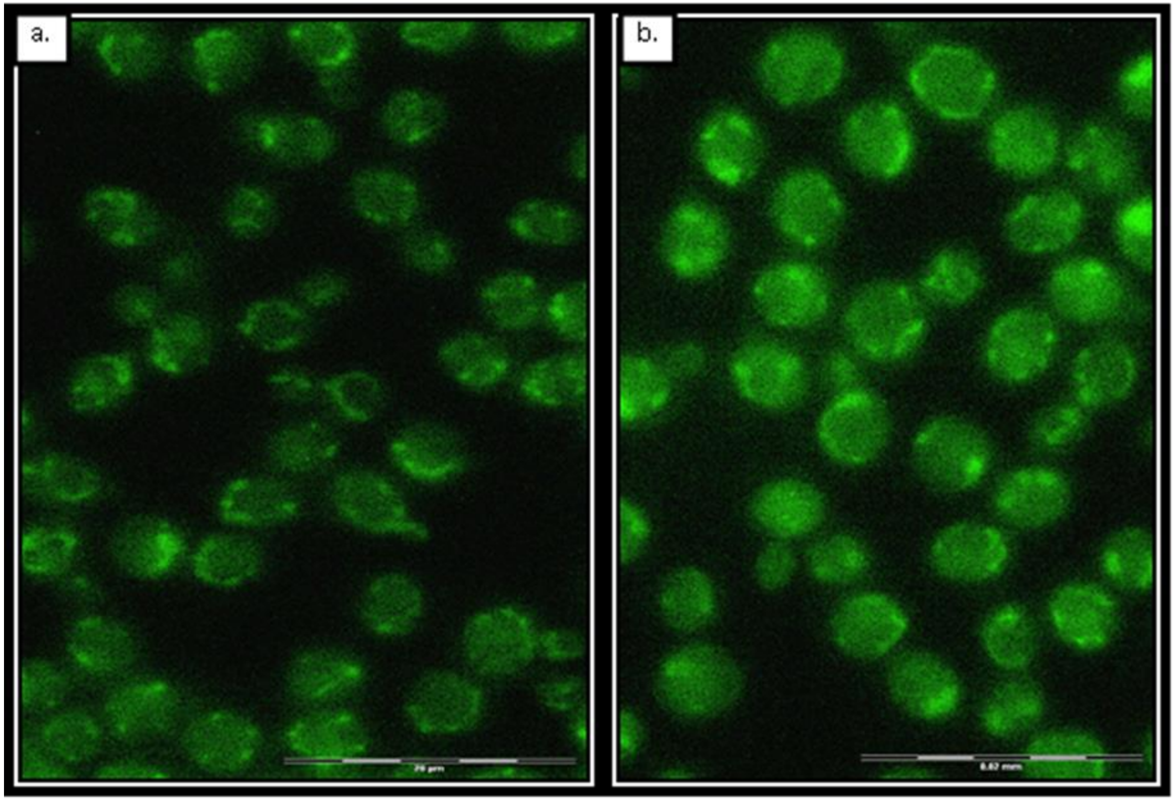
Quantification of chitin

Cell wall chitin of different yeast strains

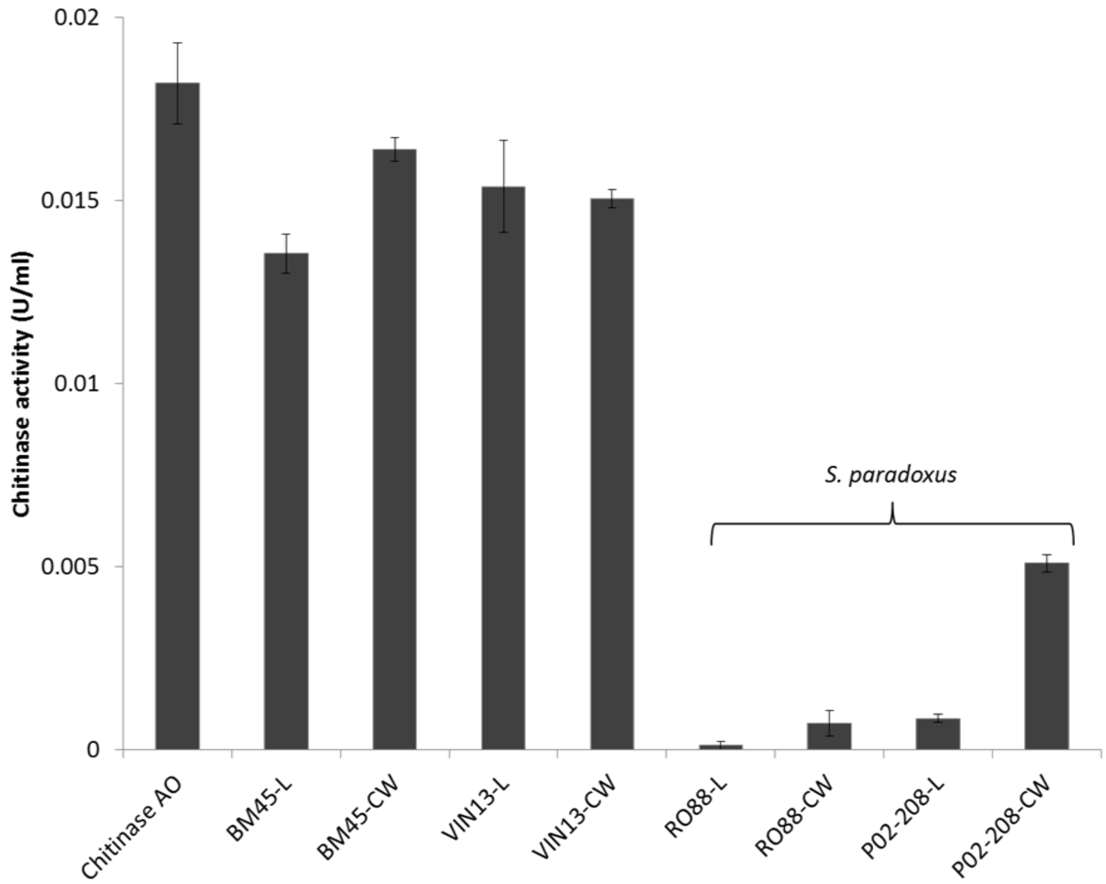


Do grape chitinases bind to yeast cell walls?

GFP-grape chitinase fusion

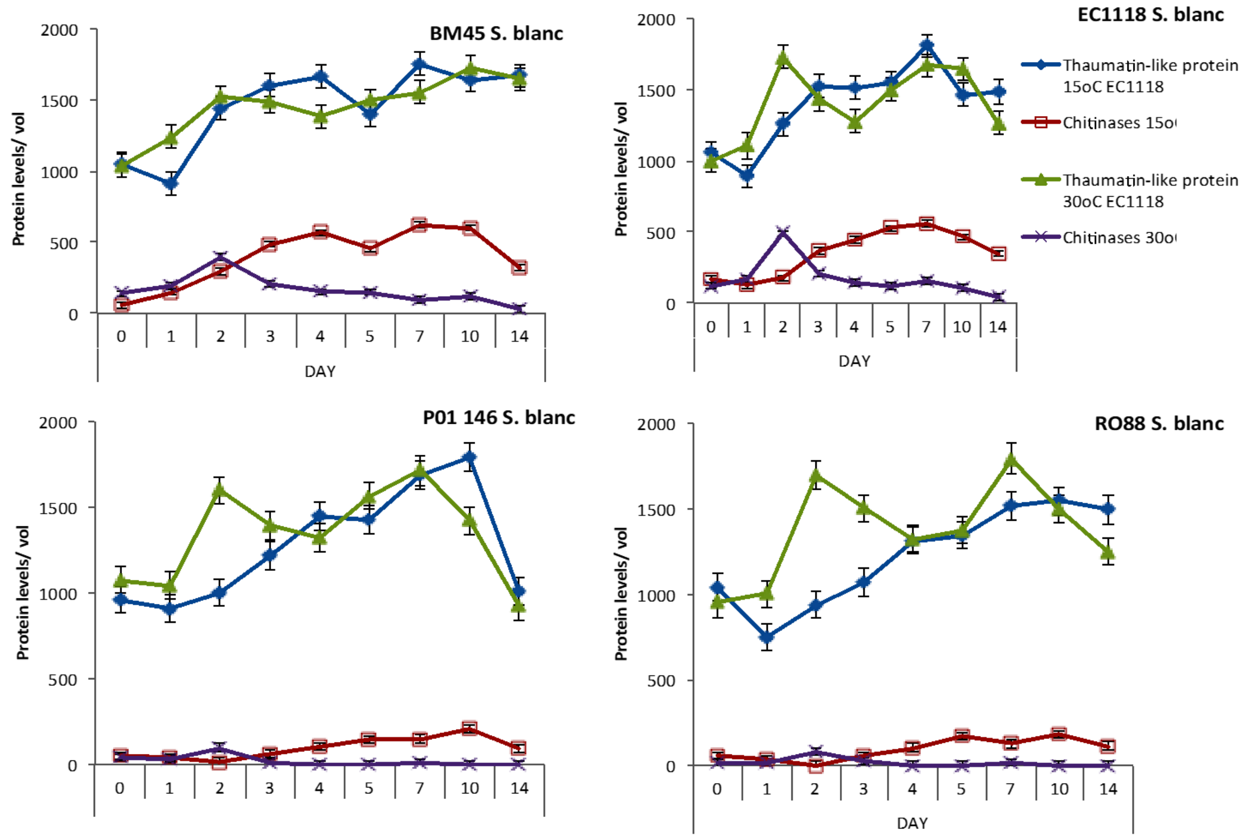


More evidence:
Incubate purified grape chitinase in the presence of different yeast strains



And more evidence
Chitinases and thaumatin-like proteins during fermentation

a.



Ndlovu et al. 2019

Conclusion

- Clear correlation between haze protection and chitin levels of yeast strains
- Chitin levels have significantly higher impact on haze reduction than mannoproteins
- Grape chitinases bind yeast cell walls
  - Lateral chitin

- Can we produce yeast with higher chitin levels?
  - Either as fermenting strain or as a yeast-additive

Vielen Dank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