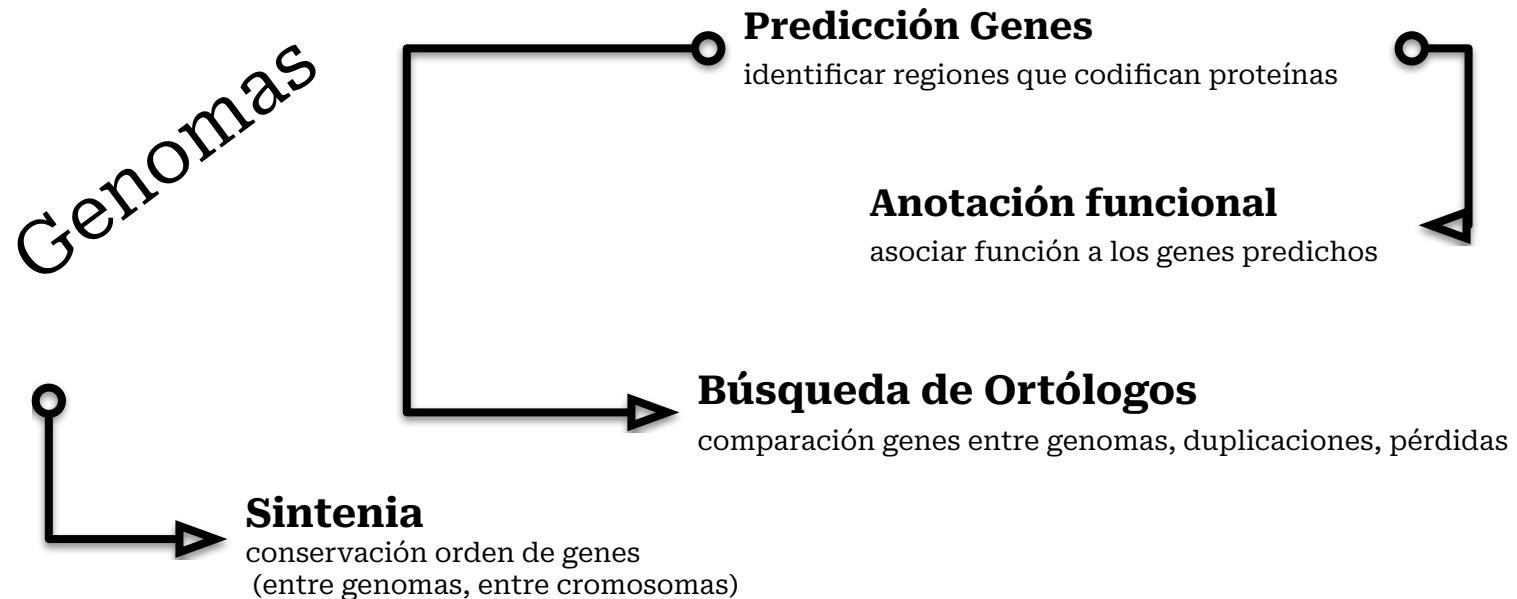


# **Curso Evolución**

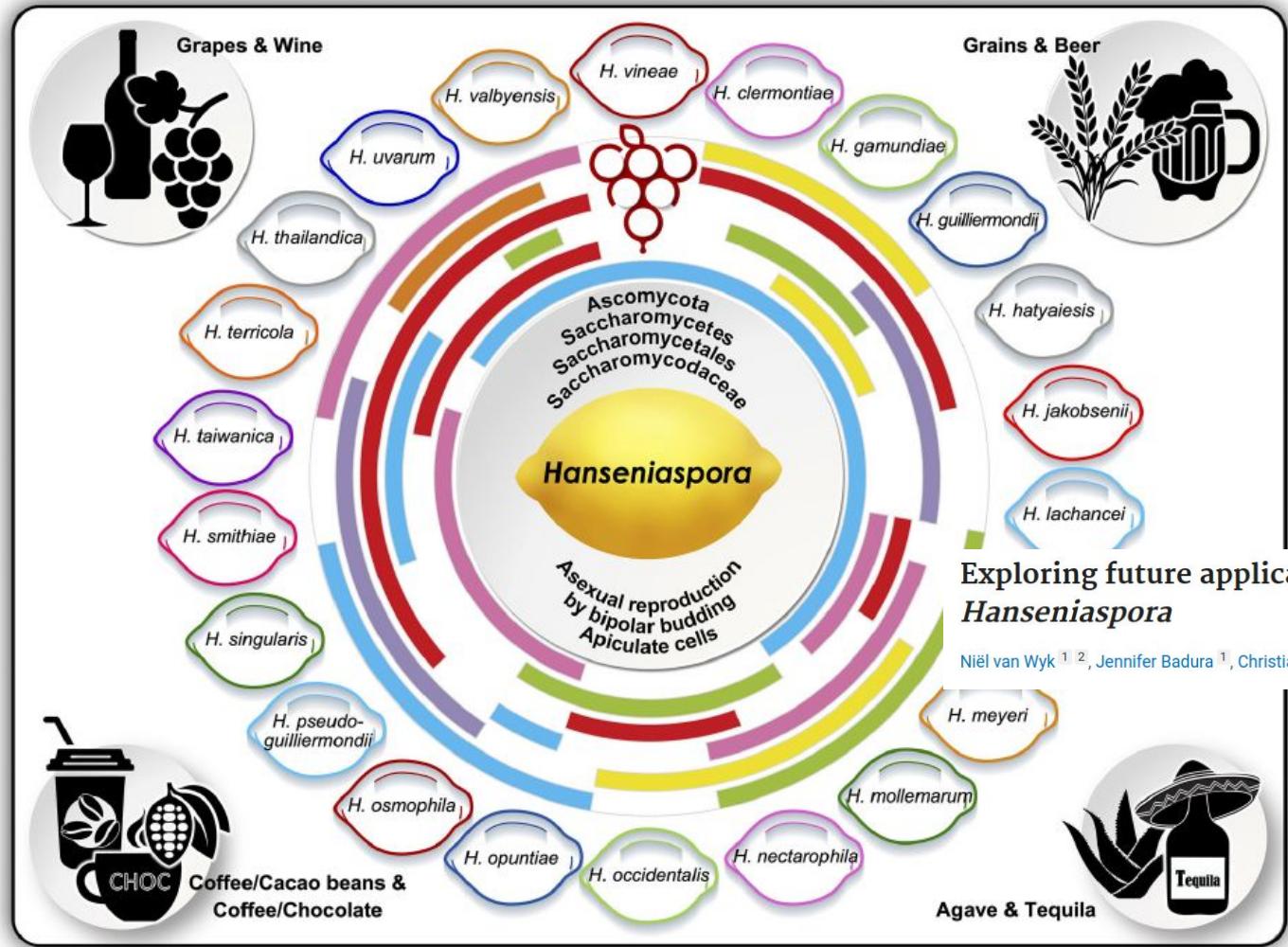
## **Práctico 10 Genómica**



# Diagrama de pipeline a seguir...(o que queremos)







## Exploring future applications of the apiculate yeast *Hanseniaspora*

Niël van Wyk <sup>1</sup> <sup>2</sup>, Jennifer Badura <sup>1</sup>, Christian von Wallbrunn <sup>1</sup>, Isak S Pretorius <sup>2</sup>

***Hanseniaspora uvarum*****Increase**

- Terpenes, C<sub>13</sub>-norisoprenoids, ethyl esters in Cabernet Sauvignon
- Linalool, hotrienol, and terpineol in Muscat
- Ethyl acetate in Pinot Grigio, Verdicchio, and Pinotage
- Polyphenolic and volatile compounds in Aglianico
- Acetate esters in Macabeo
- Isoamyl acetate in an unspecified grape must
- 2-phenethyl acetate in Trebbiano

**Decrease**

- Amyl alcohols in Trebbiano
- Volatile acidity in Negromaro
- Ethanol in Malbec, Pinotage, and Sauvignon Blanc
- Higher alcohols, ethyl esters, and isoamyl acetate in unspecified grape must

***Hanseniaspora osmophilic*****Increase**

- 2-phenylethyl acetate in Muscat and Bobal
- Isoamyl acetate and isoamyl alcohol in Verdicchio

Flavour  
contribution of  
*Hanseniaspora*

***Hanseniaspora vineae*****Increase**

- Linalool, hotrienol and terpineol in Muscat
- Anthocyanins, terpenes, and 2-phenylethyl acetate in Albilllo and Tempranillo
- 2-phenylethyl acetate in Macabeo
- Acetate esters, cis-rose oxide, β-damascenone, and phenylacetaldehyde in Vidal Blanc icewine
- 2-phenethyl acetate, monoterpane, and ethyl acetate in Pinotage and Sauvignon Blanc
- Glycerol, acetate ester and ethyl ester in Chardonnay

**Decrease**

- Ethanol in Pinotage and Sauvignon Blanc
- Alcohols and fatty acids in Chardonnay

***Hanseniaspora guilliermondii*****Increase**

- 2-phenethyl acetate in Bobal
- 2-phenethyl acetate in an unspecified grape must
- 2-phenethyl acetate and in Tinta Roriz
- 2-phenethyl acetate in Trebbiano

**Decrease**

- Amyl alcohols in Trebbiano
- Higher alcohols in an unspecified grape must
- Free fatty acids in Tinta Roriz

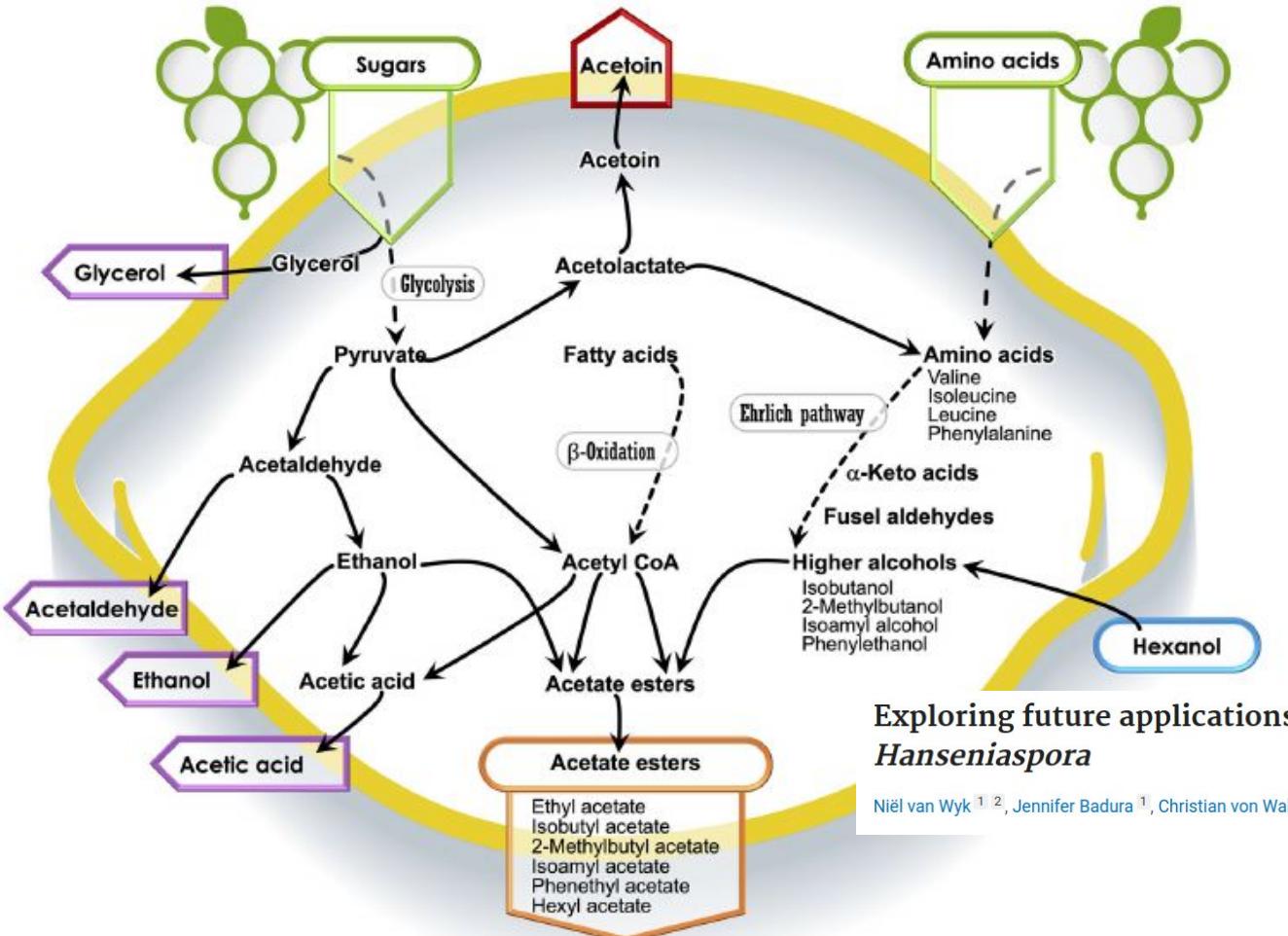
***Hanseniaspora opuntiae*****Increase**

- 2-phenylethanol, 3-methyl-butanol, and phenylacetaldehyde in Cabernet Sauvignon
- Increase in ethyl acetate and citronellol in Pinotage and Sauvignon Blanc

**Decrease**

- Ethanol in Pinotage and Sauvignon Blanc

Niël van Wyk <sup>1,2</sup>, Jennifer Badura <sup>1</sup>, Christian von Wallbrunn <sup>1</sup>, Isak S Pretorius <sup>2</sup>

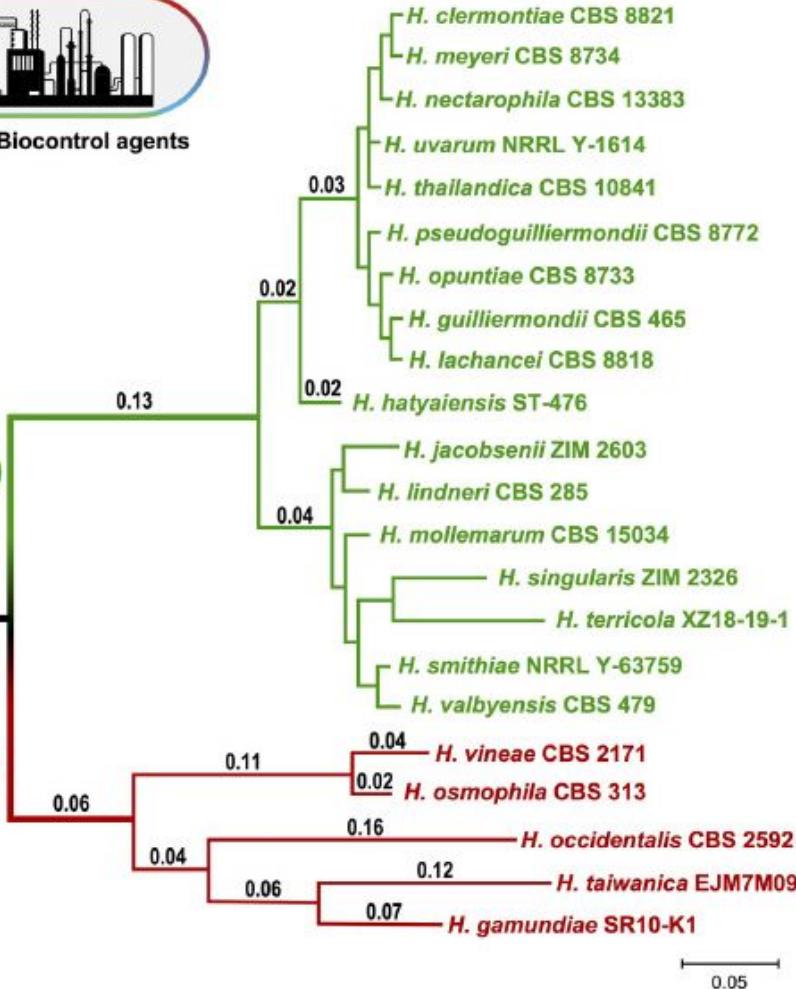
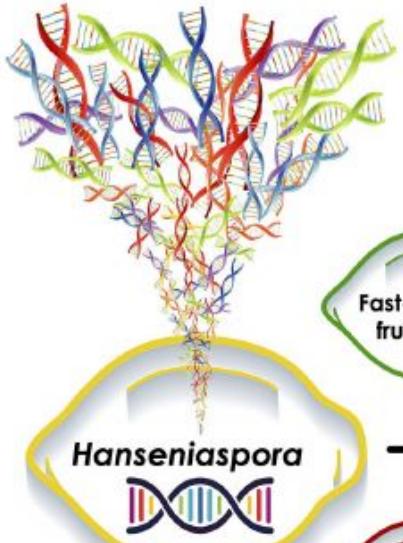


Exploring future applications of the apiculate yeast  
*Hanseniaspora*

Niel van Wyk <sup>1, 2</sup>, Jennifer Badura <sup>1</sup>, Christian von Wallbrunn <sup>1</sup>, Isak S Pretorius <sup>2</sup>



Fermentation agents Whole-cell biocatalysts Biocontrol agents





Applied and Environmental  
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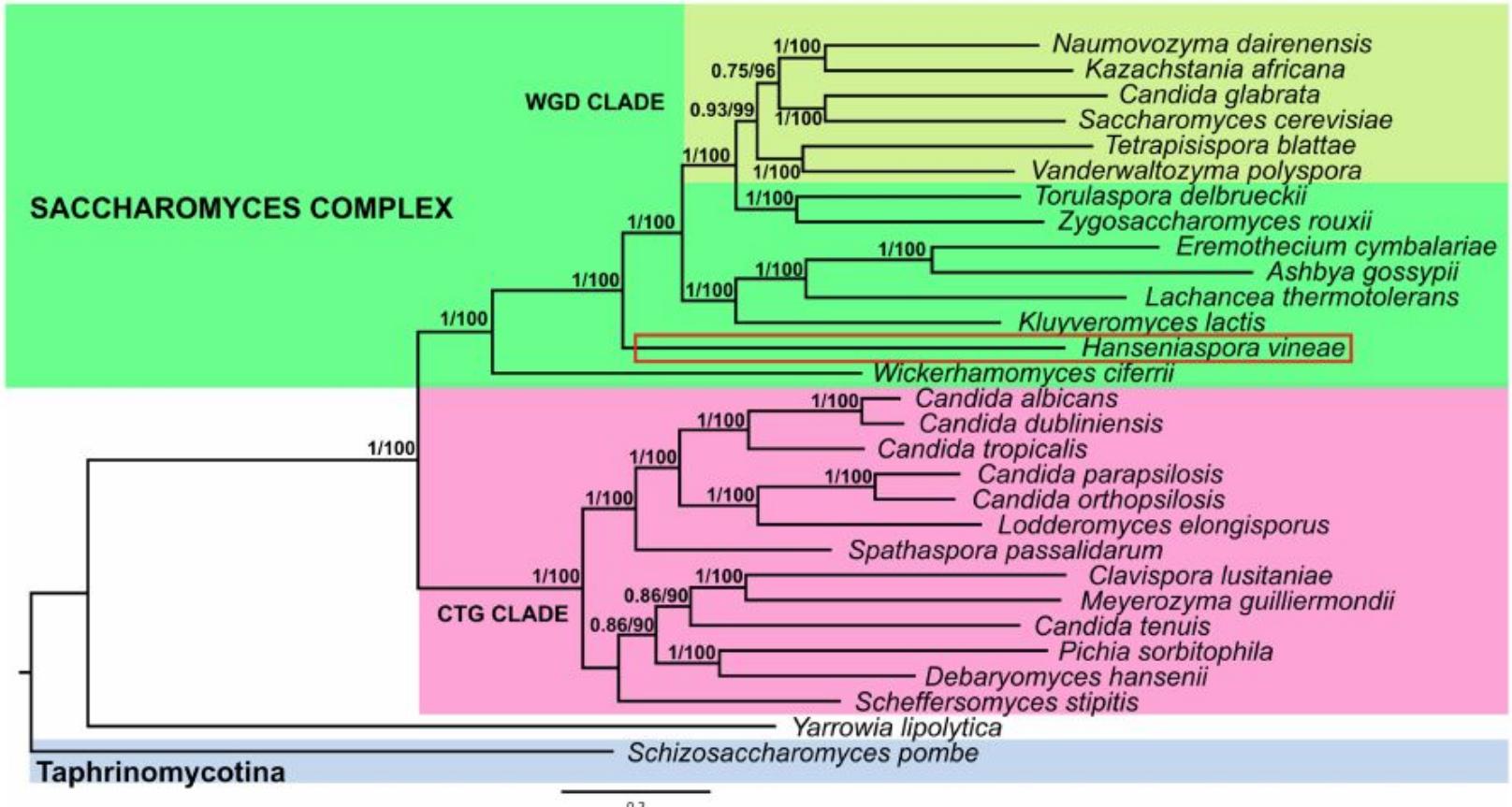
GENETICS AND MOLECULAR BIOLOGY



# Genomic and Transcriptomic Basis of *Hanseniaspora vineae*'s Impact on Flavor Diversity and Wine Quality

Facundo Giorello,<sup>a,b</sup> María Jose Valera,<sup>b</sup> Valentina Martín,<sup>b</sup> Andrés Parada,<sup>c</sup> Valentina Salzman,<sup>d</sup> Laura Camesasca,<sup>e</sup> Laura Fariña,<sup>b</sup> Eduardo Boido,<sup>b</sup> Karina Medina,<sup>b</sup> Eduardo Dellacassa,<sup>f</sup> Luisa Berna,<sup>d</sup> Pablo S. Aguilar,<sup>d,g</sup> Albert Mas,<sup>h</sup> Carina Gaggero,<sup>e</sup> Francisco Carrau<sup>b</sup>

**ABSTRACT** *Hanseniaspora* is the main genus of the apiculate yeast group that represents approximately 70% of the grape-associated microflora. *Hanseniaspora vineae* is emerging as a promising species for quality wine production compared to other non-*Saccharomyces* species. Wines produced by *H. vineae* with *Saccharomyces cerevisiae* consistently exhibit more intense fruity flavors and complexity than wines produced by *S. cerevisiae* alone. In this work, genome sequencing, assembling, and phylogenetic analysis of two strains of *H. vineae* showed that it is a member of the *Saccharomyces* complex and it diverged before the whole-genome duplication (WGD) event from this clade. Specific flavor gene duplications and absences were identified in the *H. vineae* genome compared to 14 fully sequenced industrial *S. cerevisiae* genomes. The increased formation of 2-phenylethyl acetate and phenyl-propanoids such as 2-phenylethyl and benzyl alcohols might be explained by gene duplications of *H. vineae* aromatic amino acid aminotransferases (AR08 and AR09) and phenylpyruvate decarboxylases (AR010). Transcriptome and aroma profiles under fermentation conditions confirmed these genes were highly expressed at the beginning of stationary phase coupled to the production of their related compounds. The extremely high level of acetate esters produced by *H. vineae* compared to that by *S. cerevisiae* is consistent with the identification of six novel proteins with alcohol acetyltransferase (AATase) domains. The absence of the branched-chain amino acid transaminases (BAT2) and acyl coenzyme A (acyl-CoA)/ethanol O-acyltransferases (EEB1) genes correlates with *H. vineae*'s reduced production of branched-chain higher alcohols, fatty acids, and ethyl esters, respectively. Our study provides sustenance for understanding and potentially utilizing genes that determine fermentation aromas.



Posición filogenética de *H. vineae* (Giorello et al. 2019)

**TABLE 1** Yeast strains analyzed in this work

Species	Strain	Ploidy	Source	BioSample ID from NCBI database <sup>a</sup>	Use
<i>H. vineae</i>	T02/19AF	Haploid	Uruguayan Tannat grape vines	<a href="#">SAMN02644989</a>	Genomic transcriptomic and phenomic study
<i>H. vineae</i>	T02/05AF	Haploid	Uruguayan Tannat grape vines	<a href="#">SAMN04487210</a>	Genomic study
<i>S. cerevisiae</i>	BY4742	Haploid	Laboratory strain, derived from S288c	<a href="#">SAMN03020230</a>	FCM analysis
<i>S. cerevisiae</i>	BY4743	Diploid	Laboratory strain, derived from S288c	<a href="#">SAMN01822968</a>	FCM analysis
<i>S. cerevisiae</i>	Montrachet 522	Diploid	Fortified wines, CA	<a href="#">SAMN03325349</a>	Flavor compound analysis
<i>S. cerevisiae</i>	S288c	Haploid	Laboratory strain, CA	<a href="#">SAMD00065885</a>	Genomic comparison
<i>S. cerevisiae</i>	AWRI1631	Haploid	Australian derivative of South African commercial wine strain N96	<a href="#">SAMN02953734</a>	Genomic comparison
<i>S. cerevisiae</i>	AWRI796	Diploid	South African wine strain	<a href="#">SAMN04286136</a>	Genomic comparison
<i>S. cerevisiae</i>	BC187	Diploid	Derivative of CA wine barrel isolate	<a href="#">SAMEA687137</a>	Genomic comparison
<i>S. cerevisiae</i>	DBVPG6044	Diploid	West African isolate	<a href="#">SAMEA687132</a>	Genomic comparison
<i>S. cerevisiae</i>	EC1118	Diploid	Commercial wine strain	<a href="#">SAMEA2272624</a>	Genomic comparison
<i>S. cerevisiae</i>	L1528	Diploid	Chilean wine strain	<a href="#">SAMN03020223</a>	Genomic comparison
<i>S. cerevisiae</i>	LalvinQA23	Diploid	Portuguese Vinho Verde white wine strain	<a href="#">SAMN02981266</a>	Genomic comparison
<i>S. cerevisiae</i>	M22	Diploid	Italian vineyard isolate	<a href="#">SAMN00189351</a>	Genomic comparison
<i>S. cerevisiae</i>	PW5	Diploid	Nigerian Raphia palm wine isolate	<a href="#">SAMN00199004</a>	Genomic comparison
<i>S. cerevisiae</i>	RM11-1A	Haploid	Natural isolate collected from a vineyard, CA		
<i>S. cerevisiae</i>	T73	Near-diploid	Spanish red wine strain		
<i>S. cerevisiae</i>	Vin13	Diploid	South African white wine strain		
<i>S. cerevisiae</i>	VL3	Diploid	French white wine strain		
<i>S. cerevisiae</i>	YJM269	Diploid	Austrian wine from Blauer Portugieser grapes isolate		

<sup>a</sup>ID, identifier; NCBI, National Center of Biotechnology Information.



# Genomas de levaduras

# Genes asociados a fermentación y sabor

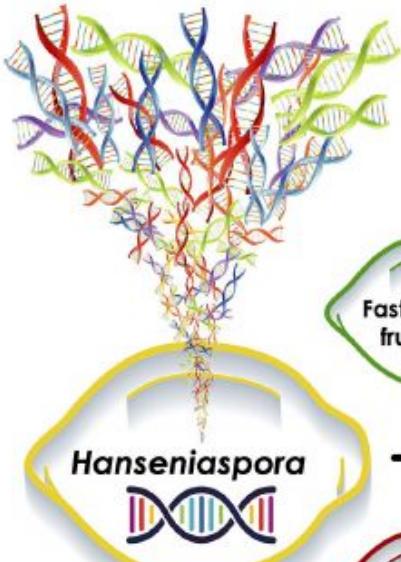
**TABLE 3** Comparison of genes involved in biosynthesis routes for key flavor compound production in *S. cerevisiae* and *H. vineae*

Biosynthesis route	Enzymatic activity	Genes identified (% amino acid identity with <i>S. cerevisiae</i> homologous protein) <sup>a</sup>
Higher alcohols	Aromatic amino acid transferases	3×ARO8 (45.51, 59.84, 56.06), 4×ARO9 (42.70, 35.27, 36.08, 36.91)
	Branched-chain amino acid transferases	<u>BAT1</u> (78.84), <u>BAT2</u>
	Decarboxylase	2×ARO10 (34.10, 30.99), 2×PDC1 (80.46, 50.66), <u>PDC5</u> , <u>PDC6</u> , <u>THI3</u>
	Alcohol dehydrogenase	2×ADH1 (77.71, 78.74), <u>ADH2</u> , 2×ADH3 (74.79, 74.80), <u>ADH4</u> , <u>ADH5</u> , 4×ADH6 (44.74, 44.47, 44.74, 44.06), <u>ADH7</u> , <u>SFA1</u> (68.16), 4×GRE2 (44.74, 50.73, 47.51, 43.02), <u>YPR1</u> , <u>PAD1</u> , <u>SPE1</u> , 3×OYE2 (55.10, 58.06, 57.25), <u>HOM2</u> (78.24)
Acetate esters	Aryl alcohol dehydrogenase	<u>AAD3</u> , <u>AAD4</u> , <u>AAD6</u> , <u>AAD10</u> , <u>AAD14</u> , <u>AAD15</u> , <u>AAD16</u>
	Regulation	<u>ARO80</u> (34.80), <u>GAT2</u> , <u>GLN3</u> , <u>GZF3</u> , <u>DAL80</u>
Ethyl esters	Alcohol acetyl transferases	<u>ATF1</u> , <u>ATF2</u> (26.58), 4×SLI1 (22–24), g4599.t1
	Ethanol O-acyltransferase and esterase	<u>EEB1</u> , <u>EHT1</u> (51.35), <u>MGL2</u> (30.06), <u>AAD</u> , <u>IAH1</u> (54.67)
Volatile organic acids	Aldehyde dehydrogenase	2×ALD2 (40.55, 44.01), <u>ALD3</u> , <u>ALD4</u> , <u>ALD5</u> (53.45), <u>ALD6</u> (55.07)
	Synthesis of chorismate, phenylalanine, tryptophan, and tyrosine	<u>ARO1</u> (66.79), <u>ARO2</u> (80.59), <u>ARO3</u> (77.03), <u>ARO4</u> (83.51), <u>TRP2</u> (70.84), <u>TRP3</u> (69.14), <u>ARO7</u> (67.97), <u>PHA2</u> (41.99), <u>TYR1</u> (62.37)
Benzyl alcohol/benzaldehyde synthesis	Mandelate pathway	2×ARO10 (34.10, 30.99), 2×PDC1 (80.46, 50.66), <u>SCS7</u> (66.50), <u>ALD6</u> (55.07), 2×ALD2 (40.55, 44.01), <u>DLD1</u> (53.00), <u>DLD2</u> (70.00), <u>DLD3</u>

<sup>a</sup>Homologous genes in *H. vineae* are not underlined, and the copy numbers (e.g., 2×) are indicated as prefixes for repeated genes. Predicted amino acid sequences from the genome of *H. vineae* were compared with protein homologs found in *S. cerevisiae*. Underlined genes represent absent homologous genes in *H. vineae*.



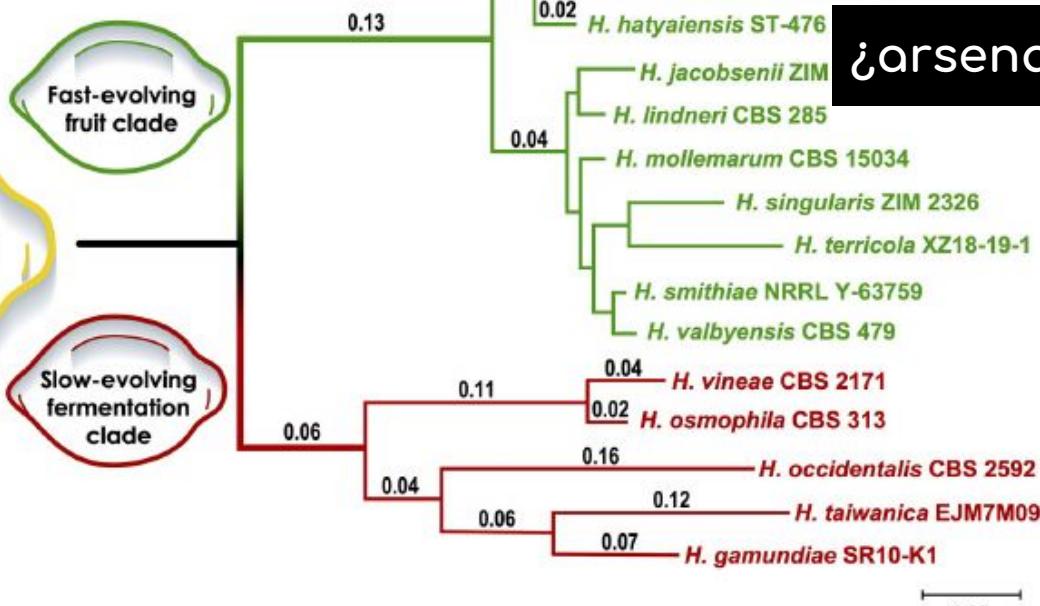
Fermentation agents Whole-cell biocatalysts Biocontrol agents



Fast-evolving  
fruit clade

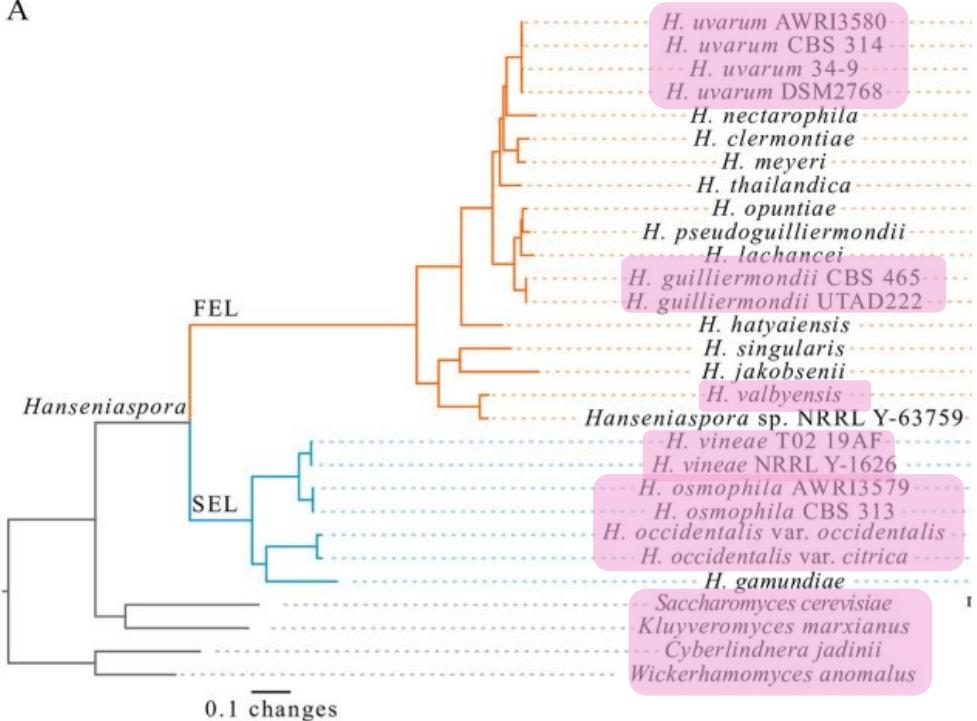
*Hanseniaspora*

Slow-evolving  
fermentation  
clade



¿arsenal difiere?

A

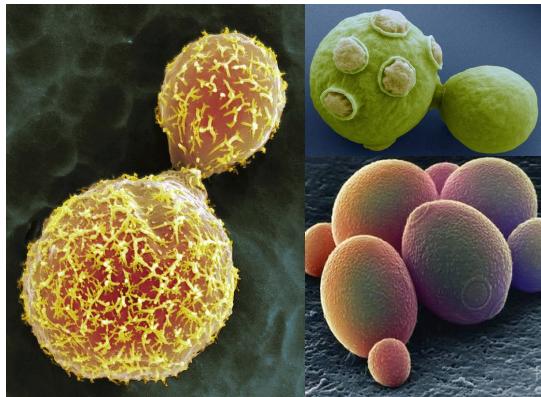


## Spp a incluir hoy

- Hanseniaspora uvarum*
- Hanseniaspora guilliermondii*
- Hanseniaspora valbyensis*
- Hanseniaspora vineae*
- Hanseniaspora occidentalis*
- Hanseniaspora osmophila*
- Cyberlindnera jadinii*
- Kazachstania servazzii*
- Saccharomyces cerevisiae*
- Wickerhamomyces anomalus*

# Diagrama de pipeline a seguir...(o que queremos)

## Genomas



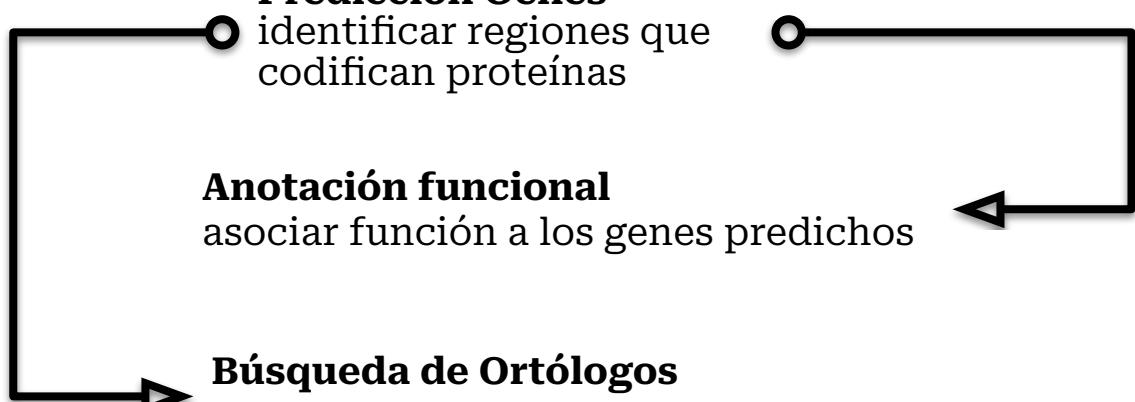
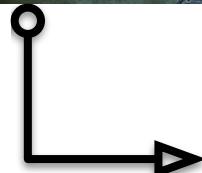
- **Predicción Genes**  
identificar regiones que codifican proteínas

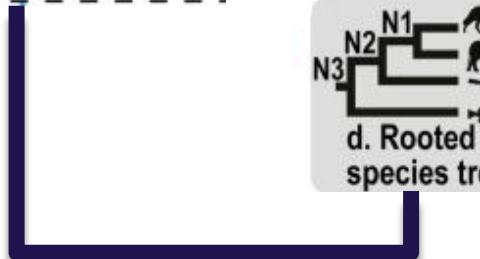
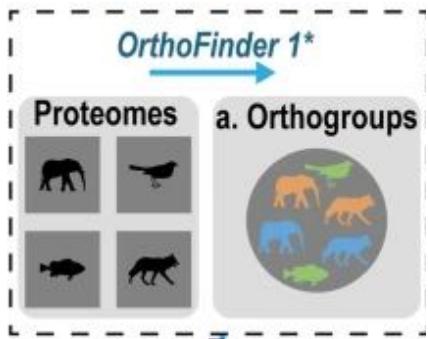
- **Anotación funcional**  
asociar función a los genes predichos

- **Búsqueda de Ortólogos**  
comparación genes entre genomas

## Sintenia

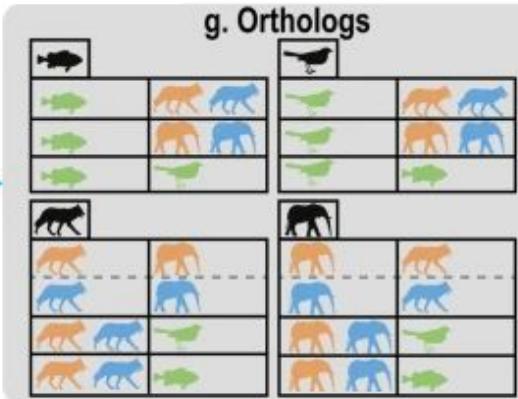
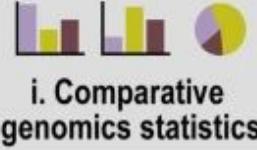
conservación orden de genes  
(entre genomas, entre cromosomas)





alineamientos

¿duplicación?  
¿pérdida?

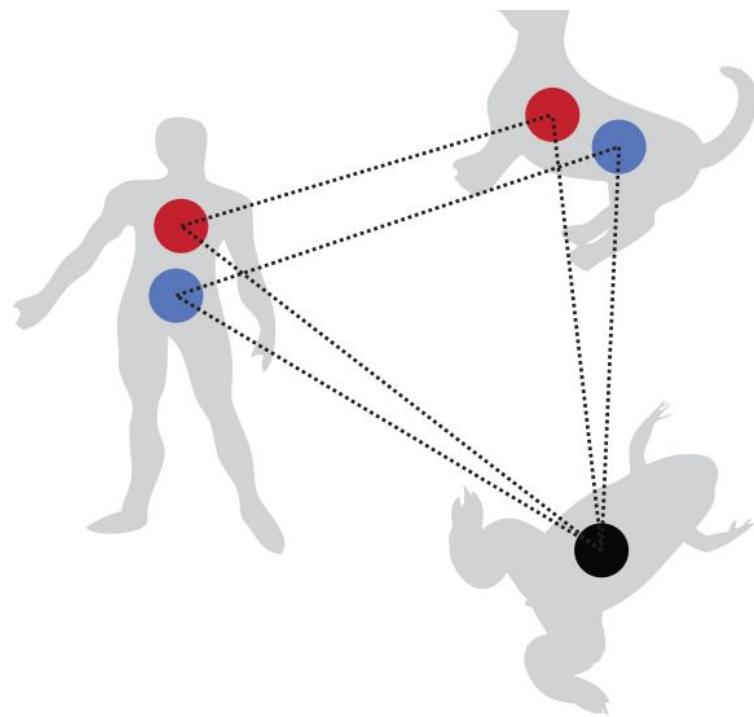
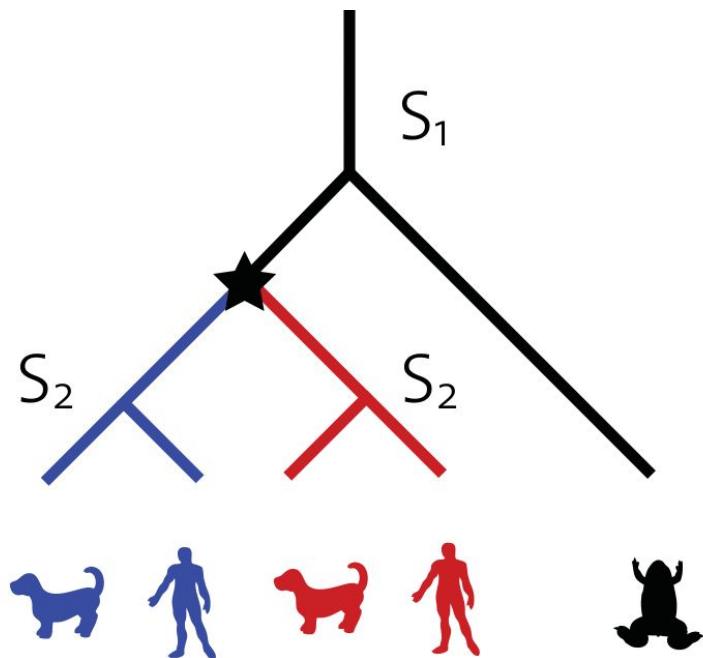


h. Gene Duplication Events

N1	n2	100%	orange	orange	blue
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variación en número copias

# para qué sirve reconocer ortólogos ahora



<https://omabrowser.org/oma/type/>

# categorías COG, funcionales

## CELLULAR PROCESSES AND SIGNALING

- [D] Cell cycle control, cell division, chromosome partitioning
- [M] Cell wall/membrane/envelope biogenesis
- [N] Cell motility
- [O] Post-translational modification, protein turnover, and chaperone-mediated protein folding
- [T] Signal transduction mechanisms
- [U] Intracellular trafficking, secretion, and vesicular transport
- [V] Defense mechanisms
- [W] Extracellular structures
- [Y] Nuclear structure
- [Z] Cytoskeleton

## INFORMATION STORAGE AND PROCESSING

- [A] RNA processing and modification
- [B] Chromatin structure and dynamics
- [J] Translation, ribosomal structure and biogenesis
- [K] Transcription
- [L] Replication, recombination and repair

## METABOLISM

- [C] Energy production and conversion
- [E] Amino acid transport and metabolism
- [F] Nucleotide transport and metabolism
- [G] Carbohydrate transport and metabolism
- [H] Coenzyme transport and metabolism
- [I] Lipid transport and metabolism
- [P] Inorganic ion transport and metabolism
- [Q] Secondary metabolites biosynthesis, transport, and catabolism

## POORLY CHARACTERIZED

- [R] General function prediction only
- [S] Function unknown

[https://ecoliwiki.org/colipedia/index.php/Clusters\\_of\\_Orthologous\\_Groups\\_\(COGs\)](https://ecoliwiki.org/colipedia/index.php/Clusters_of_Orthologous_Groups_(COGs))

<http://clovr.org/docs/clusters-of-orthologous-groups-cogs/>

The End