

Indigenous yeast biodiversity revealed by bioprospecting methods in South West Switzerland

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INTRODUCTION & AIM

Yeasts colonize various natural and anthropogenic habitats, notably vineyards and wineries. There is a growing interest in indigenous yeast starters, reflective of the local yeast biodiversity and potentially adapted to cultivated grape varieties. **Native yeasts** participate to the microorganism's diversity of distinct niches in the vineyards and wineries and could be linked to the notion of “terroir”. They could be a unique microbial signature of the “**terroir**” and therefore an invaluable resource for winemakers. The present **bioprospection** reported here covered **8 vineyards and their associated wineries** across 4 Swiss cantons (Geneve, Vaud, Neuchâtel, and Valais) in South-Western Switzerland.

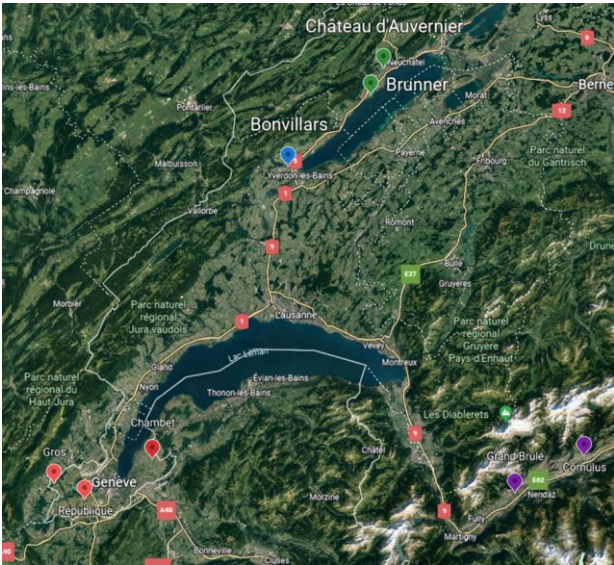


Figure 1: Sampled vineyards and wineries
Geneva (red)
Vaud (blue)
Neuchâtel (green)
Valais (violet)

METHOD

Collection of samples

Saccharomyces yeasts were collected from winery equipment and surfaces using sterile swabs, with five samples per area. In the vineyard, non-*Saccharomyces* yeasts were sampled from vine bark, flowers, insects, and berries, with five samples per type in each parcel.



Figure 2: Samples types (left to right)
Vine bark
Flowers
Insects
Whole berries

Selective liquid medias

Samples were inoculated into YPD medium containing chloramphenicol to inhibit bacteria and ethanol to select for yeasts (3% ethanol for vineyard, 6% for winery) and incubated at 22°C for 48 hours before plating.

Isolation of colonies

Cultures were diluted and plated onto WLN agar. Colonies were selected according to color, shape, and growth characteristics. Morphologically distinct colonies were subcultured to obtain pure cultures.

Genetic identification by PCR

After DNA extraction, the D1/D2 domain of 26S rDNA was amplified by PCR, purified and Sanger sequenced. Species identity was obtained by sequence comparison to the GenBank database.

RESULTS & DISCUSSION

A total of 221 yeast isolates were genetically identified, encompassing 25 genera and 45 species.

Table 1: Yeast occurrence by viticultural domain

Genus	Yeast species	Wine estate									Total
		République (GE)	Chambet (GE)	Gros (GE)	Bonvillars (VD)	Brunner (NE)	Auvernier (NE)	Cornulus (VS)	Grand Brûlé (VS)		
Aureobasidium	Aureobasidium pullulans	0	0	2	0	0	0	0	0	2	
Candida	Candida boidinii	0	0	0	0	0	1	0	0	1	
	Candida friedrichii	0	0	0	1	0	0	0	0	1	
	Candida norvegica	0	0	0	0	0	1	0	0	1	
	Candida pseudofarinosa	0	0	0	0	1	1	0	0	2	
	Candida tschuyiae	0	0	0	1	0	0	0	0	1	
Citeromyces	Citeromyces matritensis	0	0	0	1	0	0	0	0	1	
Curvibasidium	Curvibasidium cygneicollum	0	0	2	0	4	0	0	0	6	
Cystobasidium	Cystobasidium pinicola	0	0	0	0	0	0	1	2	3	
Debaryomyces	Debaryomyces hansenii	1	0	0	0	0	0	0	0	1	
Filobasidium	Filobasidium magnum	0	1	0	0	0	0	0	0	1	
Hanseniaspora	Hanseniaspora osmophila	0	0	0	0	0	1	0	0	1	
	Hanseniaspora uvarum	4	3	8	5	0	6	0	0	26	
Hyphopichia	Hyphopichia burtonii	0	0	1	0	0	0	0	0	1	
Kazachstania	Kazachstania servazii	3	0	0	0	0	0	0	0	3	
Kwonilella	Kwonilella europaea	0	0	0	0	1	0	0	0	1	
Lachancea	Lachancea thermotolerans	0	0	2	0	0	0	1	0	3	
Metschnikowia	Metschnikowia fructicola	2	0	1	2	0	0	2	0	7	
	Metschnikowia gruessii	1	0	1	0	0	0	0	0	2	
	Metschnikowia korensis	0	0	1	0	0	0	0	0	1	
	Metschnikowia pulcherrima	9	0	0	3	0	0	4	1	17	
	Metschnikowia reukaufii	7	5	3	2	1	0	2	1	21	
Meyerozyma	Metschnikowia viticola	0	4	0	0	0	1	0	0	5	
	Meyerozyma caribbica	0	0	2	0	1	0	0	0	3	
	Meyerozyma guilliermondii	0	1	1	0	0	0	0	0	2	
Papiliotrema	Papiliotrema terrestris	0	0	0	0	0	0	0	1	1	
Pichia	Pichia kluyveri	0	1	0	0	0	0	0	0	1	
	Pichia manshurica	0	0	0	0	0	1	0	0	1	
	Pichia membranifaciens	0	1	0	3	0	2	3	0	9	
Rhodospiridium	Rhodospiridium babjevae	0	1	0	0	0	0	0	0	1	
Rhodotorula	Rhodotorula graminis	2	0	1	0	0	1	0	0	4	
	Rhodotorula mucilaginosa	1	0	0	0	0	0	0	0	1	
Saccharomyces	Saccharomyces cerevisiae	4	2	0	11	1	13	1	0	32	
Solicozozyma	Solicozozyma aeria	1	0	0	0	0	0	0	0	1	
Sporidiobolus	Sporidiobolus pararoseus	0	0	5	0	0	0	0	0	5	
Starmerella	Starmerella apicola	0	0	0	1	0	0	0	1	2	
	Starmerella bombi	2	1	0	0	0	0	1	1	5	
	Starmerella bombicola	4	4	0	1	1	2	2	4	18	
	Starmerella orientalis	1	0	0	0	0	0	1	0	2	
	Starmerella sorbosivorans	0	0	0	0	0	0	2	0	2	
Torulaspora	Starmerella vitiae	1	0	0	0	0	0	0	0	1	
	Torulaspora delbrueckii	2	0	0	1	0	2	1	4	10	
Vishniacozyma	Vishniacozyma carnescens	0	1	0	0	0	0	0	0	1	
	Vishniacozyma victoriae	0	1	1	0	0	0	0	0	2	
Wickerhamomyces	Wickerhamomyces anomalus	0	4	0	0	0	3	2	0	9	
25 genera	45 species	45	30	31	32	10	35	23	15		

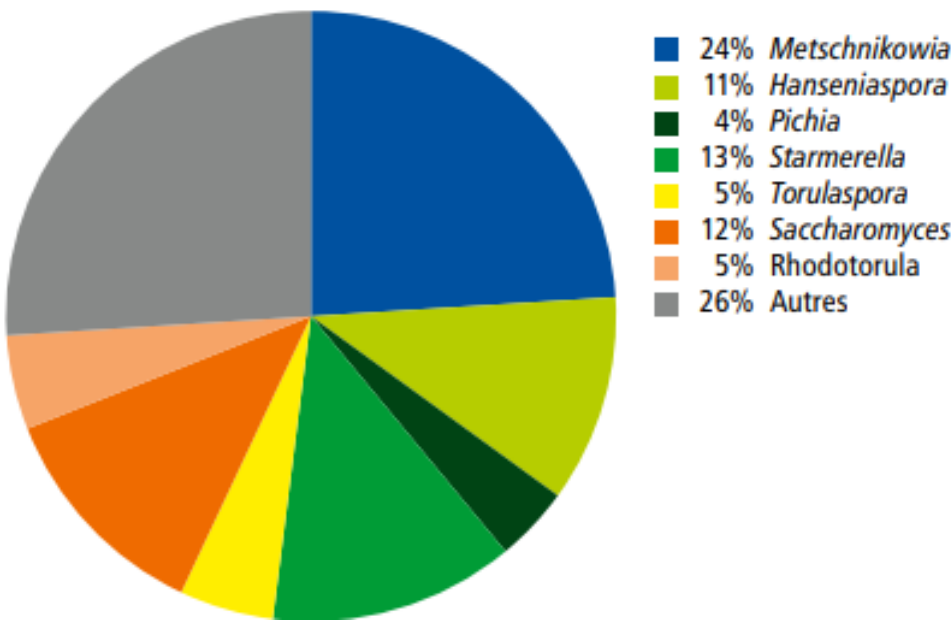


Figure 3: Main yeast genera identified in eight vineyards and wineries of Western Switzerland

CONCLUSION

This approach revealed a rich diversity of indigenous yeasts with a potential to enhance and diversify wine flavors. Some of the non-*Saccharomyces* species found here are already used commercially, highlighting the potential of these yeasts as valuable resources for winemaking.

FUTURE WORK / REFERENCES

The *Metschnikowia* subset of this yeast collection has undergone characterization in co-fermentations with *S. cerevisiae* (doi:10.3390/foods12244485). Characterization studies, including micro-fermentations alongside chemical and sensory analyses, will be extended to additional species. Another subset of has been evaluated as biocontrol agents against *Botrytis cinerea*, some yeasts showed a very strong effect against.