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REDUCTION OF VOLATILE ACIDITY OF WINES BY ISOLATED AND COMMERCIAL YEAST STRAINS

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An enological problem

- **Acetic acid is the main component of volatile acidity, and critical for wine quality;**
- **This acid is mainly produced by bacterial spoilage and *Botrytis cinerea* infections of grapes; also formed by yeasts during alcoholic fermentation.**
- **Above a certain limit (0.8 $g.l^{-1}$), acetic acid has a detrimental organoleptical effect (acidic wine);**

Available solutions?

Stabilization followed by mixture with other wines

Distillation

Nanofiltration and Reverse osmosis

"Remostagem" or refermentation

The “remostagem”procedure

- The acidic wine (1/3) is mixed with freshly crushed grapes or incubated with the residual marc from a finished wine fermentation (2/3);
- The volatile acidity of this mixture should not exceed 0.6 $g.l^{-1}$;
- Spontaneous fermentation (indigenous yeast species) reduce volatile acidity;
- The volatile acidity of the newly made wine rarely exceeds 0.3 $g.l^{-1}$.

(Ribéreau-Gayon *et al*., 2000)

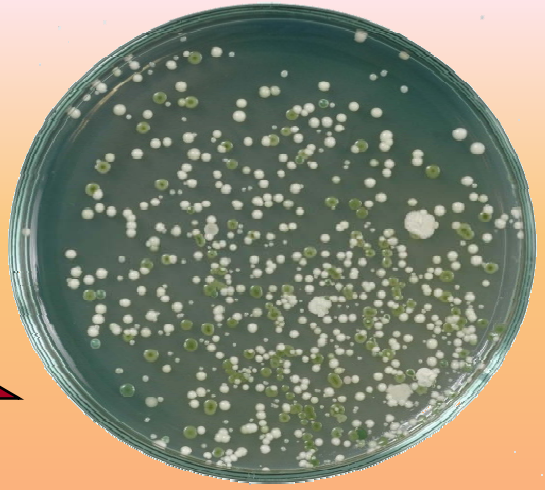
The aim of the study

- Isolate and characterize yeasts species able to reduce the acetic acid content of wines with high volatile acidity.
- Develop a controlled biological deacidification procedure.

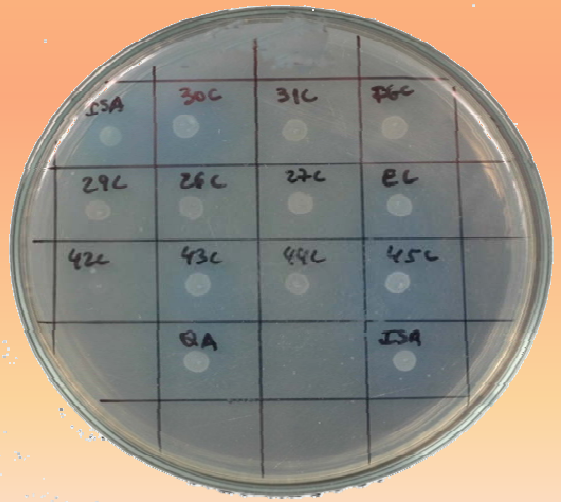
Strategy of yeast isolation and selection

“Remostagem” of a spoiled wine

Isolation of 135 isolates



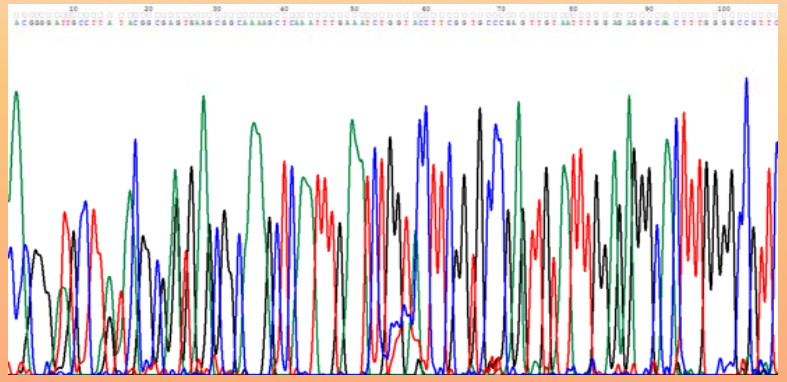
Screening of acetic acid utilization in a selective medium (Schuller *et al*., 2000)



Selected isolates
30C, 43C, 45C and 44C

Identification:
D1/D2 region amplification and sequencing

D1/D2 variable domain at the 5' end of the 26S rDNA (nucleotides 63–642 for *Saccharomyces cerevisiae*) was amplified with primers NL-1 and NL-4 (O´Donnell, 1993).



30C
43C } *S. cerevisiae* (99% - 100% identity)
45C

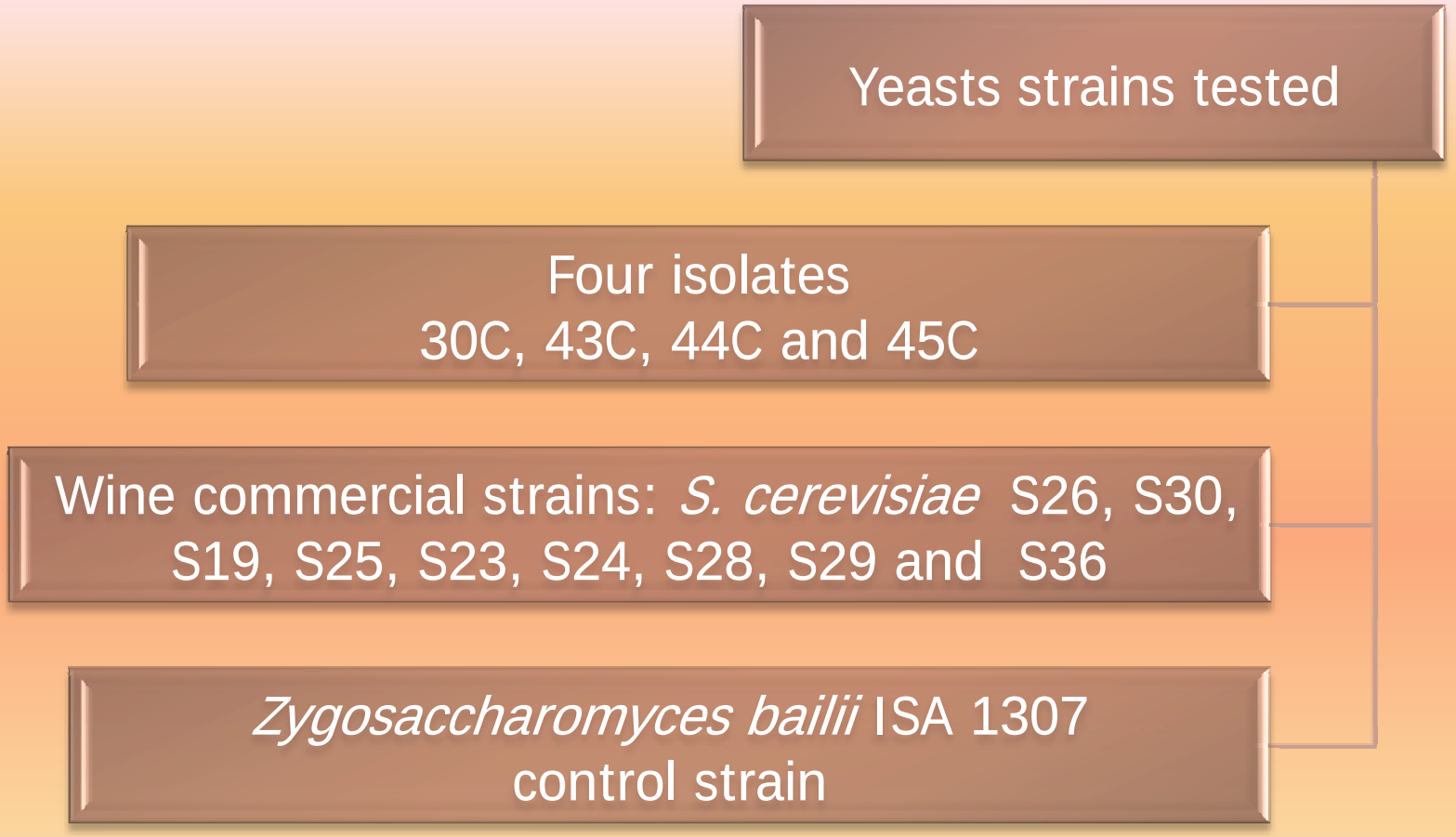
44C *Lachancea thermotolerans* NRRL Y-8284 (99% identity)

Microsatellite amplification

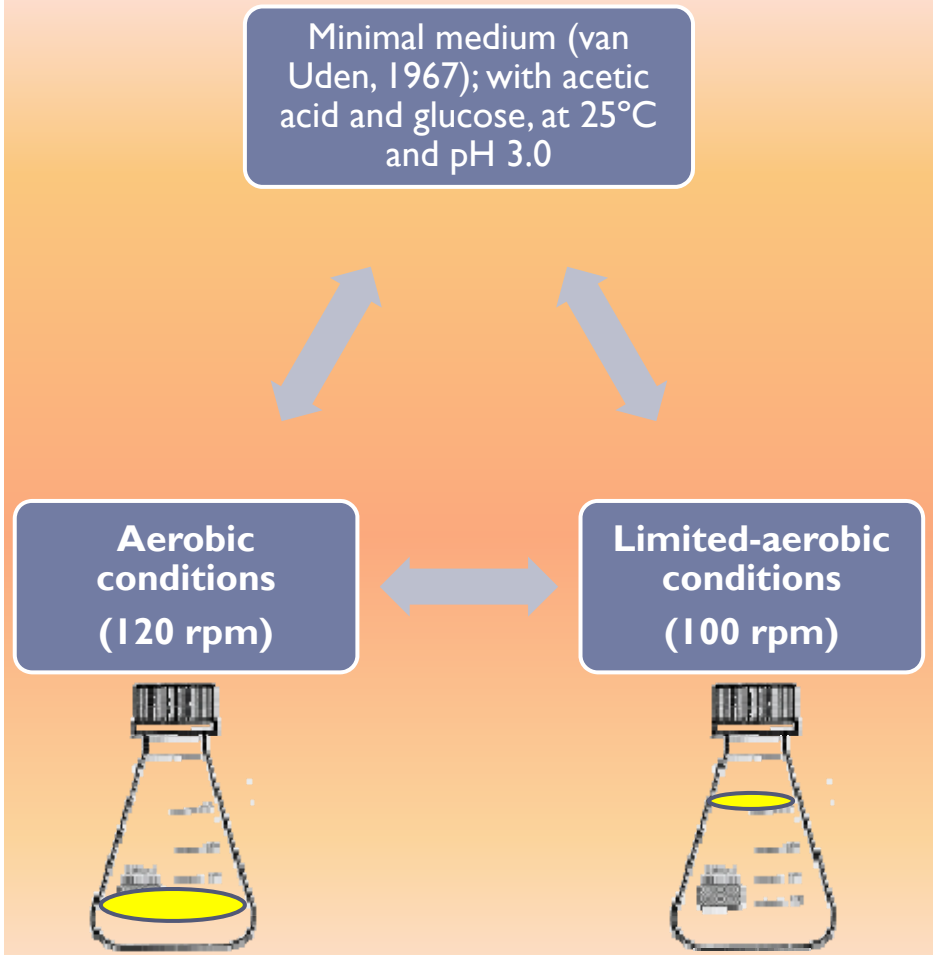
Allelic diversity of *S. cerevisiae* strains 30C, 45C and 43C. Numbers indicate the length (bp) of alleles for the six microsatellite loci ScAAT1 to ScAAT6

	Microsatellite (bp)					
Strain number	ScAAT1	ScAAT2	ScAAT3	ScAAT4	ScAAT5	ScAAT6
30C	171	381	271	329	216	259
45C	171	381	271	329	216/219	259
43C	158	378	247	308	219	259

Evaluation of acetic acid degradation



Evaluation of acetic acid degradation



Aerobic conditions	acetic acid (0.5%, v/v) glucose (0.5%, w/v)	Strains 30C, 43C, 44C, 45C, S26 and ISA 1307
Limited aerobic conditions	acetic acid (0.5%, v/v) glucose (0.75%, w/v)	
	acetic acid (0.5%, v/v) glucose (5%, w/v)	
	acetic acid (0.25%, v/v) glucose (0.75%, w/v)	Nine commercial strains

Consumption of acetic acid and glucose by the four yeast isolates in comparison with *S. cerevisiae* strain S26 and *Z. bailii* ISA 1307

	Aerobic conditions		**Limited-aerobic conditions**			
	Glucose (0.5%, w/v)		Glucose (0.75%, w/v)		Glucose (5%, w/v)	
Yeasts	Acetic acid (0.5%, v/v)		Acetic acid (0.5%, v/v)		Acetic acid (0.5%, v/v)	
strains	Glucose ($g.l^{-1}$)	**Acetic acid** ($g.l^{-1}$)	Glucose ($g.l^{-1}$)	**Acetic acid** ($g.l^{-1}$)	Glucose ($g.l^{-1}$)	**Acetic acid** ($g.l^{-1}$)
ISA 1307	0^{a}	**0 (72 h) ^{a}***	0^{a}	**0.02 ± 0.03 ^{a}**	0^{a}	**1.92 ±0.03 ^{b}**
S26	0^{a}	**0 (144) ^{a}***	0^{a}	**2.09 ± 0.09 ^{b}**	0^{a}	**4.41± 0.03 ^{d,e}**
30C	0^{a}	**0 (192 h) ^{a}***	0^{a}	**4.40 ± 0.04 ^{b,e}**	0^{a}	**4.90± 0.04 ^{e}**
43C	0^{a}	**0 (168 h) ^{a}***	0^{a}	**2.02 ± 0.09 ^{b}**	0^{a}	**4.77 ± 0.02 ^{e}**
44C	0^{a}	**0 (216 h) ^{a}***	0^{a}	**3.99 ± 0.13 ^{c,d}**	15.11 ± 0.06^{b}	**3.59± 0.06 ^{c}**
45C	0^{a}	**0 (168 h) ^{a}***	0^{a}	**4.01 ± 0.08 ^{c,d}**	0^{a}	**4.71± 0.01 ^{d,e}**

* Time needed to exhaust acetic acid from the medium.

Consumption of acetic acid ($g.l^{-1}$), after 336 and 504 hours, by nine commercial strains and *Z. bailii* ISA 1307 in MM containing acetic acid 0.25% (v/v) and glucose 0.75% (w/v), under limited-aerobic conditions, at 25ºC and pH 3.0.

	Yeast strains									
Time	ISA 1307	S26	S24	S23	S25	S19	S28	S29	S30	S36
336 h	0 ± 0^{b}	0.02 ± 0^{b}	$1.56 \pm 0.23^{a,c}$	2.13 ± 0.28^{a}	1.96 ± 0.07^{a}	2.53 ± 0.07^{a}	2.12 ± 0.21^{a}	$1.59 \pm 0^{a,c}$	$0.70 \pm 0.23^{b,c}$	2.48 ± 0^{a}
504 h	0 ± 0^{a}	0 ± 0^{a}	$0.31 \pm 0.02^{a,b}$	$0.46 \pm 0.07^{a,b,c}$	$0.79 \pm 0.10^{b,c}$	1.49 ± 0.39^{d}	$0.76 \pm 0.23^{b,c}$	0.12 ± 0.04^{a}	0 ± 0^{a}	$0.92 \pm 0.11^{c,d}$

Strains S29 and S30 showed the most similar behavior to S26 and were therefore included in further experiments.

Simulation assays of a “remostagem” process

***Yeasts strains*: 43C, 44C, 45C, S26, S29, S30, and ISA 1307**

Culture medium:

2/3 MM

+

1/3 acidic white wine.

Volatile acidity adjustment to 1.13 $g.l^{-1}$ acetic acid
pH 3.5, temperature of 25ºC
Initial ethanol concentration: 4% (v/v) or 10% (v/v)
Initial glucose concentration: 13% (w/v) or 3.3% (w/v)
Pre-inoculum: 10 ml overnight culture

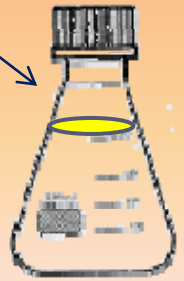
Glucose 13% (w/v) • Ethanol 4% (v/v)

- Aerobic
- Limited-aerobic

Glucose 3.3% (w/v) • Ethanol 10% (v/v)

- Aerobic
- Limited- aerobic

120 rpm



100 rpm

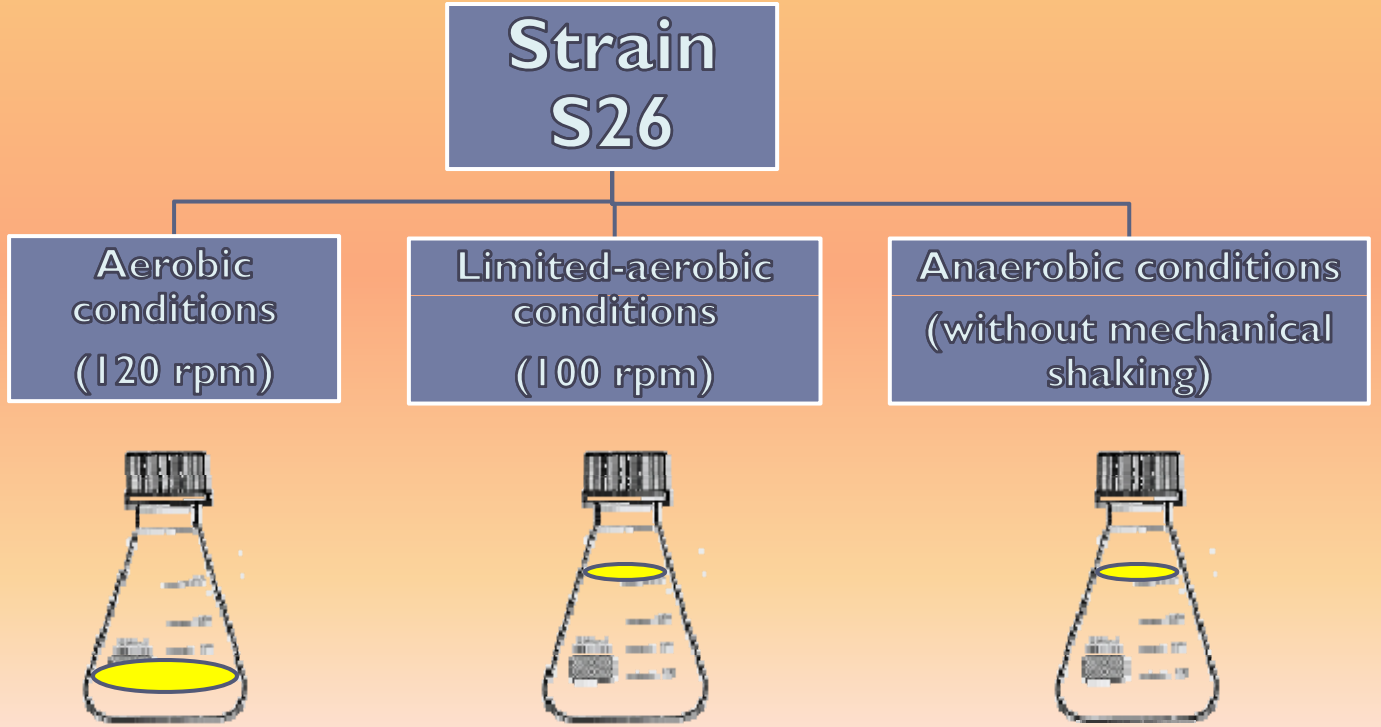
Percentage of acetic acid and glucose consumption after refermentation of wine-supplemented culture medium containing glucose 13% (w/v) and ethanol 4% (v/v) or glucose 3.3% (w/v) and ethanol 10% (v/v) (48 and 72 hours of incubation, respectively)

	Glucose (13%,w/v) and Ethanol (4%, v/v)		Glucose (3.3%,w/v) and Ethanol (10%, v/v)	
	Aerobic conditions	Limited-aerobic conditions	Aerobic conditions	Limited-aerobic conditions
Yeast strains	**Acetic acid** Glucose	**Acetic acid** Glucose	**Acetic acid** Glucose	**Acetic acid** Glucose
ISA 1307	**94.8 ± 3.30** ^{h} 52.4 ± 2.62 $^{e, f}$	**40.9 ± 9.80** $^{e, f}$ 38.8 ± 6.36 $^{d, e}$	**71.2 ± 3.02** ^{g} 23.1 ± 5.60 $^{a, b, c}$	**41.6 ± 2.64** $^{e, f}$ 39.4 ± 2.10 $^{d, e}$
44C	**94.6 ± 4.79** ^{h} 58.5 ± 8.60 ^{f}	**15.25 ± 3.30** $^{a, b, c}$ 31.0 ± 5.69 $^{c, d}$	**28.1 ± 1.70** $^{c, d, e}$ 16,4 ± 1.76 $^{a, b}$	**17.4 ± 7.16** $^{b, c, d}$ 30.4 ± 5.79 ^{c}
43C	**0 ± 0** ^{a} 100 ± 0 ^{g}	**31.2 ± 9.70** $^{c, d, e, f}$ 96.94 ± 3.17 ^{g}	**36.4 ± 9.88** $^{e, f}$ 40.7 ± 7.42 $^{d, e}$	**37.5 ± 3.17** $^{e, f}$ 100 ± 0 ^{g}
45C	**16.0 ± 4.06** $^{a, b, c}$ 100 ± 0 ^{g}	**40.3 ± 6.60** $^{e, f}$ 97.4 ± 2.28 ^{g}	**33.4 ± 6.88** $^{d, e, f}$ 23.8 ± 6.61 $^{a, b, c}$	**40.1 ± 6.58** $^{e, f}$ 100 ± 0 ^{g}
S26	**46.8 ± 4.99** ^{f} 100 ± 0 ^{g}	**45.9 ± 5.60** ^{f} 87.7 ± 10.72 ^{g}	**86.7 ± 2.63** $^{g, h}$ 100 ± 0 ^{g}	**44.6 ± 3.58** $^{e, f}$ 100 ± 0 ^{g}
S30	**8.6 ± 4.44** $^{a, b}$ 100 ± 0 ^{g}	**39.9 ± 5.70** $^{e, f}$ 98.2 ± 3.15 ^{g}	**36.3 ± 4.91** $^{e, f}$ 31.7 ± 5.40 $^{c, d}$	**35.1 ± 6.37** $^{e, f}$ 100 ± 0 ^{g}
S29	**31.4 ± 2.47** $^{c, d, e, f}$ 92.7± 1.15 ^{g}	**82.5 ± 3.03** $^{g, h}$ 56.8 ± 4.65 ^{f}	**9.6 ± 3.03** $^{a, b}$ 17.3 ± 2.86 $^{a, b}$	**43.3 ± 4.75** $^{e, f}$ 14.85 ± 4.98 ^{a}

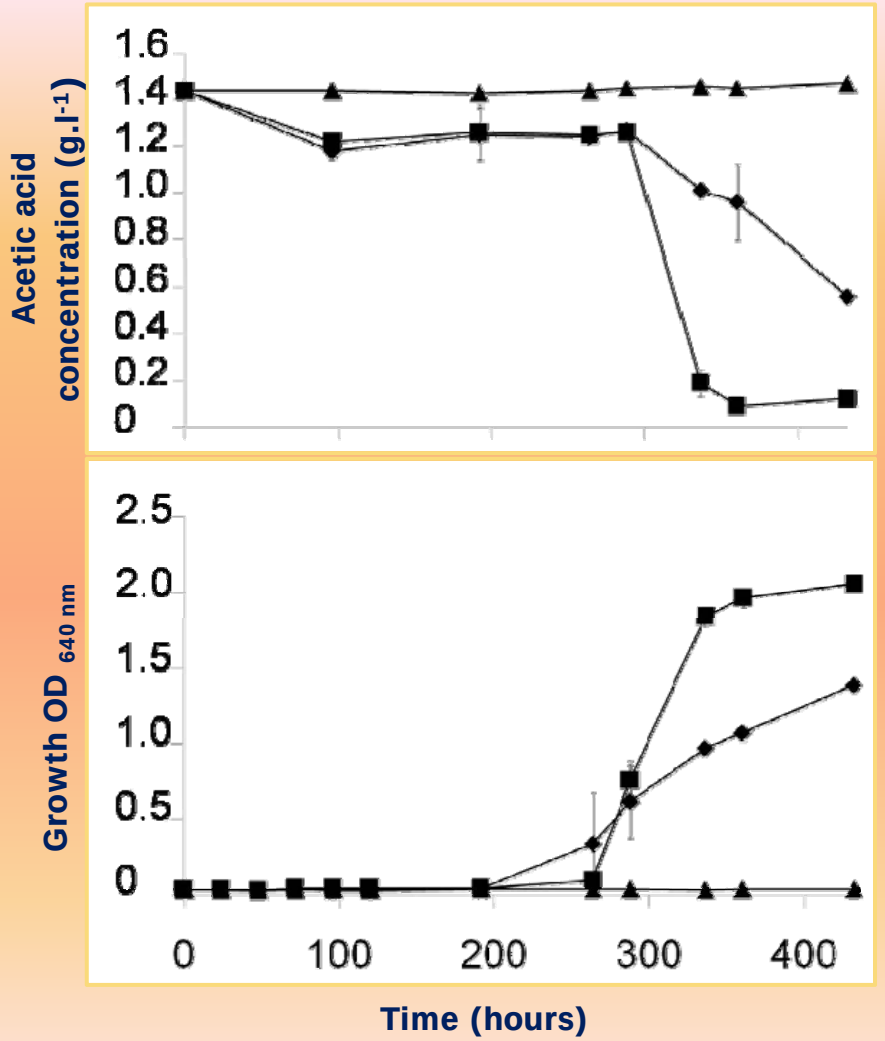
Removal of acetic acid from an acidic wine under different oxygenation conditions by strain S26

Culture medium:
Acidic white wine.

Volatile acidity :1.44 $g.l^{-1}$ acetic acid
pH 3.55, temperature of 25ºC
Initial ethanol concentration:10.4% (v/v)
Residual sugars: 1.10 $g.l^{-1}$
Pre-inoculum: 10 ml overnight culture



Removal of acetic acid from an acidic wine under different oxygenation conditions



Growth ($OD_{640\ nm}$) of the *S. cerevisiae* strain S26 and acetic acid consumption ($g.l^{-1}$) under aerobic (■), limited-aerobic (♦) and anaerobic conditions (▲).

Final values of acetic and ethanol, after 432 hours

Aeration conditions	Final ethanol degree % (v/v)	Final volatile acidity ($g.l^{-1}$)	Percentage of acetic acid consumption
Aerobic	6.5±0.21	0.12±0.04	89.6±2.97
Limited-aerobic	9.0±0.28	0.56±0.06	61.5±4.45
Anaerobic	8.6±0.14	1.47±0.00	0

Removal of acetic acid from an acidic wine for different initial ethanol/acetic acid concentrations by the strains S26 and S29

Culture medium:
Acidic white wine.

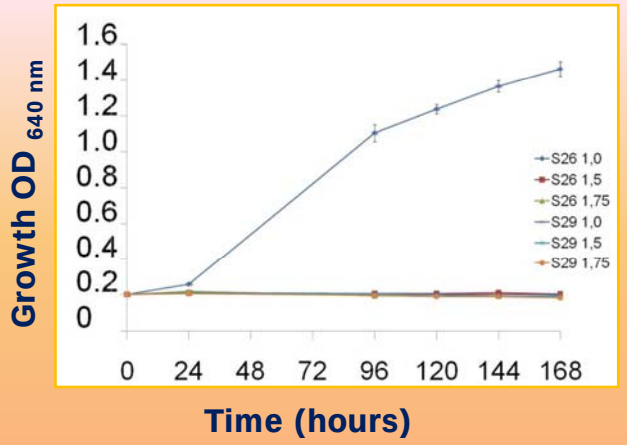
pH 3.5, temperature of 25ºC.
Residual sugars: 1.15 $g.l^{-1}$
Pre-inoculum: 10 ml overnight culture

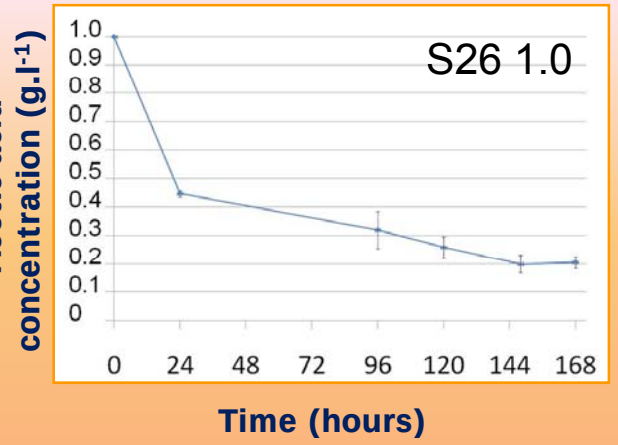
The ethanol effect

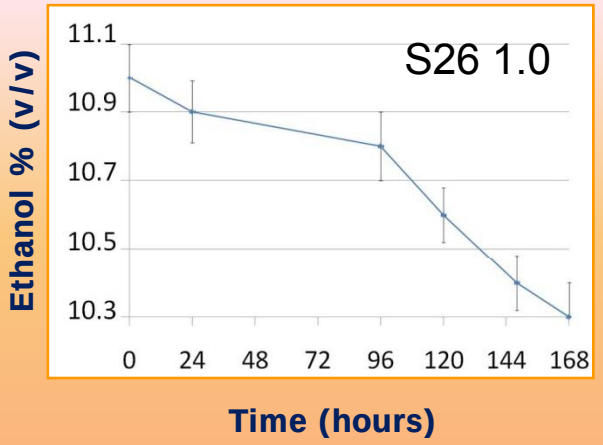
- 12% of ethanol in combination with 1.0,1.5 or 1.75 g.l $^{-1}$ of acetit acid were toxic for both yeasts.

- After 48 hours, no growth had occur, the cells where dead and there was no consumption of acetic acid.

Ethanol 11% (v/v)
The effect of the initial concentration of acetic acid



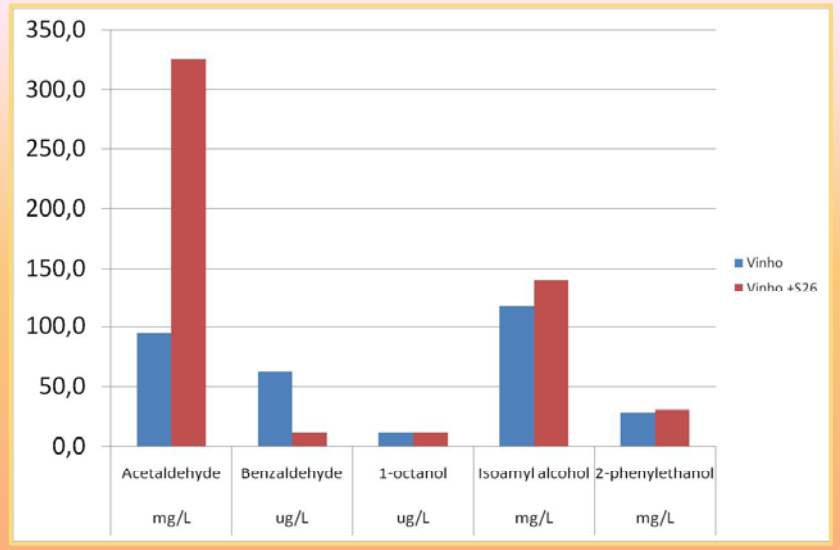


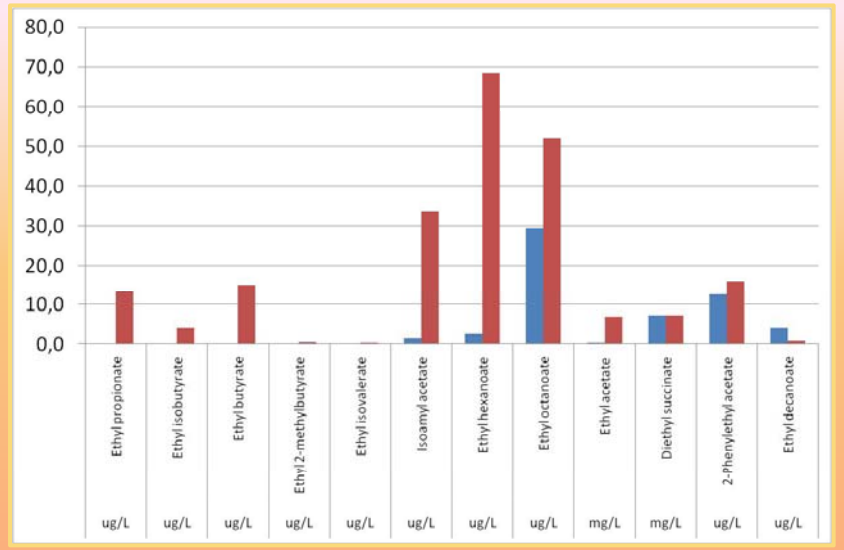


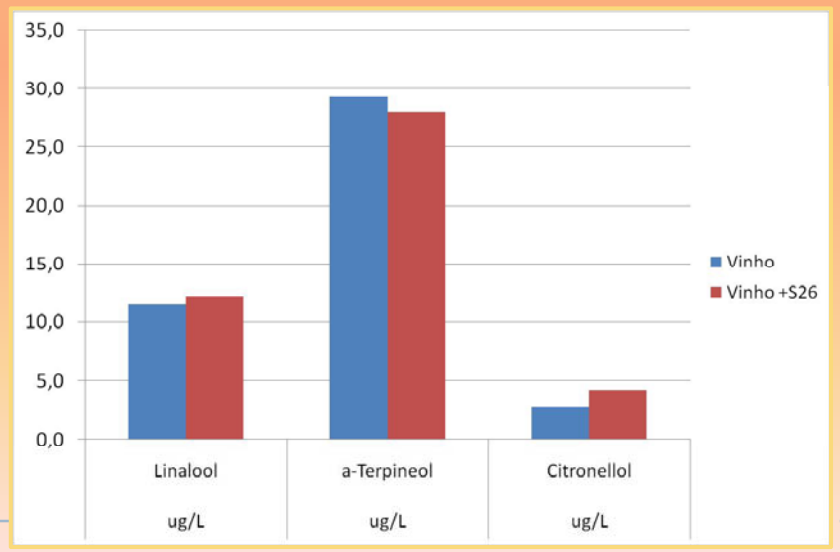
Final analysis of the wines obtained after 168 hours

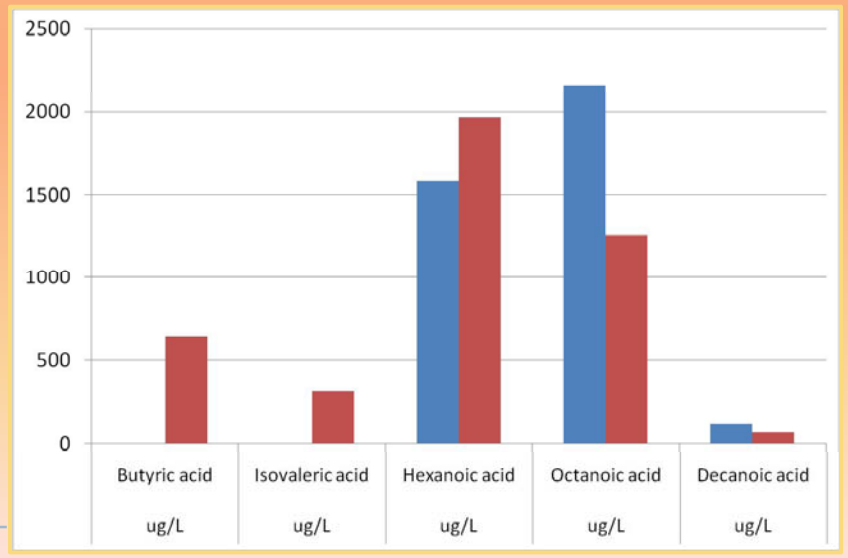
Strains	Ethanol	pH	Acetic acid (g.l-1)	Titratable acidity (g.l-1)	Total SO2 (mg.l-1)	Free SO2 (mg.l-1)	cfu
S26 1.0	10.3±0.1	3.68±0.03	0.22±0.03	3.77±0.15	74.77±1.43	0.0±0.0	10x10^6
S26 1.5	9.7±0.4	3.58±0.01	1.13±0.06	5.37±0.06	59.90±1.43	0.0±0.0	0
S26 1.75	9.8±0.2	3.57±0.01	1.37±0.02	5.87±0.38	66.86±0.41	0.0±0.0	0
S29 1.0	9.8±0.2	3.61±0.02	0.52±0.05	4.60±0.10	64.75±0.98	0.0±0.0	0
S29 1.5	9.7±0.2	3.60±0.01	1.37±0.05	5.50±0.40	66.93±9.40	0.0±0.0	0
S29 1.75	10.0±0.1	3.58±0.01	1.49±0.02	5.80±0.20	65.18±3.82	0.0±0.0	0

GC-MS Analysis of wine obtained with S26 strain









Sulfur dioxide is mainly used in the following cases:

- In the must of white wines, in order to avoid the activation of alcoholic fermentation and to allow the decanting of solid parts;
- Before the start of alcoholic fermentation in order to select yeasts and, in case of red wines, to favor a better extraction of color and tannins from the skins;
- Every time the wine comes in contact with the air - such as decanting, clarifying, filtering and bottling - therefore avoiding oxidation and development of unwanted bacteria or yeasts.

Removal of acetic acid from an acidic wine for different initial SO_2 concentrations by the strains S26 and S29

Culture medium:
Acidic white wine.

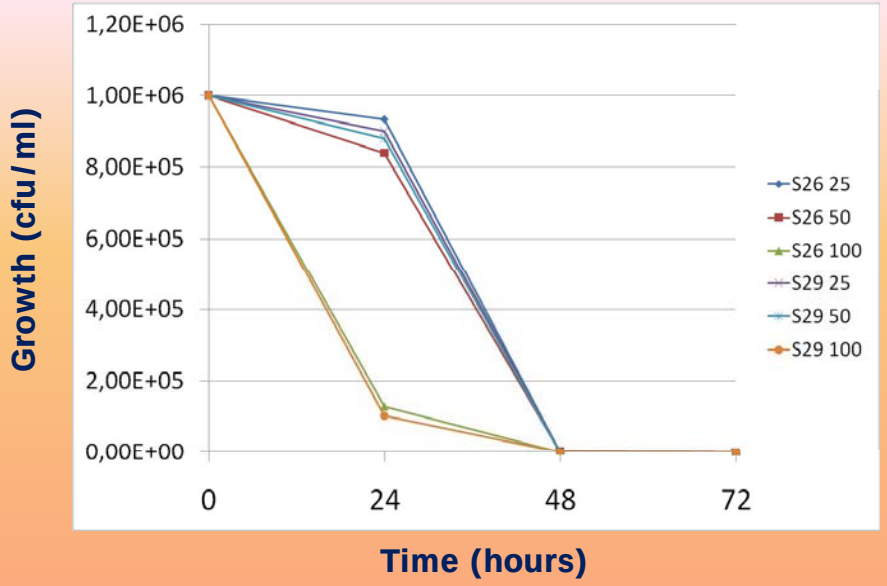
pH 3.5, temperature of 25ºC
Residual sugars: 1.15 $g.l^{-1}$
Total SO_2 70.3 $mg.l^{-1}$ / Free SO_2 3.2 $mg.l^{-1}$
Pre-inoculum: 10 ml overnight culture

100 rpm

11% (v/v) ethanol
1.0 $g.l^{-1}$ acetic acid

- SO_2 25 $mg.l^{-1}$
- SO_2 50 $mg.l^{-1}$
- SO_2 100 $mg.l^{-1}$

The effect of SO_2 initial concentration…



Strong anti-oxidant properties, combines itself with oxygen.

Antiseptic capability.

SO_2 combines with acetaldehyde, sugars, aldehydes and ketones

Final analysis of the wines obtained at the end of 72 hours

Strains	Ethanol	pH	Acetic acid (g.l-1)	Titratable acidity (g.l-1)	Total SO2 (mg.l-1)	Free SO2 (mg.l-1)	cfu
S26 25	10.6±0.2	3.49±0.01	0.99±0.03	5.21±0.04	93.68±8.71	2.17±0.65	0
S26 50	10.6±0.1	3.49±0.00	0.95±0.04	5.25±0.05	122.26±2.75	1.32±0.89	0
S26 100	10.6±0.1	3.47±0.01	0.99±0.03	5.14±0.04	173.01±2.18	0.96±0.32	0
S29 25	10.7±0.1	3.49±0.01	1.00±0.02	5.06±0.10	103.28±2.83	1.86±0.51	0
S29 50	10.5±0.1	3.49±0.01	0.94±0.03	5.13±0.03	123.14±2.62	2.84±0.59	0
S29 100	10.6±0.1	3.47±0.01	1.00±0.02	5.23±0.02	171.45±1.03	2.34±1.82	0

Final Remarks

- Generally, the *S. cerevisiae* strains characterized herein, are capable to remove acetic acid independently of the relative amounts of glucose and ethanol:
  - *S. cerevisiae* strain S26 is the most efficient acid degrading strain in a refermentation process containing low glucose/high ethanol concentrations, under aerobic conditions.
  - *S. cerevisiae* strain S29 is the most efficient acid degrading strain in a refermentation process containing high glucose/low ethanol initial concentrations, with low oxygen availability.
  - Acetic acid removal efficiencies were obtained for initial concentrations about two-fold higher (1.1 g l^{-1}) than the values proposed for a typical refermentation assay (0.6 g.l^{-1}) and the desired acetic acid reduction occurs in less than 72.
- *L. thermotolerans* 44C displays a behaviour similar to the reference strain *Z. bailii* ISA 1307 both regarding acetic acid and glucose degradation in the presence of high glucose/low ethanol concentrations, under aerobic conditions.

Final Remarks

- *S. cerevisiae* can decrease volatile acidity of wines with an elevated content of acetic acid (1.0 to 1.44 $g.l^{-1}$) and low residual sugar (1.1 $g.l^{-1}$), even without further sugar addition, in conditions where oxygen is limited (strain S26) with an initial ethanol concentration of 11% (v/v).

- High ethanol concentrations (12%, v/v) in combination with 1.0,1.5 or 1.75 $g.l^{-1}$ of acetic acid inhibit the ability of strains S26 and S29 to remove acetic acid from acidic wines.

- High levels of SO_2 inhibit acetic acid consumption by yeasts probably due to is strong anti-oxidant and antiseptic properties.

Future perspectives

- Evaluate the capacity of encapsulated *S. cerevisiae* S26 and S29 to perform biological deacidification of wines with excessive levels of acetic acid either directly or through a “remostagem” process;
- Evaluate the fermentative profiles and the organoleptical properties of the wines deacidified by those strains;
- Scale-up of the optimized “remostagem” process.

Acknowledgements

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