Isolation of yeast strains with ability to reduce volatile acidity of wines

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Main Goal

Acetic acid is the main component of volatile acidity, and is critical for wine quality. Its concentration in wines is approximately 0.5 gl^{-1}, and legally, must remain below 1 $g.l^{-1}$. This acid is mainly produced by bacterial spoilage in *Botrytis cinerea* infected grapes but is also formed by yeasts during alcoholic fermentation. *S. cerevisiae* is a yeast species that can use acetic acid as a sole carbon and energy source. The aim of the present study was to isolate and characterize indigenous yeasts species from typical refermentation processes that can be used as starters in an efficient and controlled biological procedure to decrease volatile acidity of acidic wines.

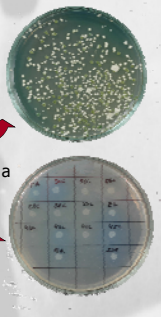
Experimental Approach and Results

Refermentation of a spoiled wine

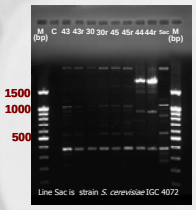
135 isolates

Screening of acetic acid utilization in a selective medium [1]

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



Characterization by RAPD (primer T3B) [2] and identification by amplification and sequencing of the D1/D2 domain of the large subunit (26S) ribosomal DNA [3]. Three of the isolates (30C, 43C and 45C) were identified as *Saccharomyces cerevisiae* and one (44C) as *Lachancea thermotolerans*. Sequence similarity search was done using GenBank BLASTN search [4].



Acetic acid utilization was then assessed in a minimal mineral medium [5] containing acetic acid (0.5% v/v) and glucose (from 0.5% w/v up to 5% w/v) at 25ºC and pH 3.0. The strains *Zygosaccharomyces bailii* ISA 1307 and *S. cerevisiae* IGC 4072 were used as control strains.

Culture medium

Aerobic conditions — 120 rpm

Limited-aerobic conditions — 100 rpm

All the strains tested (Table 1) excepting *S. cerevisiae* IGC 4072, were able to exhaust acetic acid from the medium, under aerobic conditions and for 0.5% (w/v) of glucose. Oxygen limitation and a slight increase in glucose concentration (0.75%, w/v) led to a decrease of the acid consumption rates by all strains excepting *Z. bailii* ISA 1307. Under these conditions *Z. bailii* ISA 1307 and isolate 43C behaved significantly different ($P \leq 0.05$) and degraded about 100 and 60 % of the initial acetic acid after 312h, respectively, whereas the other strains displayed low acid removal percentages (Table 1).

Table 1 - Consumption of acetic acid and glucose by the four yeast isolates in comparison with *S. cerevisiae* strains IGC 4072, and *Z. bailii* ISA 1307, in minimal media with different initial concentrations of glucose (0.5% to 5% w/v) and acetic acid (0.5% v/v), under aerobic and limited-aerobic conditions after 216h (aerobic conditions) and 312h (limited aerobic conditions)

	Aerobic conditions			Limited-aerobic conditions		
Yeasts strains	Glucose (0.5% w/v)		Glucose (0.75% w/v)		Glucose (5% w/v)	
	Glucose (gl^{-1})	Acetic acid (gl^{-1})	Glucose (gl^{-1})	Acetic acid (gl^{-1})	Glucose (gl^{-1})	Acetic acid (gl^{-1})
ISA 1307	0	0 (72 h)*	0	0.02 ± 0.03	0	1.92 ± 0.03
IGC 4072	0	4.0 ± 0.11 (216)*	0	3.00 ± 0.07	0	4.96 ± 0.13
30C	0	0 (192 h)*	0	4.40 ±0.04	0	4.90 ± 0.04
43C	0	0 (168 h)*	0	2.02 ±0.09	0	4.77 ± 0.02
44C	0	0 (216 h)*	0	3.99 ± 0.13	15.11 ± 0.06	3.59 ± 0.06
45C	0	0 (168 h)*	0	4.01 ± 0.08	0	4.71 ± 0.01

* Time needed to exhaust acetic acid from the medium

The two *S. cerevisiae* isolates (43C and 45C), the *L. thermotolerans* isolate (44C) and the strain *Z. bailii* ISA 1307 were further tested under conditions simulating refermentation processes. In order to simulate a refermentation of a wine with excessive amounts of acetic acid, mixtures were prepared containing 2/3 of a mineral medium [4] and 1/3 of acidic white wine. Volatile acidity (V. a.) of the mixture was adjusted to 1.12 - 1.14 gl^{-1}. and the pH to 3.5. The medium containing wine 1 (ethanol, 4% v/v) was further supplemented with glucose (13 %, w/v) in order to simulate a refermentation of an acidic wine with must from the beginning of fermentation. The mineral medium containing wine 2 (ethanol, 10% v/v) was supplemented with glucose (3.3% w/v) in order to simulate a refermentation of an acidic wine with the residual marc from a finished wine fermentation.

Culture medium:
2/3 MM medium [4]
+
Glucose
(13% or 3.3% w/v)
+
1/3 acidic white wine

V. a. adjustment to 1.14 or 1.12 gl^{-1} acetic acid
pH 3.5, temp 25ºC
Initial ethanol concentration: 4% or 10% (v/v)

Pre-inoculum:
10 ml overnight culture

Aerobic conditions — 120 rpm

Limited-aerobic conditions — 100 rpm

Under aerobic conditions and in the presence of high glucose and low ethanol initial concentration, all strains consumed acetic acid simultaneously with glucose (Table 2). *L. thermotolerans* 44C, behaved similarly to *Z. bailii* ISA 1307. Under limited aerobic conditions there were no differences in percentage of acid removal (~50%) between *Z. bailii* ISA 1307 and the *S. cerevisiae* isolates 43C and 45C, after 48h. Regarding refermentation assays, with acidic wine containing media with low glucose and high ethanol initial concentrations, under aerobic conditions (Table 2), the strain *Z. bailii* ISA 1307 appears again faster than *S. cerevisiae* isolates 43C and 45C with an acid removal after 72h of about 50%, comparatively to about 30%, respectively. This observation indicates that while glucose/ethanol concentration affects acid removal by *Z. bailii* ISA 1307 it does not affect acid removal by *S. cerevisiae* 43C and 45C. The percentages of acid removal by *S. cerevisiae* 43C and 45C under these low glucose and high ethanol concentrations appear not affected by oxygen limitations [6]. Therefore, under these latter conditions *S. cerevisiae* 43C and 45C appear equally efficient as *Z. bailii* ISA 1307.

Table 2 – Comparison of acetic acid (A) and glucose (G) consumption (%) for each strain tested in the refermentation simulation assays, after a given incubation time (T), and maximum values of acetic acid consumption achieved (A_{max}) and correspondent glucose consumption (GA_{max}) at given incubation times (T_{max}).

	Glucose 13% (w/v) and ethanol 4% (v/v)								Glucose 3.3% (w/v) and ethanol 10% (v/v)							
	Aerobic conditions				Limited-aerobic condition				Aerobic conditions				Limited-aerobic conditions			
Yeast strains	A G	T (h)	A_{max} GA_{max}	T_{max} (h)	A G	T (h)	A_{max} GA_{max}	T_{max} (h)	A G	T (h)	A_{max} GA_{max}	T_{max} (h)	A G	T (h)	A_{max} GA_{max}	T_{max} (h)
ISA 1307	91.2	48	91.2	48	52.6	48	67.5	168	52.7	72	96.4	120	29.5	72	91.9	408
	35.6		35.6		8.2		88.8		4.3		61.8		11.7		100	
43C	17.5	48	34.2	72	53.5	48	53.5	48	33.0	72	33.0	72	34.8	72	34.8	72
	86.1		98.2		71.1		71.1		100		100		83.9*		83.9*	
44C	6.1	48	99.1	264	0	48	35.1	168	18.8	72	83.9	336	22.3	72	48.2	408
	1.8		98.0		0		2.3		4.9		100		8.3		100	
45C	16.7	48	16.7	48	52.1	48	52.1	48	28.6	72	28.6	72	34.8	72	34.8	72
	90.1		90.1		61.4		61.4		100		100		74.6*		74.6*	

* These strains completely depleted glucose from the medium after 96 h.

Final Remark

The data obtained, in this work, show that *S. cerevisiae* isolates can be used to decrease excessive volatile acidity of acidic wines. A more widespread analysis of others *S. cerevisiae* strains will be done to determine how general the phenomenon is.

Acknowledgements

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