volatile acidity of wines

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Introduction

The level of acetic acid, the main component of volatile acidity, is critical for wine quality. Its concentration in wines is approximately 0.5 gl^{-1} and, legally, must remain below 1.0 gl^{-1}. This acid is mainly produced by bacterial spoilage and can be also formed by yeasts during alcoholic fermentation. Acetic acid produced during the first step of fermentation is partially metabolized by wine yeasts at the middle/end of this process in the presence of residual sugars. Acetic acid seems to be converted to acetyl CoA and used in the lipid biosynthesis (Ribéreau-Gayon *et al.*, 2000). Winemakers use a procedure called "remostagem" to lower the acetic acid of wines with high volatile acidity (higher than 0.8 gl^{-1}). The acidic wine is mixed with freshly crushed grapes in a proportion of no more than 20-30% (v/v). The volatile acidity of this mixture should not exceed 0.6 gl^{-1}. The volatile acidity of the newly made wine rarely exceeds 0.3 gl^{-1}. The aim of this study is to select wine yeasts capable of decreasing the volatile acidity of spoiled wines.

Materials and Methods

From a group of 135 isolates colected during a "remostagem" procedure, four wild yeasts were selected based on their ability to consume acetic acid in the presence of glucose in a solid media at pH 4.0 or 6.0 (Schuller, 1998).

The four strains were further analyzed regarding acetic acid and glucose consumption, specific growth rate and ethanol production in comparison to the commercial strains Lalvin QA23, *Saccharomyces cerevisiae* IGC 4072 and *Zygosaccharomyces bailii* ISA 1307, using minimal media containing acetic acid (0.5% v/v) and glucose (0.5% w/v to 5% w/v) at 25ºC and pH 3.0. *Zygosaccharomyces bailii* ISA 1307 was used as a control since it has been described to use acetic acid in the presence of glucose (Sousa *et al.*, 1998). The consumption of acetic acid was tested in aerobic (100 ml of minimal media in a 250 ml Erlenmeyer flask, at 120 rpm) and limited aerobic conditions (230 ml of minimal media in a 250 ml Erlenmeyer flask, at 100 rpm).

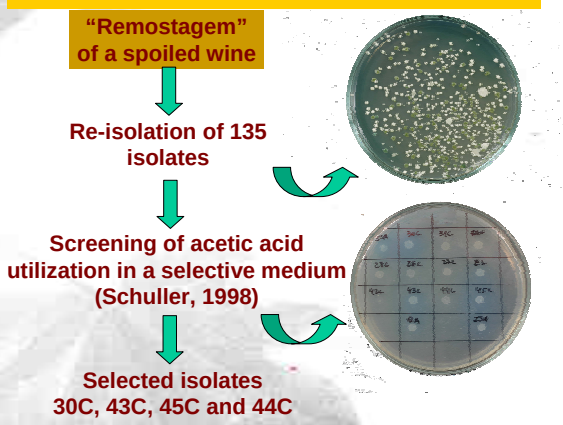
Concentrations of glucose, acetic acid and ethanol were measured by HPLC using an HPX-87H Ion Exclusion Column. The samples were collected and previously filtered before injection.

To perform the molecular characterization of the four wild yeasts, DNA extraction followed performances previously described (Kaiser, C. *et al.*, 1999) and the oligonucleotide used as single primer for PCR-fingerprinting was T3B (5'- AGG TCG CGG GTT CGA ATC C-3').

Amplification reactions were performed in a Perkin Elmer Cetus DNA thermal cycler 480 using a final volume of 25 µl containing 1µl of template DNA, 2.5 µl 10x amplification buffer, 1.5 µl $MgCl_2$ 25 mM, 0.5 µl T3B primer (50pmol/µl), 0.5 µl dNTP mix 10 mM, 0.2 µl Taq DNA polymerase (REAGENTE 5) (5U/µl) and 18.8 µl deionised water. The thermal cycler was programmed for 1 cycle of 10 min at 95ºC, followed by 35 cycles of 30 sec at 95ºC, 30 sec at 52ºC and 1 min at 72ºC set, followed by 1 cycle of 8 min at 72ºC, and finally 1 cycle at 4ºC.

PCR products were separated on a 1.2% agarose gel in 0,5x TBE buffer. PCR fingerprinting profiles were visualized and captured with a Biocapt Transiluminator Vilber Lourmat and respective software.

Strategy of yeast isolation and selection



Results and Discussion

Molecular characterization

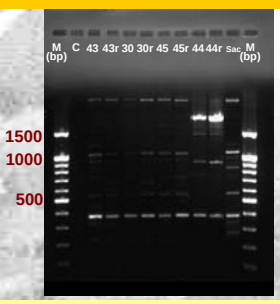


Figure 1. Agarose gel showing the PCR fingerprinting profiles of four wild strains (43, 30, 45 and 44) with one replica **(r).** The strains 43, 30 and 45 exhibit a *Saccharomyces* profile. Strain 44 is a non-*Saccharomyces* species. Lane **C** is the control without DNA and lane **Sac** is *S. cerevisiae* IGC 4072.

Consumption of acetic acid and glucose by the isolated strains

Table 1. Consumption of acetic acid and glucose by the four isolates in comparison with *S. cerevisiae* strains QA23, 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 and final pH values, after 168 h (aerobic conditions) or 312 h (reduced aerobic conditions). For higher glucose levels (2.5% and 5.0%) acetic acid was not consumed, with the exception of *Z. bailii* 1307 and strain 44C.

	Aerobic conditions			Limited aerobic conditions								
	0.5% (v/v) acetic acid 0.5% (w/v) glucose			0.5% (v/v) acetic acid 0.75% (w/v) glucose			0.5% (v/v) acetic acid 2.5% (w/v) glucose			0.5% (v/v) acetic acid 5% (w/v) glucose		
Yeasts	Glucose (gl^{-1})	Ac. acid (gl^{-1})	pH	Glucose (gl^{-1})	Ac. acid (gl^{-1})	pH	Glucose (gl^{-1})	Ac. acid (gl^{-1})	pH	Glucose (gl^{-1})	Ac. acid (gl^{-1})	pH
Z. bailii 1307	0	0	2,60	0	0,02	2,61	0	1,92	2,66	0	1,92	2,58
IGC 4072	0	4,00	3,01	0	3,00	2,81	0	4.99	2,77	0	4,96	2,65
QA 23	0	0	2,65	0	2,09	2,86	0	4,76	2,76	0	4,41	2,70
43C	0	0	2,72	0	2,02	2,78	0	5,00	2,76	0	4,77	2,65
45C	0	0	2,71	0	4,01	2,81	0	5,00	2,73	0	4,71	2,58
30C	0	0	2,75	0	4,40	2,82	0	5,00	2,71	0	4,90	2,60
44C	0	0	2,73	0	3,99	2,83	0	4,85	2,77	15,11	3,59	2,60

Strain QA 23 revealed to be more efficient than strain 43 C. *Z. bailii* ISA1307 used acetic acid simultaneously with glucose as described by Sousa *et al.* (1998).

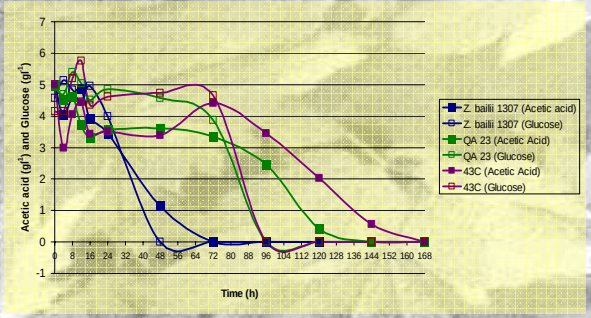


Figure 2. Acetic acid and glucose consumption in minimal media containing acetic acid 0.5% (v/v) and glucose 0.5% (w/v), under aerobic conditions, at 25ºC and pH 3.0.

Strains 43 C and QA 23 displayed identical ability to degrade acetic acid although not so efficient as strain *Z. bailii* ISA 1307, since they did not exhaust acetic acid.

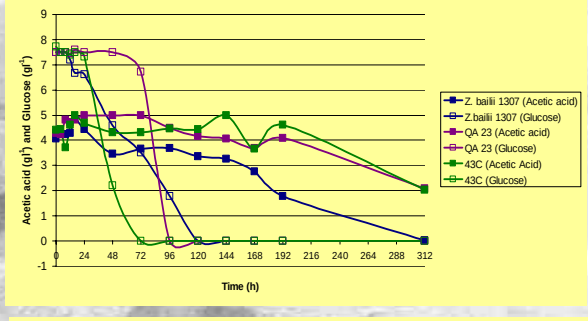


Figure 3. Acetic acid and glucose consumption in minimal media containing acetic acid 0.5% (v/v) and glucose 0,75% (w/v), under limited aerobic conditions, at 25ºC and pH 3.0.

Final Remarks

We have demonstrated that all strains metabolise acetic acid more efficiently under aerobic conditions. Though none of the isolated strains is so efficient as *Z. bailii* ISA 1307 and able to consume glucose and acetic acid simultaneously, the *Saccharomyces* strains 43C and QA 23 reveal more efficient than *S. cerevisiae* IGC 4072 and were able to remove 60% of acetic acid after 312 h, under limited aerobic conditions. Lower initial glucose concentrations favour acetic degradation. Further assays will be carried out to assess the behaviour of the isolated studies in acidic wines.

References

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