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REVIEW PAPER

Oenological versatility of *Schizosaccharomyces* spp.

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Abstract The biodiversity of non-*Saccharomyces* yeasts is currently a topic of great interest. The possibility of their use in winemaking has led to much research into the metabolic and structural properties of some of these yeasts, such as those belonging to *Torulaspora*, *Pichia*, *Hanseniaspora* and *Hansenula*. The present work reviews our knowledge of the genus *Schizosaccharomyces*, the use of which in winemaking has recently been discussed at the International Organisation of Vine and Wine. However, despite offering the advantage of malic dehydrogenase activity, plus a wall structure that ensures the autolytic release of mannoproteins and polysaccharides during ageing over lees, only one commercial strain of *Schizosaccharomyces pombe* is currently available.

Keywords Wine · *Schizosaccharomyces* spp. · Biological deacidification · Demalication ageing over lees · Ethyl carbamate

Background

The market is making increasing demands for new strains of yeast capable of producing wines with novel properties [1–4]. Strains that afford winemakers precise control over fermentation are, therefore, now being sought [5]. For example, the use of certain non-*Saccharomyces* yeasts with the ability to reduce the malic acid content of wine, such as *Schizosaccharomyces* spp., is now being viewed with much interest (Fig. 1) [6–11].

Although *Schizosaccharomyces* is used in the production of rum and cocoa liquors in Madagascar [12–18], non-*Saccharomyces* yeast genera have traditionally been regarded as wild or spoilage organisms in wine [19–23]. Certainly, they are commonly isolated from wine vats in which fermentation has run into problems, and from wines suffering from strong organoleptic and chemical deviations through the appearance of acetic acid, H_2S, acetaldehyde, acetoin and ethyl acetate. However, many studies have been performed over the last 10 years to better determine the true impact of these yeasts on the volatile composition and sensorial characteristics of wine with the aim of eventually employing them in winemaking [9, 24–28]. Their use in mixed or sequential fermentations is now seen as a potential way of improving the complexity and aromatic typicity of wines [29–31]. In fact, a commercial kit is already available for the sequential inoculation of *Torulaspora delbrueckii* and *Saccharomyces cerevisiae* (LEVEL2^{TM}, Lallemand). The induction of controlled maloalcoholic fermentation (total or partial) through the use of *Schizosaccharomyces* spp. is also awakening interest as a way of reducing the 'green apple sourness' that malic acid brings to wine. The genetic modification of *Saccharomyces* spp. has been investigated as a means of bringing this about [32–35], but the use of genetically modified organisms (GMOs) is controversial and, in fact, restricted at the industrial level by European legislation (CE No 479/2008).

In recent years, *Schizosaccharomyces* spp. immobilised in alginate beads [4, 33, 34, 36], mixed with *Saccharomyces* or employed sequentially with the latter [10, 11] as a means of mitigating its scant oenological aptitude [21] have all been successfully used to remove malic acid from wine.

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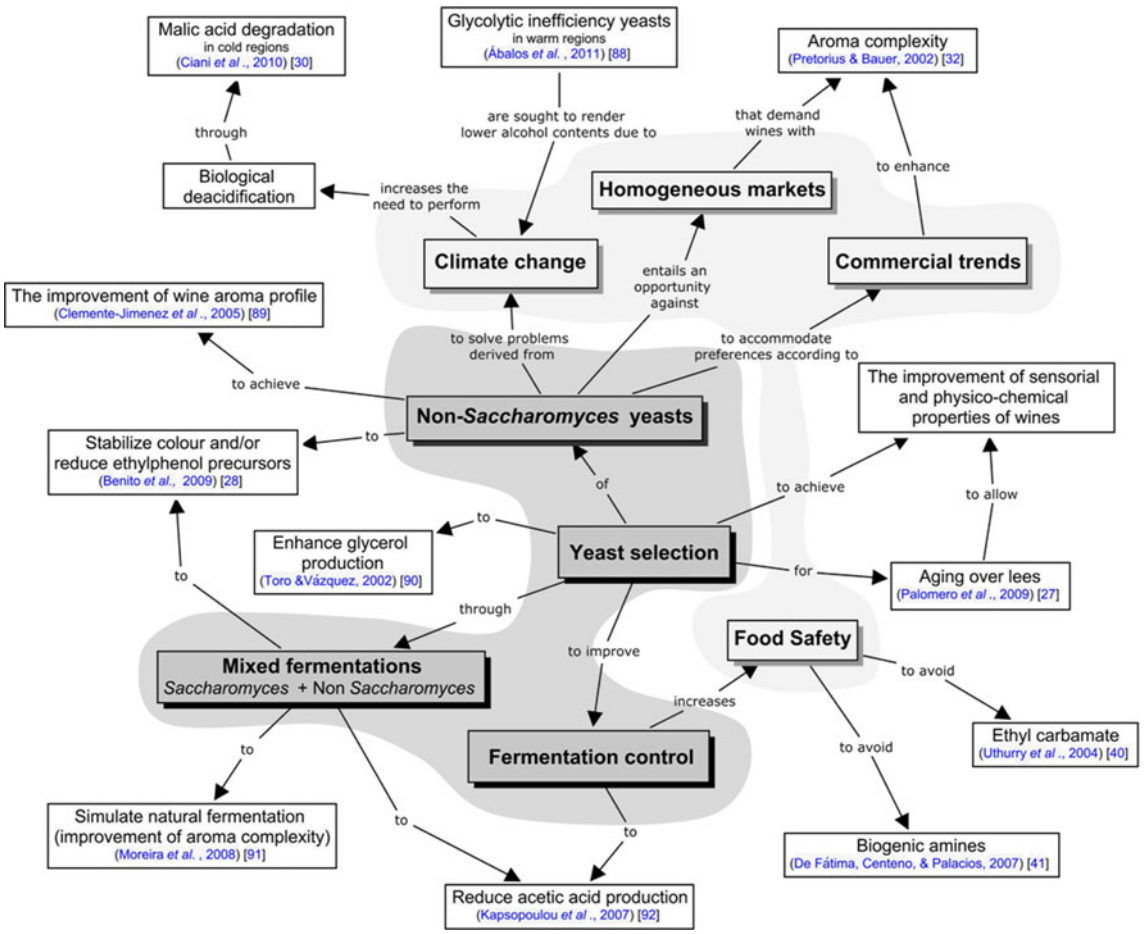

Fig. 1 Re-evaluation of the role of non-*Saccharomyces* yeasts in winemaking. *Light grey area* encompasses novel problems in viticulture, oenology and wine marketing; *darker grey area* encompasses new tools and new ways of solving different issues

Experiments have also been performed to determine the capacity of different strains of *Schizosaccharomyces* spp. to eliminate high levels of gluconic acid, a main factor determining the food safety of grapes [38]. The urease activity attributed to *Schizosaccharomyces* [39] could also be used to reduce high levels of ethyl carbamate in wine through the removal of its urea precursor [40]. Recent studies have also looked into the effectiveness of malic deacidification (or demalication) by selected strains of *Schizosaccharomyces* spp. immobilised in alginate beads [41].

Taxonomy, morphology and physiology of *Schizosaccharomyces* spp.

The Dutch school of Lodder [42] and Kreger van Rij [43] recognised four species belonging to *Schizosaccharomyces*: *Schizosaccharomyces pombe* Lindner (1883) [44], *Schizosaccharomyces octosporus* Beijerinck (1894) [45], *Schizosaccharomyces japonicus* var. *versatilis* Wickerhan and Duprat (1945) [46] and *Schizosaccharomyces malidevorans* Rankine and Fornachon (1964) [47]. The corresponding classification criteria essentially involved the number of spores per ascus and the capacity to ferment maltose, melibiose and raffinose.

Recent findings suggest the genus *Schizosaccharomyces* to contain three species: *Schizosaccharomyces japonicus*, *Schizosaccharomcyes octosporus* and *Schizo. pombe* [48]. These are found in areas that have temperate to very hot climates.

The type species *Schizo. pombe* has elongated cylindrical cells of dimensions 3–5 × 6–16 μm. They exist either as single cells or in pairs (Fig. 2). *Schizo. pombe* is an ascosporogenic or sporulating yeast belonging to the family *Saccharomycetaceae*. It reproduces vegetatively by binary fission via the formation of a wall at the centre of the cell (Fig. 2). Pseudomycelia can be formed, but no film is produced on the surface of liquid media. Its cells do not assimilate nitrates, nor do they possess β-glucosidase, an enzyme required for breaking down arbutin. The species' fermentative power is high, producing 10°–12.6° of alcohol in anaerobiosis and 13°–15° with slight aeration [49].

Schizosaccharomyces pombe is capable of metabolising malic acid to produce ethanol and CO_2 [50] (Fig. 3). Chalenko (1941) [51] isolated a synonym of *Schizo. pombe*—*Schizosaccharomyces acidovorans* (*acidodevoratus*)—that removed practically all the malic acid from culture media.

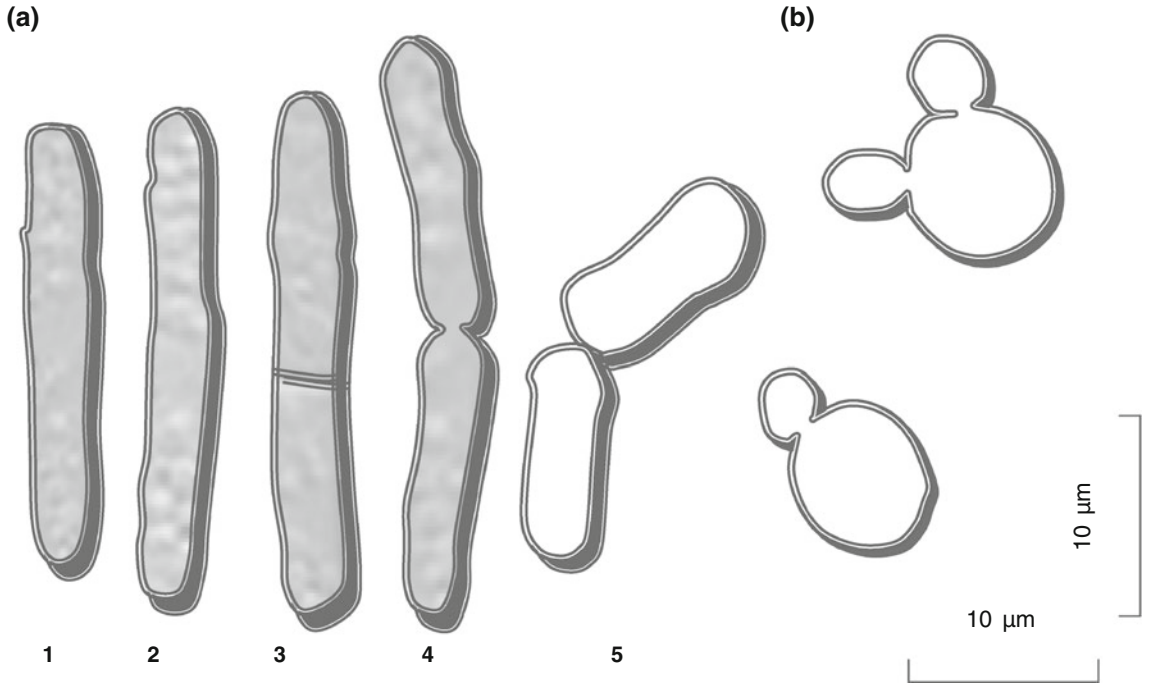

Fig. 2 Vegetative reproduction and morphology of **a** *Schizosaccharomyces* spp. and **b** *Saccharomyces cerevisiae*. *1–2* Yeast cells grow mainly by extension at their tips. *3–4* Septum formation in *Schizo. pombe*. *5* Binary fission completed

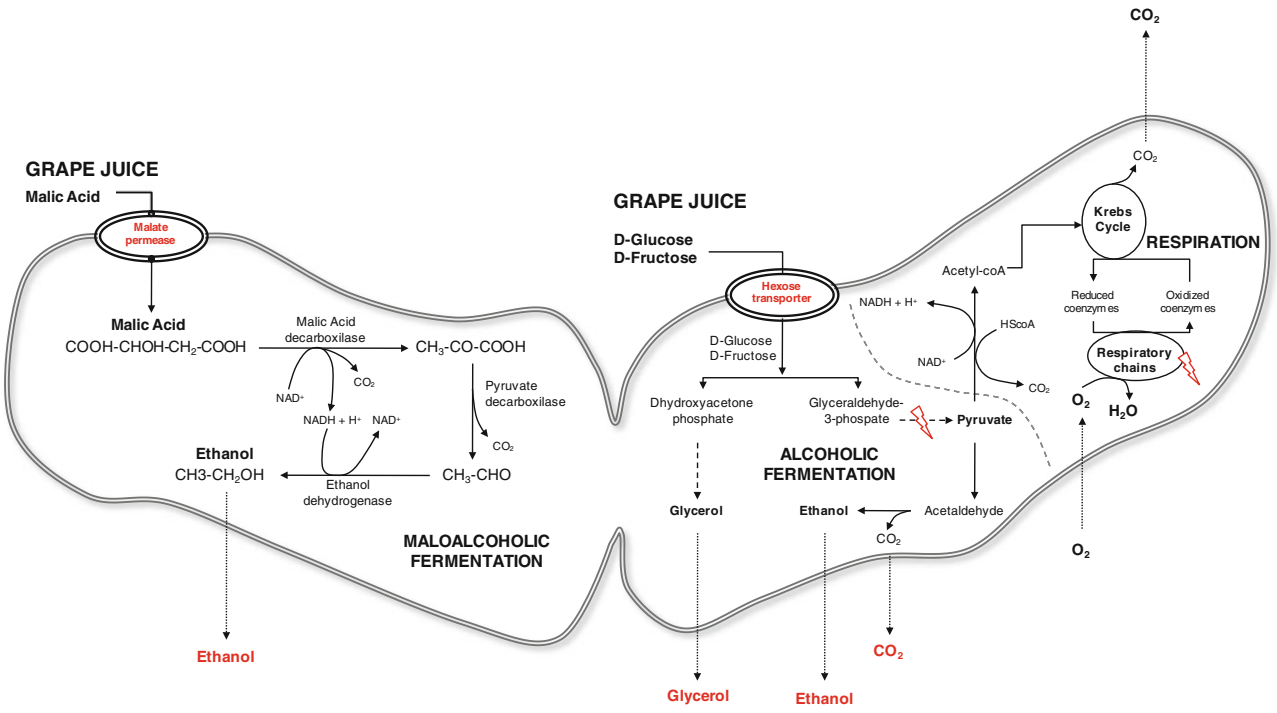

Fig. 3 Respiratory and fermentative metabolism of *Schizosaccharomyces* spp.

Industrial potential of maloalcoholic fermentation by *Schizosaccharomyces* spp.

Malic acid is one of the main organic acids present in grape must. Indeed, alongside tartaric acid it makes up 70–90 % of its total acidity, significantly influencing the final organoleptic characteristics of any ensuing wine [52, 53]. Its elimination is particularly necessary in red musts from areas with colder climates. Under such conditions, where growth cycles are short, grapes accumulate excessively high quantities of malic acid. Many authors have reported that malic acid can be metabolised by different species of yeast found in fermenting grape must, such as *Hansenula anomala* [54], *Candida sphaerica* [55], *Pichia stipitis* and *Pachysolen tannophilus* [56]. However, its reduction does not surpass 20–25 % of the initial concentration since the use of this carbon source is inhibited in the presence of sugar [5]. *Schizosaccharomyces* spp., in contrast, can reduce malic acid concentrations by 75–100 % (Table 1). Mayer and Temperli (1963) [57] were the first to show (via the measurement of the CO_2 released into a Warburg apparatus and the amount of ethanol formed) that *Schizosaccharomyces* spp. undertook maloalcoholic fermentation. One molecule of alcohol and two of CO_2 are produced for every malic acid molecule transformed (Fig. 3). As a first step, malic acid is broken down via malic acid decarboxylase into pyruvic acid in the presence of Mn^{2+}/Mn^{3+} ions. This pyruvic acid then enters the alcoholic fermentation pathway; it is first decarboxylated to acetaldehyde and then reduced to form ethanol (Fig. 3). Under anaerobic conditions, the degradation of 2.33 g/L of malic acid generates 0.1 % v/v of alcohol [58]. This ability could be of great use in the wine industry [6, 52, 59–64]. Indeed, a commercial strain of *Schizo. pombe*, used in an immobilised form, is now available for the removal of malic acid (ProMalic®; Proenol, http://www.proenol.pt/files/products/ProMalic_09_2008.pdf). The marketing of *Schizosaccharomyces* strains as dry, active yeast for demalication was approved back in 2003 at the 83rd Generally Assembly of the OIV in Paris (OENO/MICRO/97/75/Stage 7), yet the above strain remains the only one commercially available, suggesting that this potential resource remains largely unexplored.

Until now, the lactic acid bacteria *Oenococcus oeni* and *Lactobacillus plantarum* have been the most commonly used organisms for 'demalicating' musts and wines [65, 66], although not without difficulties. Indeed, demalication using these organisms is one of the most complicated processes in winemaking [67] (Table 2). Using *Schizosaccharomyces* yeasts, particularly *Schizo. pombe* and *Schizo. malidevorans*, for this task is easier since they grow more readily in musts and wines (Table 2). Their use also avoids the production of biogenic amines, unwanted by-products of lactic acid bacteria [41] (Table 1). Further, the immobilisation of *Schizosaccharomyces* spp. in alginate beads has been shown to offer good control over the breakdown of malic acid into ethanol. In addition, no post-demalication filtering is needed to remove any cellular remains, as would be the case if cells in free suspension were used [7, 68].

The traditional view of *Schizo. pombe* as a spoilage organism of wines and other beverages [21–23] has led some authors to recommend demalication be performed using other *Schizosaccharomyces* spp., followed by the use of *Saccharomyces* spp. for the main process of alcoholic fermentation [69, 70]. This limits the time that large populations of *Schizosaccharomcyes* spp. are allowed to exist, which seems to allow wines to be produced without olfactory problems [37].

Schizosaccharomyces pombe and ageing over lees

The structure and composition of the cell walls of *Schizosaccharomyces* spp. [71] render them interest for use in ageing over lees, an important technique employed in the production of high quality wines [72]. The polysaccharide fraction released from the walls through the action of the cells' own β-glucanases and wall mannosidases [73] has an important influence on the sensorial and physicochemical properties of wines aged by this technique.

The qualitative composition and the organisation of the wall polysaccharides differ between yeast species, although only a few species have been studied in any detail, and even fewer studies have investigated the distribution of the different components [74]. Weijman and Golubev [75] distinguished three types of yeast cell wall, two of which are of interest from an oenological viewpoint: the '*Saccharomyces* type' (with glucose and mannose) and the '*Schizosaccharomyces* type' (with galactose, glucose and mannose). Structurally, the wall of *S. cerevisiae* is largely made up of β-1,3-glucan with lateral β-1,6-branches [76]. These fibres are entwined with small quantities of chitin [77] to form the three-dimensional structure upon which the wall proteins and glucomannose complexes lie [78]. After enzymatically treating *Schizo. pombe* cells, Kopecka (1995) [79] showed their walls to have an interior layer of fibrillar glucan (α-1,3-glucan with lateral β-1,6-branches) and an exterior layer of amorphous glucans (largely β-1,3-glucan with some β-1,6-branches) with α-galactomannose residues.

Ageing over lees experiments with *Schizo. pombe* showed this yeast to have a complex wall polysaccharide profile, and that high molecular weight biopolymers were rapidly released from the walls during cellular autolysis (Fig. 2) [27]. These wall fragments showed good properties in terms of maintaining wine pigments in colloidal suspension, the

Table 1 Demalication using *Schizosaccharomyces* spp. described in the literature

Authors	Medium	Strains and sources	Culture	Results	Comments
Snow and Gallander [86]	Seyval blanc (85 %) and Aurora (15 %) musts 204 g/L reducing sugars 5.2 g/L malic acid pH = 3.2–3.56	*Sacch. cerevisiae* (Montrachet strain) Source not specified *Schizo. pombe* (UCD 592) University of California, Davis	Partial fermentation assays with *Schizo. pombe* over 1, 2, 4 and 6 days	$T_{1\ day}$ → malic acid degraded = 2.3 g/L (44.23 %) $T_{2\ day}$ → malic acid degraded = 3 g/L (57.69 %) $T_{4\ day}$ → malic acid degraded = 4.98 (95.76 %) $T_{6\ day}$ → malic acid degraded = 5.1 g/L (98.07 %)	Sensory evaluation revealed the wines produced by partial fermentation to be of better quality than those only fermented with *Schizo. pombe*
Magyar and Panyik [7]	Red *Vitis vinifera L. cv.* Blaufrenkish must 182 g/L reducing sugars 4.6 g/L malic acid pH = 3.39	*Schizo. pombe* RIVE 4-4-3 From Dr. Minarik, Bratislava, Czechoslovakia *Schizo. pombe* Y00315 NCAIM Budapest, Hungary Selected *Sacch. cerevisiae* Not specified	Sequential fermentation with *Schizo. pombe* trapped in Ca-alginate gel with different contact times (40, 48, 88 h)	$T_{40\ h}$ → malic acid degraded = 1.81 g/L (39.34 %) $T_{48\ h}$ → malic acid degraded = 2.55 g/L (55.43 %) $T_{40\ h}$ → malic acid degraded = 3.19 g/L (69.34 %)	–
	Partially fermented wines from red *Vitis vinifera L.cv.* Blaufrenkish must 5, 15, 50 g/L reducing sugars 4,6 g/L malic acid pH = 3.39	*Schizo. pombe* RIVE 4-4-3 From Dr. Minarik, Bratislava, Czechoslovakia *Schizo. pombe* Y00315 NCAIM Budapest, Hungary	Contact with immobilised *Schizo. pombe* cells (40, 30, 164 h) with no *Sacch. cerevisiae* inoculation to complete fermentation	$T_{40\ h,\ 50\ g/L\ sugar}$ → malic acid degraded = 2.99 g/L (65.00 %) $T_{30\ h,\ 15\ g/L\ sugar}$ → malic acid degraded = 2.42 g/L (47.39 %) $T_{164\ h,\ 5\ g/L\ sugar}$ → malic acid degraded = 3.95 g/L (85.86 %)	Demalication activity decreased with lower glucose and higher alcohol content
Taillandier et al. [87]	Semi-synthetic100 g/L glucose 8 g/L malic acid pH = 3	*Schizo. pombe* (G_2) Institut Coopératif du Vin (Montpellier, France) *Sacch. cerevisiae* Lalvin K1 Lallemand Inc. (Montreal, Canada)	Sequential inoculation with T_{delay} = 4, 8, 12, 16 h	T_{delay} = 4 h → malic acid degraded = 6.7 g/L (83.75 %) T_{delay} = 8, 12, 16 h → malic acid degraded = 8 g (100.00 %)	*Schizosaccharomyces* exhibited an amensal effect against *Saccharomyces*

Table 1 continued

Authors	Medium	Strains and sources	Culture	Results	Comments
Gao and Fleet [62]	Synthetic phosphate-tartrate-malate buffer 250 g/L glucose 3 g/L malic acid pH = 3.5	*Schizo. pombe* AWRI 160 Australian Wine Research Institute (AWRI) *Schizo. malidevorans* AWRI 158 Australian Wine Research Institute (AWRI)	High density cell suspension inoculation	*Schizo. pombe* AWRI 160 malic acid degraded after 48 h 2.85 g/L (95.00 %) *Schizo. malidevorans* AWRI 158 malic acid degraded after 48 h 2.94 g/L (99.00 %)	–
Thornton and Rodríguez [67]	*Vitis vinifera L.cv.* Chardonnay, Semillon and Cabernet grape musts 182–238 g/L reducing sugars 3.5–10 g/L malic acid pH = 3.2–3.56	*Schizo. malidevorans* UV mutant Australian Wine Research Institute (AWRI) *Sacch. cerevisiae Prise de Mousse* EC1118 Lallemand Inc. (Montreal, Canada)	Mixed and sequential inoculations with T_{delay} = 33–48 h	Complete degradation within 21–73 h	The wines produced lacked obvious organoleptic defects
Silva et al. [37]	Lab-scale conditions, store-bought white grape juice 165 g/L reducing sugars 8 g/L malic acidpH = 2.8	*Schizo. pombe* (G_2) Institut Coopératif du Vin (Montpellier, France)	Immobilised cells in double-layer Ca-alginate beads	Complete degradation within 50 h	Immobilisation did not alter the demalicating activity of the cells
	Winemaking conditions White *Vitis vinifera L.cv.* Azal must 200 g/L reducing sugars 8.4 g/L malic acid pH = 3.12	*Schizo. pombe* (G_2) Institut Coopératif du Vin (Montpellier, France) Selected *Sacch. cerevisiae* Source not specified	Sequential inoculation with immobilised *Schizo. pombe* cells in double-layer Ca-alginate beads at T_{delay} = 113 h	T_{delay} = 113 h → malic acid consumption = 6.4 g/L (76.19 %)	The wines made using *Schizo. pombe* were always more highly rated than control wine during sensory evaluation

Table 1 continued

Authors	Medium	Strains and sources	Culture	Results	Comments
Fátima, et al. [41]	*Vitis vinifera L.cv*. Albariño must No specified sugar content 8.5 g/L malic acid pH = 3.28	*Schizo. pombe* (Promalic; Proenol) Institut Coopératif du Vin (Montpellier, France) Selected *Sacch. cerevisiae* Source not specified	Sequential inoculation with *Schizo. pombe* and then *Sacch. cerevisiae* 2 days later	Reduction of malic acid concentration to 3 g/L (final desired content) in 13 days	Induced demalication using *Schizo. pombe* as a method to avoid any trace of biogenic amine production
	Vitis vinifera L.cv. Albariño must No specified sugar content 6.1 g/L malic acid pH = 3.13	*Schizo. pombe* (Promalic; Proenol) Institut Coopératif du Vin (Montpellier, France) Selected *Sacch. cerevisiae* Source not specified	Mixed inoculation of both yeasts	Reduction of malic acid concentration to 3.5 g/L (final desired content) in 7 days	

Table 2 Factors affecting malolactic and maloalcoholic fermentation

Commercial malolactic bacteria *O. oeni*, *L. plantarum*	Maloalcoholic yeasts *Schizo. pombe*, *Schizo. malidevorans*
Advantages	Advantages
Control of malolactic fermentation	More reliable growth in wine environment
	Better prospects of faster growth
	Ease of culture and handling
	Simple growth requirements
	High resistance to SO_2
	Can grow at very low pHs
	Grows over a wide range of temperatures
Disadvantages	Disadvantages
Failure to grow	Not commercially acceptable because of adverse effects on wine sensorial quality
Variations in time to malic acid depletion	Lack of selected strains
Low resistance to SO_2	
High sensitivity to temperature	
Complex growth requirements	
Excessive lactic acid and derivative production when high initial concentrations of malic acid are present; this can affect wine sensorial quality by leaving a 'sour milk' taste.	
Production of aromas and/or flavours detrimental to wine quality[a]	
Production of metabolites detrimental to wine safety (biogenic amines, ethyl carbamate)[a]	

[a] Non-commercial or wild malolactic bacteria

anthocyanins adsorbed onto the walls of the living yeasts being released with these post-autolysis wall fragments [27, 80]. The selection of appropriate *Schizo. pombe* strains holds the promise of being able to notably reduce the length of time red wines need to adequately age, as well as reducing the microbiological risks associated with the technique.

Other possible applications

Some authors have investigated the capacity of *Schizosaccharomyces* to eliminate gluconic acid, a compound that poses a major food safety problem, generally produced when grapes suffer attack by fungi such as *Botrytis* or *Aspergillus,* etc. during ripening [81]. The latter authors reported some strains to reduce must gluconic acid concentrations, but not enough for them to be of industrial interest (Table 2). The urease activity attributed to *Schizosaccharomyces* spp. [39, 82, 83] may also offer food safety advantages by reducing ethyl carbamate in wine through the removal of its urea precursor [40].

One of the main factors affecting the quality of red wine is its colour. Novel yeast selection criteria highlight the importance of acquiring strains that can increase the formation or pyranoanthocyanins [71, 80]. Fermentation with *Schizosaccharomyces* spp. could improve the production of these highly stable pigments (mainly vitisin A and B) [84]. The presence of these long-lasting and highly resistant pigments becomes particularly important when wines are aged in oak barrels over long periods of time [72]. During ageing, monomeric anthocyanins and their derived pigments slowly disappear while the more stable pyranoanthocyanins remain, resulting in a gradual increase in their proportion. The enhancement of the production of these types of wine colour-related pigments using mixed or sequential fermentations with *Schizosaccharomyces* spp. could, therefore, be of great interest when attempting to improve the chromatic properties of wine.

Another finding of interest is that wines obtained using *Schizosaccharomyces* spp. (both in mixed and sequential fermentations) presented lesser amounts of ethanol after sugar depletion was complete (submitted for publication). This glycolytic inefficiency could bring a key to solve excessive alcohol wine content, a situation that is now becoming more and more usual in warm viticultural regions [85].

Challenges for the future

It would be of great interest to select different *Schizosaccharomyces* strains with qualities of winemaking interest. However, it would first be necessary to develop selective media that could be used to isolate them; to date, no such media are available.

Conclusion

Schizosaccharomyces spp. may offer winemakers opportunities to reduce unwanted compounds in musts and wines, such as malic acid, gluconic acid and ethyl carbamate. The composition and structure of the cell walls of these yeasts may also offer advantages in the ageing of red wines over lees. Their use would also allow demalication without the production of biogenic amines, a problem associated with the traditional employment of lactic acid bacteria for this task. The selection of *Schizosaccharomyces* spp. strains may allow these functions to be optimised. However, much work would be first needed to develop the selective media that would enable their isolation.

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