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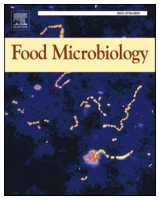

Genetic bases for the metabolism of the DMS precursor *S*-methylmethionine by *Saccharomyces cerevisiae*

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ABSTRACT

Dimethyl sulfide (DMS) is a sulfur containing volatile that enhances general fruity aroma and imparts aromatic notes in wine. The most important precursor of DMS is *S*-methylmethionine (SMM), which is synthesized by grapes and can be metabolized by the yeast *S. cerevisiae* during wine fermentation. Precursor molecules left after fermentation are chemically converted to DMS during wine maturation, meaning that wine DMS levels are determined by the amount of remaining precursors at bottling.

To elucidate SMM metabolism in yeast we performed quantitative trait locus (QTL) mapping using a population of 130 F2-segregants obtained from a cross between two wine yeast strains, and we detected one major QTL explaining almost 30% of trait variation. Within the QTL, gene YLL058W and SMM transporter gene *MMP1* were found to influence SMM metabolism, from which *MMP1* has the bigger impact. We identified and characterized a variant coding for a truncated transporter with superior SMM preserving attributes. A population analysis with 85 yeast strains from different origins revealed a significant association of the variant to flor strains and minor occurrence in cheese and wine strains.

These results will help selecting and improving *S. cerevisiae* strains for the production of wine and other fermented foods containing DMS such as cheese or beer.

1. Introduction

The perception of wine is tightly linked to its aroma, a complex blend of compounds with low boiling points, which are therefore volatile. These molecules can emerge from the liquid in the wine glass to become detectable by the human nose (Swiegers et al., 2005). Over 1000 aroma compounds have been found and described in wine (Tao and Li, 2009). Depending on their origin, these compounds can be divided into classes (Styger et al., 2011). Varietal aromas are contributed by the grapes and are distinctive for each grape variety. Fermentative aromas are produced by yeast and bacteria during alcoholic and malolactic fermentation. Post-fermentative aromas develop during conservation and aging of wine due to occurring transformations. However, these classes are not strictly separated. Varietal aroma contributors, for example, may exist as odorless precursors in the grape berry, which are then transformed to odorous compounds during harvesting, winemaking or ageing (Liu et al., 2017).

Dimethyl sulfide (DMS), a well described sulfur-containing odorant (Anness and Bamforth, 1982), is one of the most important aroma compounds released from grape derived precursors during the maturation of wine (Simpson, 1979). The olfactory perception threshold of DMS was described to be 27 μg/L in red wines (Beloqui et al., 1996) and 25 μg/L in white wines (Spedding and Raut, 1982). DMS is known to confer pleasant notes to Cabernet-Sauvignon (De Mora et al., 1987) and Shiraz red wines (Segurel, 2005). Furthermore, different white wines with small additions of DMS were preferred over their non-treated

Abbreviations: AdoMet, S-adenosyl methionine; DMS, Dimethyl sulfide; GMO, Genetically modified organism; LOD, Logarithm of odds; pDMS, DMS potential; QTL, Quantitative trait locus; RHA, Reciprocal hemizygosity analysis; SMM, S-methylmethionine.

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counterparts (Spedding and Raut, 1982). In low concentrations, DMS enhances the berry fruit aroma of wines (De Mora et al., 1987; Escudero et al., 2007; Lytra et al., 2016) and was reported to impart additional truffle and black olive notes (Segurel et al., 2004). In fact, DMS is a characteristic aroma molecule of black and summer truffles (Culleré et al., 2010). At higher concentrations and in certain wines, the presence of DMS is perceived less pleasant (Spedding and Raut, 1982). The compound can impart vegetal or molasses notes (Mestres et al., 2000). Correspondingly, DMS was described as a characteristic flavor compound in many raw and processed vegetables, such as tomatoes, asparagus, broccoli or cooked corn (Buttery et al., 1971; Dignan and Wiley, 1976; Tulio et al., 2002; Ulrich et al., 2001).

Different grape derived precursor molecules are able to form DMS in wine and therefore determine the DMS potential (pDMS). Besides from dimethyl sulfoxide (DMSO), yeast can generate DMS from the sulfur amino acid cysteine, and the sulfur containing amino acid derivatives cystine and glutathione (Anness and Bamforth, 1982; de Mora et al., 1986). With a vapor pressure of 53 kPa at 20 °C, DMS is relatively volatile (Lestremau et al., 2003) and molecules produced during fermentation are mostly driven off by CO_2, which results in low DMS concentrations observed just after fermentation (Dagan, 2006). The main precursor of DMS, however, was identified to be *S*-methylmethionine (SMM), an amino acid derivative accounting for more than 70% of formed DMS (Loscos et al., 2008). The synthesis of SMM is only reported in plants, where it is synthesized from methionine and *S*-adenosyl methionine (AdoMet), an intermediate of methionine metabolism (Mudd and Datko, 1990). SMM plays an important role for the phloem sulfur transport (Bourgis et al., 1999) and in addition acts as a precursor for the biosynthesis of dimethylsulfoniopropionate, an osmoprotectant (Trossat et al., 1998).

The uptake of SMM into yeast is achieved by the high affinity SMM permease, encoded by gene *MMP1,* and by another low affinity transporter system that could also account for the low affinity uptake of AdoMet (Rouillon et al., 1999). Inside the cell, SMM is metabolized, together with homocystein, into two molecules of methionine by the SMM-homocystein methyl-transferase Mht1 (Fig. 1) (Thomas et al., 2000). SMM can therefore be used as a sulfur source by the yeast (Rouillon et al., 1999).

The genes *MMP1* and *MHT1* are clustered in the genome of *S. cerevisiae* and oriented in opposite direction, being only separated by a *cis*-acting regulator sequence for the MET gene network (Thomas et al., 2000), a set of genes implicated in the steps of methionine biosynthesis (Thomas and Surdin-Kerjan, 1997). *MHT1* is regulated along with other MET genes, and both genes, *MMP1* and *MHT1*, are repressed by methionine and require the Met4 transcription factor for activation (Thomas et al., 2000).

The presence of DMS in wine mainly depends on its release from conserved precursor molecules through a chemical process during wine

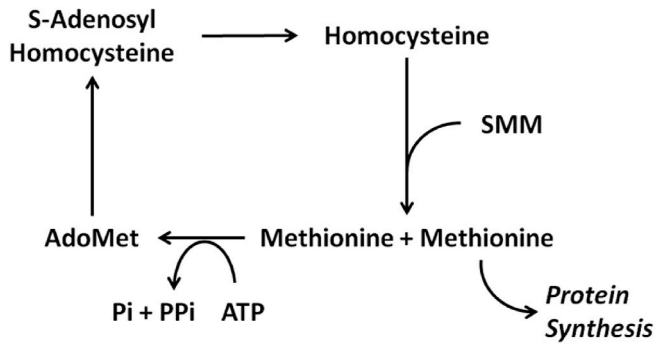

Fig. 1. Metabolism of SMM by *S. cerevisiae*. Two molecules of methionine are synthesized from SMM and homocysteine; One methionine molecule is recycled to homocysteine, the other methionine molecule can be used for protein synthesis; adapted from Thomas et al. (2000).

aging (Segurel et al., 2005), but only a small proportion of pDMS is recovered in young wines. The recovery rate depends on numerous factors, such as the yeast strain, yeast assimilable nitrogen content in must and the general winemaking process (Dagan and Schneider, 2012). Nevertheless, the remaining pDMS leads to the release of DMS during maturation, and DMS levels far exceeding the olfactory threshold were detected in older vintages (Dagan, 2006; Lee et al., 1993). Although the release of DMS is dependent on wine storage conditions - mostly storage temperature - the final level is determined by the pDMS content at bottling (Fedrizzi et al., 2007; Marais, 1979).

As the decrease of SMM during fermentation is mainly a yeast metabolic process dependent on several genes, the genetic properties of yeast are expected to account for trait variation, and differences between yeast strains in pDMS preservation could be observed (Dagan and Schneider, 2012; Deed et al., 2019). One approach to link genetic to phenotypic variation is quantitative trait locus (QTL) mapping, which has already been successfully applied in yeast to decipher enological important traits (Ambroset et al., 2011; Bartle et al., 2021; Brice et al., 2014a; Eder et al., 2018; Kessi-Pérez et al., 2020; Martí-raga et al., 2017; Marullo et al., 2019, 2007; Noble et al., 2015; Salinas et al., 2012; Steyer et al., 2012; Stovicek et al., 2015). In addition to studying the formation of fermentative aroma, Steyer et al. (2012) used a QTL mapping approach to assess the role of yeast metabolism on the transformation of grape derived aroma molecules during wine fermentation, and they found 5 genomic regions influencing the alteration of grape terpenols. However, the impact of yeast genetic properties on the metabolic reduction of pDMS has never been investigated until now.

The aim of this study was to understand the impact of *S. cerevisiae* genetic variation on the metabolism of SMM, the main determinant for pDMS, during wine fermentation. To this end, we pursued a QTL mapping approach with a set of 130 F2-segregants previously used to detect genomic regions influencing fermentative aroma formation and modeled intracellular metabolic fluxes (Eder et al., 2018, 2020). We detected a locus in the yeast genome with a dominant effect on SMM preservation and could validate two genes within the QTL, YLL058W (reserved name *HSU1*) and *MMP1*, to have an impact on the trait. We showed that the SMM-transporter gene *MMP1* has the major impact on SMM metabolism, and we identified and characterized an Mmp1 variant with superior preservation properties. The detection of *MMP1* and YLL058W variants offer new perspectives to select commercially available yeast starter cultures for the production of more aromatic wines and other fermented foods, or to improve commercial *S. cerevisiae* strains through non-genetically modified organisms (GMO) methods, like breeding via marker-assisted selection.

2. Materials and methods

2.1. Media

Yeast strains were cultured at 28 °C in yeast extract peptone dextrose (YPD) media, containing 10 g/L yeast extract, 20 g/L peptone and 20 g/L glucose. For solid YPD media, 1.5% agar was added. Selective YPD media contained 200 μg/mL geneticin (G418), 200 μg/mL nourseothricin (clonNAT) or 200 μg/mL hygromycin B.

Wine fermentations were carried out in synthetic grape must (MS200) as described by Bely et al. (1990). The synthetic must contains 100 g/L of each sugar, glucose and fructose, and 200 mg/L of assimilable nitrogen. The composition of amino acids and ammonium hereby mimics the nitrogen content of standard grape juice.

2.2. Strain selection, segregant generation and genotyping

The haploid *S. cerevisiae* strains MTF2621 (4CAR1 [Δ*ho*::Neo^r]) and MTF2622 (T73 [Δ*ho*::Nat^r]) were selected for the study according to their different need for nitrogen during wine fermentation. The requirement of nitrogen had been estimated by using an approach based

on the addition of nitrogen to keep the CO_2 production rate constant during nitrogen limitation (Brice et al., 2014b). The strain MTF2622 belongs to the phylogenetic clade of wine strains. The strain MTF2621, however, belongs to the group of champagne strains, which originated through crossings between strains of the wine and flor clades (Coi et al., 2016). For this study, we used a population of 130 segregants of the F2 generation obtained from a cross between these two strains (Eder et al., 2018). These strains have been genotyped by whole genome sequencing in order to generate a marker map for QTL mapping.

2.3. Phenotyping of strains

Segregant fermentations were carried out in duplicates with the parent strains as controls. Strains were precultured overnight in 50 mL YPD media under shaking and cell density was determined using a Multisizer™ 3 Coulter Counter (Beckman Coulter, Brea, CA). Sterilized 300-mL glassware mini fermenters were filled with 280 mL of SM200, supplemented with 500 μg/L *S*-methylmethionine chloride and closed with an air lock. Fermenters were inoculated to a cell density of 1×10^6 cells/mL and incubated at 24 °C under stirring (300 rpm). Flasks were weighted two times daily to determine CO_2 production, which directly reflects sugar consumption.

Samples to assess pDMS reduction were taken when approx. 80% of the sugars were depleted in order to determine the concentration of *S*-methylmethionine near the end of fermentation. These samples were then analyzed for pDMS by eliminating formed DMS, reacting SMM to DMS by heat alkaline treatment and determining DMS by solid phase micro extraction and GC-MS (Nyseos, Montpellier, France). pDMS concentrations are given as equivalent of DMS formation.

Samples to determine SMM and nitrogen compound uptake were taken in triplicates approximately every 2 h during the first 24 h of fermentation, and at the end after 28 h. pDMS was determined as described above. Amino acids were quantified with the Biochrom 30+ Physiological HPLC System (Serlabo, Entraigues-sur-la-Sorgue, France), which relies on the derivatization of amino acids with ninhydrin, forming a purple compound detectable at 570 nm proportional to the concentration of the amino acid. Proline and hydroxyproline, which do not possess an amine but an imine function, form a yellow compound detectable at 440 nm. For quantification, a physiological standard was used. This standard contains one volume of acidic and neutral amino acids (A6407, Sigma-Aldrich, St Louis, MO), one volume of basic amino acids (A1585, Sigma-Aldrich), one volume of glutamine at 2500 nmol/mL and two volumes of lithium citrate.

2.4. QTL mapping

The phenotyping and genotyping data were used to identify QTLs in the yeast genome that influence SMM metabolism during wine fermentation. The statistical analyses were carried out using the programming language R v3.2.3 (www.r-project.org) with the R/qtl v1.40-8 and R/eqtl v1.1-7 libraries (Broman et al., 2003). QTL mapping was performed with two different phenotype models, the normal model based on Haley-Knott regression and a non-parametric analysis. While in our case the normal model relies on statistics equivalent to a *t*-test and therefore performs best with residual variation (i.e., the phenotype distribution within each of the genotype groups at a putative QTL position) that follows a normal distribution, the non-parametric analysis requires less assumptions about data distribution (Broman and Sen, 2009). The mapping returned logarithm of odds (LOD) scores for each marker and pseudo-marker every 2.5 cM (interval mapping method). The span of each QTL was derived from the 1-LOD support interval, the region in which the LOD score is within 1 unit of the peak LOD score. In case the same locus was detected with both phenotype models, the result with the higher LOD score was presented, assuming that the corresponding model fitted the underlying data better.

2.5. Reciprocal hemizygosity analysis

Molecular dissection of QTLs was performed using reciprocal hemizygosity analysis (RHA) (Steinmetz et al., 2002; Warringer et al., 2017). The gene sequences of selected target genes were deleted in both parent strains by homologous recombination with a disruption cassette carrying the hygromycin B resistance gene (Hph^r). The disruption cassette was generated by PCR of the plasmid pAG32 (#35122, https://www.addgene.org/) with the primers del_(GENE)_fw/del_(GENE)_rv (Additional file 1). Transformants were selected for positive integration by plating them on YPD-agar plates containing hygromycin B. Correct gene deletion was verified by PCR using the primers test_[GENE]_fw/Hygro_rv (Additional file 1), which bind in the upstream region of the deleted gene and within the deletion cassette. Deleted parent strains were then mated with the respective undeleted parent to obtain a heterozygote that is hemizygous for the target gene. The obtained strains were phenotyped in biological triplicates and technical duplicates as previously described in section 2.3. Significance of the impact of allelic variation on the trait was evaluated by Student's *t*-test.

2.6. Allelic swap

The impact of the allelic variants on the phenotype was validated by exchanging the corresponding sequence between the parent cells, using the CRISPR/Cas9 toolbox developed by Mans et al. (2015). This approach has the advantage that genes and even SNPs can be rapidly exchanged in a marker- and scarless way. The *SpCas9* expression cassette was transformed into both parent strains via homologous recombination replacing *GAL1*. For the allele swap, the allelic gene variants were amplified with Phusion DNA polymerase according to the protocol (Thermo Fisher Scientific, Waltham, MA), using primers CasRep_MMP1_fw/CasRep_MMP1_rv (Additional file 1). An allele specific guide-RNA was designed and transformed into the parent cells where it induced the Cas9 mediated double-strand break within the variant sequence, while the corresponding allele of the other parent was provided as repair fragment. Positive sequence exchange was verified by allelic PCR, using a forward primer in the upstream region of the gene (CasRep_MMP1_fw) and an allele specific reverse primer on the targeted SNP position within the gene (tal_MMP1_1, tal_MMP1_2). The obtained strains were phenotyped in biological triplicates and technical duplicates as previously described in section 2.3 to validate the impact of the allelic variants on the trait.

3. Results and discussion

3.1. Phenotyping of strains

First, the ability of both parental strains to metabolize SMM, the precursor of DMS, was compared. The content of pDMS in synthetic must before fermentation (as equivalent of DMS formation) was determined as 282.2 ± 48.0 μg/L. The two parent strains consumed the majority of pDMS after 80% of fermentation but differed significantly in their ability to reduce the precursor. While the residual concentration of pDMS was 73.04 ± 18.30 μg/L for strain MTF2621, almost no precursor was left by strain MTF2622 (6.54 ± 6.95 μg/L pDMS).

As DMS is mainly originating from amino acid related compounds, we wanted to assess whether the consumption of pDMS is related to the consumption of certain amino acids. This could indeed indicate a common regulation or reveal the involvement of an amino acid transporter in pDMS uptake. To this end, we compared for both parent strains the uptake of nitrogen sources to the decrease of pDMS in the medium during the first 28 h of fermentation (Fig. 2).

Interestingly, the parental strains show a different behavior in their consumption of nitrogen sources. Strain MTF2621 consumed all available nitrogen within 28 h, while MTF2622 achieved this within 23 h of fermentation. A rapid decrease in pDMS could be observed for both

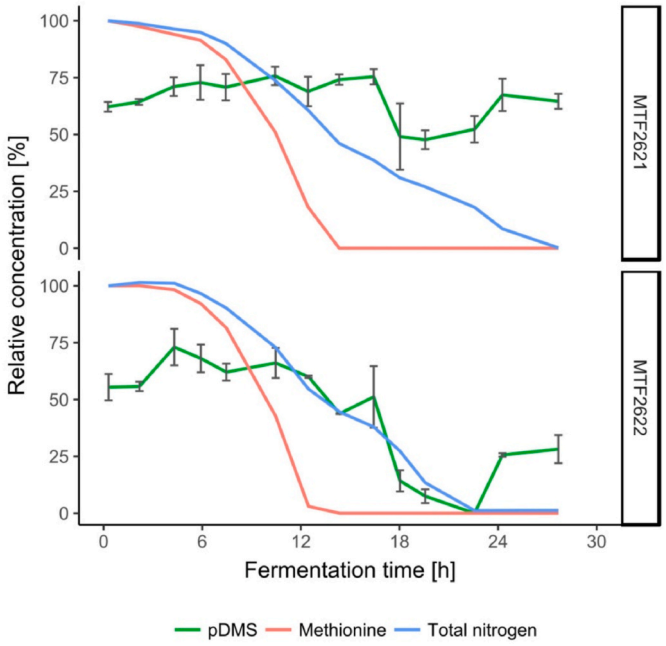

Fig. 2. Determined concentrations of pDMS (green) over the first 28 h of fermentation for the parental strains MTF2621 (top) and MTF2622 (bottom). Values for methionine (red) and the amount of total available nitrogen (blue) are shown for comparison. Concentrations are given in relation to the starting concentration determined in uninoculated synthetic must. Error bars for pDMS indicate standard error. Error bars for methionine and total nitrogen were omitted for visibility. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

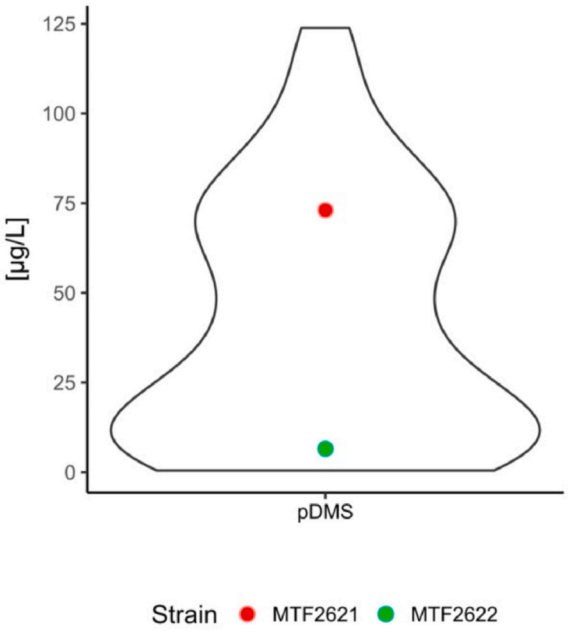

Fig. 3. Distribution of measured pDMS concentrations (as equivalent of DMS concentration) after 80% of fermentation among the population of segregants, together with the determined pDMS concentrations of parental strains MTF2621 (red) and MTF2622 (green). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

strains in the beginning of fermentation. In the first sample, taken after 20 min of inoculation, pDMS concentration was measured to be roughly 60% of the initial concentration determined in uninoculated must. A rapid decrease of pDMS after inoculation has been previously reported (Dagan and Schneider, 2012). For MTF2622, a second decrease of pDMS in the medium could be observed after 14 h. Since the decrease starts approximately with the depletion of methionine from the medium, this suggests that pDMS uptake is repressed by methionine as reported in the literature (Thomas et al., 2000). Strain MTF2622 had completely taken up all available pDMS after 23 h, which correlates to the depletion of all available nitrogen sources from the medium. However, the concentration of pDMS in the medium subsequently increased again and reached almost 30% of the initial concentration at the end of the measurement. This indicates that pDMS was actively taken up but was likely not metabolized completely and was subsequently released to the medium once all available nitrogen sources were depleted. For MTF2621, a minor decrease to 50% of the initial pDMS concentration could be observed between 16 h and 24 h of fermentation. In this case, the decrease of pDMS did not correlate with the depletion of methionine. In addition, the concentration of pDMS in the medium increased again before the depletion of all nitrogen sources and reached its initial level at the end of the measurement. This suggests that strains MTF2621 and MTF2622 differ in their ability to take up pDMS.

3.2. Genome wide identification of QTLs influencing SMM metabolism

We performed a QTL analysis in order to discover the genetic basis behind the different abilities to take up and metabolize SMM. A set of 130 F2-segregants from a cross between the parent strains MTF2621 and MTF2622 were phenotyped for their ability to reduce pDMS (Additional file 2). The distribution of remaining pDMS near the end of fermentation within the population of segregant strains revealed two sub-populations (Fig. 3). We performed a Shapiro-Wilk normality test on the phenotypic data with the R function shapiro.test(). From the output, a p-value of 8.139E-07 indicates that the distribution of the data is significantly different from a normal distribution.

A large fraction of the segregants (45.4%) had metabolized between 90 and 100% of the pDMS present in the initial must while a smaller fraction of the strains had left approximately 70.0 µg/L pDMS. These values match the determined concentrations for the parental strains, indicating the major influence of one locus on the trait. For the segregant with the highest preservation of pDMS, a concentration of 123.9 µg/L could be measured, which still corresponds to a pDMS loss of 56.1% compared to the unfermented medium.

We used the segregant marker map that we obtained from our previous study (Eder et al., 2018) to perform a linkage analysis of the segregants' ability to metabolize pDMS. One major QTL on chromosome XII was detected (Fig. 4), which matches the previous observation that the trait is mainly influences by one allele (Fig. 3).

The QTL was detected with both applied models, with the normal model yielding the higher LOD score of 9.46, meaning that approximately 29% of the trait variation can be explained by the locus. The detected region chrXII:17,035..24,535 has a size of 7.5 kB and contains four genes: *MHT1, MMP1, GTT2, and* YLL058W. Interestingly, *MHT1* and *MMP1* were described to be involved in pDMS metabolism (Rouillon et al., 1999; Thomas et al., 2000). *MHT1* is the only gene that does not contain non-synonymous SNPs between the parent strains. Two SNPs are located in the 1000-bp upstream region of the gene at positions −878 and −848, however, they are already located in *MMP1*.

The high significance of the detected region chrXII:17,035..24,535 for the trait could have masked the detection of additional QTLs with less influence (Broman and Sen, 2009). For this reason, QTL mapping was repeated with the marker chr12_20766 set as covariate. No additional QTL was found, although a region on chromosome XVI around 656,700 bp was close to being significant with an LOD score of 3.59. The region was more closely examined, but no target gene with a potential function for the trait could be identified.

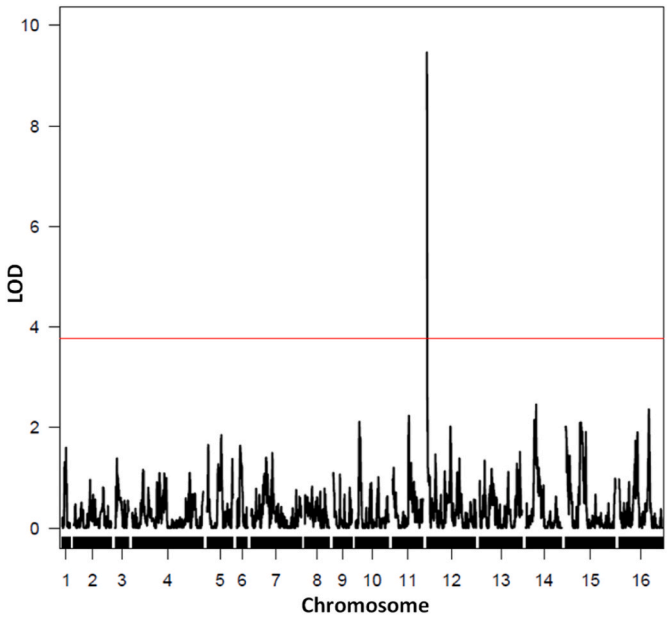

Fig. 4. Detected QTL on chromosome XII with an influence on the preservation of pDMS. Horizontal red line indicates LOD score significance threshold. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

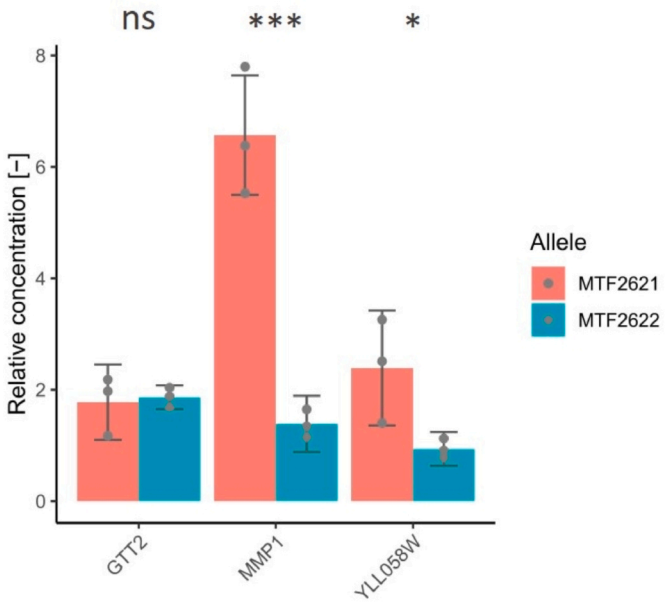

Fig. 5. Allelic impact of the genes *GTT2*, *MMP1* and YLL058W on the concentration of preserved pDMS after 80% of fermentation, assessed by RHA. Concentrations are given in relation to the undeleted parental heterozygote. Data points represent biological replicates. Error bars indicate standard error. p-value: ns > 0.05, * ≤0.05, *** ≤0.001.

3.3. *Variant of S-methylmethionine transporter Mmp1 causes pDMS preservation*

As in our previous studies that used the same set of segregants for QTL mapping (Eder et al., 2018, 2020), we focused for QTL validation on candidate genes within the QTL region that contained non-synonymous SNPs. However, it has to be emphasized that synonymous SNPs can contribute as well to phenotypic variation through impacting translation efficiency (She and Jarosz, 2018). Therefore, we cannot exclude that *MHT1*, which contains a synonymous SNP (aaG/aaA) at position 579 of the 975 bp long nucleotide sequence, contributes to the variation imparted by the QTL.

We performed reciprocal hemizygosity analysis (RHA) to evaluated the allelic effects of *MMP1*, *GTT2* and YLL058W. While no difference could be observed for the allelic variants of *GTT2*, the other two candidate genes showed significant difference between the alleles (Fig. 5).

The YLL058W allelic variants had a moderate effect, the hemizygote of the MTF2621 allele left a residual pDMS concentration at the end of fermentation more than 2.5 times higher than the MTF2622 allele. The variants differ in six non-synonymous SNPs within the coding region (Table 1), of which three lie in a predicted pyridoxal phosphate-dependent transferase domain of the protein (https://www.yeastgenome.org/). This domain is shared with several proteins involved in amino acid metabolic processes (Mozzarelli and Bettati, 2006). YLL058W encodes a protein of unknown function that shows similarity to the cystathionine gamma-synthase Str2 (Giaever et al., 2002). Str2 converts cysteine into cystathionine and is involved in the regulation of sulfur assimilation genes (Hansen and Johannesen, 2000).

The parental variants of the SMM transporter gene *MMP1* showed a bigger impact on the metabolism of pDMS. The MTF2621 allele leads to an increase of pDMS preservation of 4.7-fold in comparison to that of MTF2622. The variants differ in one non-synonymous SNP within the coding region (A536G), which results in the introduction of a STOP codon at position 179 of the 583 amino acid (AA) long polypeptide chain in strain MTF2622. This position is located within the amino acid permease sequence of the protein. Another SNP was found in the 1000bp upstream region of the gene, although no predicted transcription factor binding site was affected according to the YEASTRACT database (Teixeira et al., 2013).

Table 1
Differences in peptide chains of validated gene variants caused by non-synonymous SNPs between the parent strains. Comparison of AA identity to *S. cerevisiae* type strain S288C.

Gene	AA position	S288C	MTF2621	MTF2622
MMP1	179	W	W	STOP
YLL058W	98	K	K	E
	107	T	T	R
	131	A	A	T
	207	G	G	D
	213	P	P	S
	252	S	S	L

To assess whether the non-synonymous SNP in *MMP1* is responsible for the increased preservation of pDMS, we exchanged nucleotide 536 between the parent strains. This allelic swap resulted in strain MTF2621 *MMP1*(2622) with an eliminated early STOP codon in *MMP1* and strain MTF2622 *MMP1*(2621) with an introduced early STOP codon in *MMP1*. The elimination of the STOP codon completely inversed the phenotype of MTF2621 to MTF2622 (Fig. 6).

Strain MTF2621 *MMP1*(2622) had decreased pDMS concentration after 80% of fermentation to 18.25 μg/L, a level even lower than that showed by the parental strain MTF2622 (24.96 μg/L). This demonstrates that strains carrying this allele are not able to preserve SMM. A similar result could be seen for the opposite allelic swap: the introduction of the STOP codon within *MMP1* of MTF2622 almost completely inversed the phenotype of MTF2622 to MTF2621, increasing the preservation of pDMS over 3.6-fold from 24.96 μg/L to 90.97 μg/L. Although the difference was not significant, the concentration of preserved pDMS by MTF2622 *MMP1*(2621) was lower than for strain MTF2621 (113.0 μg/L). This could be an indication that other SNPs such the ones identified in YLL058W or *MHT1*, or external factors, like fermentation time, may also contribute to this phenotypic trait.

During fermentation, the concentration of pDMS decreased for all observed segregants, even for strains containing the truncated variant of

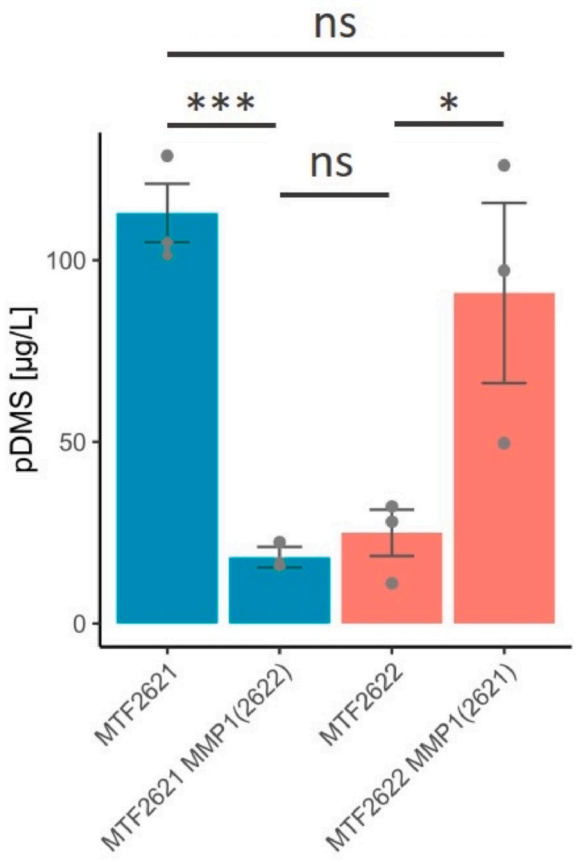

Fig. 6. Effect of the allelic swap of *MMP1* (non-synonymous SNP at nucleotide position 536) between the parent strains MTF2621 and MTF2622 on pDMS after 80% of fermentation. Data points represent biological replicates. Error bars indicate standard error. p-value: ns > 0.05, * ≤0.05, *** ≤0.001.

Mmp1 (Fig. 3). In addition, the parental strain MTF2622 showed a sharp decrease of pDMS right at the beginning of fermentation, although it contains the truncated Mmp1 transporter (Fig. 2). This could indicate the existence of other SMM transport mechanisms. For both strains, a release of pDMS could be seen after an initial uptake, indicating potential diffusion of pDMS with the concentration gradient. We cannot exclude that the observed pDMS precursor loss might also have yeast-genetic independent origins such as adsorption, degradation or evaporation.

3.4. Natural distribution of the MMP1 allelic variation across S. cerevisiae strains of different origin

To assess the distribution of the different *MMP1* alleles within *S. cerevisiae* strains of different origin, the *MMP1* nucleotide sequence was blasted against 85 yeast genomes recently sequenced in our group (Coi et al., 2017; Legras et al., 2018) or available in the *Saccharomyces* genome database (SGD) (Table 2, Additional file 3).

The *MMP1*^{G536A} mutation introduces a premature STOP codon and the resulting allele encodes a truncated protein of 179 amino acids instead of 583, which is likely not functional. This allele was found in 19 out of 85 tested strains. Out of all groups, only the flor yeast subpopulation showed a significant enrichment for the *MMP1*^{G536A} allele. All 8 flor strains screened harbor the truncated Mmp1 variant, contrasting with the high percentage (82.6%) of wine strains that possess the full-length variant. Apart from flor strains, although not statistically significant, several strains belonging to the "cheese" (50%), "bread" (33.3%), "rum" (20%), "wine" (17.4%) and the "North American oak" (12.5%) subpopulations harbor the truncated Mmp1.

Table 2
Presence of the truncated SMM transporter Mmp1, resulting from SNP G536A in the gene, in 85 assessed *S. cerevisiae* strains of different origin. The over-representation of the G536A single nucleotide variation leading to Mmp1 truncation was estimated by using a statistic based on hypergeometric distribution.

Strain origin	Full length Mmp1	Truncated Mmp1	% truncated	Enrichment *p*-value
African	4	0	0	0.36
Beer	3	0	0	0.46
Bread	2	1	33.3	0.41
Cheese	3	3	50	0.10
Clinical	1	0	0	0.78
Flor	0	8	100	1.57E-06
Laboratory	6	0	0	0.21
Oak Mediterranean	3	0	0	0.46
Oak North American	7	1	12.5	0.31
Others	8	0	0	0.12
Rum	8	2	20	0.31
Wine	19	4	17.4	0.19
Wine x flor	2	0	0	0.60
Total	66	19	22.4	

The significant concentration of the truncated Mmp1 allele in flor strains indicates a relation of this mutation and the origin. Recently, *MMP1* was also detected in a high-resolution QTL mapping study investigating survival in high ethanol environments, and a protein destabilizing amino acid exchange was proposed to promote this phenotype (Haas et al., 2019). Since flor wines, like sherry, tend to have a higher alcohol content, this could promote the selection of survival conferring *MMP1* variants. However, if the truncated Mmp1 allele detected in this study equally promotes survival in high alcohol concentrations has not been investigated.

To further evaluate the potential link between the truncated Mmp1 allele and flor strains, a phylogenetic analysis of all complete *MMP1* sequences (79) from the assessed strains was performed (Fig. 7).

Regarding the sequence of *MMP1*, the phylogenetic analysis shows a clear differentiation between two clusters, one containing flor and dairy isolates, and the other most wine alleles. This and the fact that all assessed flor strains contain the truncated Mmp1 allele would suggest that the SNP arose in flor strains. In this scenario, the occurrence of truncated Mmp1 alleles in wine strains (and others) could have been caused by gene flow from flor to wine yeast strains (as attested by the presence of the alleles of these 4 wine strains among flor alleles), an event described by Coi et al. (2017). In a possible hypothesis, the disproportionately high presence of the truncated Mmp1 variant in cheese strains could reflect human selection and represent a marker of domestication stirred by the positive flavor characteristics of elevated DMS levels in cheese. SMM is present in cow milk (Keenan and Lindsay, 1968) and DMS is an important flavor active substance in many types of cheese (Barbieri et al., 1994; Engels et al., 1997; Majcher et al., 2018; Manning, 1974). However, the majority of DMS is produced by propionic acid bacteria from methionine (Adda et al., 1982).

4. Conclusion

In this study, we confirmed the potential of QTL analysis to decipher enological important traits of *Saccharomyces cerevisiae*, in particular the ability to metabolize grape derived aroma precursors during wine fermentation. By phenotyping and genotyping a set of 130 F2-segregants deriving from a cross between two strains isolated from wine, we were able to detect a genomic region with a strong influence on the metabolism of SMM, the main precursor for aroma active volatile DMS.

Two genes in the QTL, *MMP1* and YLL058W, were found to influence pDMS and the SMM transporter gene *MMP1* was identified to be

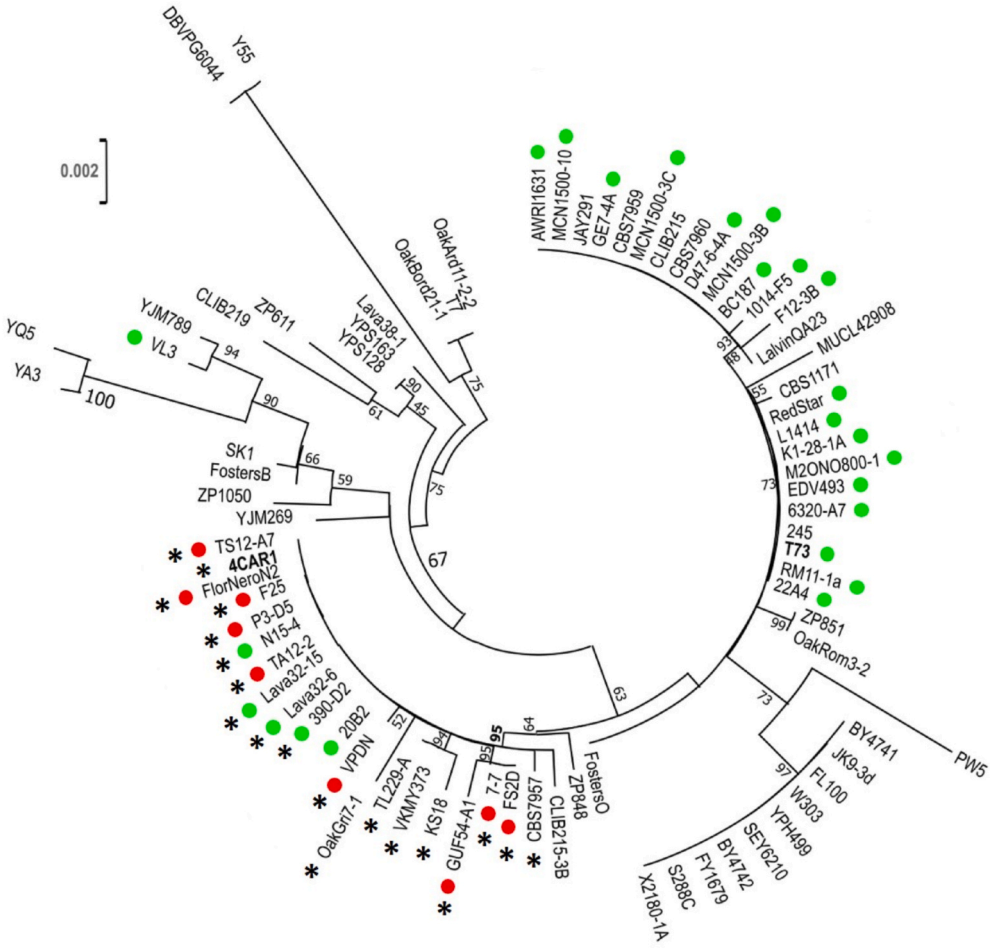

Fig. 7. Phylogenetic analysis of *MMP1* alleles from 79 strains of different origin. Wine strains are marked green and flor strains are marked red. Strains with truncated Mmp1 transporter are indicated with asterisks. Maximum likelihood tree was constructed by IQ-TREE v1.5.5 with optimal selection and 1000 bootstrap to assess branches support (Nguyen et al., 2015). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

responsible for the major part of trait variation. The MTF2621 allele of *MMP1* displays a strongly decreased ability to take up SMM from the medium and by performing allelic swap of the gene between the parent strains of the study, we identified SNP 536 G -> A to be causative for this behavior. The nucleotide change leads to the introduction of an early STOP codon at position 179 of the 583 AA long polypeptide chain, which results in the expression of a truncated SMM transporter Mmp1.

By comparing *MMP1* variants from 85 yeast strains of different origin, we could demonstrate that the truncated Mmp1 variant predominantly occurs in flor strains, therefore indicating a link to this origin. Rare findings of the described SNP in wine strains could be attributed to gene flow from flor to wine yeast strains.

These findings offer new perspectives for the management of pDMS in wine. The introduction of the truncated allele of *MMP1* into industrial strains by non-GMO methods, like breeding via marker-assisted selection, will generate superior strains for enhancing the preservation of SMM during wine fermentation and will therefore lead to a more fruity and aromatic wine. As DMS is a flavor active volatile present in other fermented foods, like beer (Anness and Bamforth, 1982) and cheese (Carbonell et al., 2002), the discovered genetic variation may therefore also be used to improve yeast starter cultures for a wide range of fermented products.

Declaration of competing interest

We see no issue relating to journal policies. The authors declare that they have no competing interest. All authors have read and approved the manuscript for submission. The content of the manuscript has not been published or submitted for publication elsewhere.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fm.2022.104041.

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