Aromatic thiols are potent aroma compounds, with a sensory perception threshold range in the parts per trillion. They are found in a wide range of foods (39), including animal products such as yoghurt and cheese, fruits, vegetables, tea, coffee, and alcoholic beverages (2, 4, 33, 35, 38). Of particular interest in wine fermentation are the aromatic volatile thiols, 4-mercapto-4-methylpentan-2-one (4MMP), 3-mercaptohexan-1-ol (3MH), and 3-mercaptohexyl acetate. These compounds impart flavors such as “grapefruit,” “passion fruit,” and “boxwood” and are major contributors to the varietal character of Vitis vinifera L. cv. Sauvignon blanc wines (17). Cysteine-S-conjugated bound forms of these free aromatic thiols are present in grape juice and are transformed into flavor-active thiol release in grape juice has been demonstrated by the constitutive expression of the Escherichia coli tnaA gene, a tryptophanase with strong CS β-lyase activity (29). Thus far, there is no direct evidence of such yeast-derived enzymatic activity releasing aromatic thiols under oenological conditions although some candidate genes have been suggested based on a gene deletion approach (15, 31). The release of aromatic thiols by other microorganisms has been linked to the activity of cystathionine β- and γ-lyases, for example, Lactobacillus casei and Lactobacillus lactis in cheese production (16, 20), Staphylococcus haemolyticus in mouth malodor (42).

Apart from its potential role in aromatic thiol release, cystathionine β-lyase (CBL; EC 4.4.1.8) is involved in the biosynthesis of methionine. CBLs catalyze the conversion of cystathionine into homocysteine in an α,β-elimination reaction, which in a later step is converted to methionine (32). This reaction is dependent on the cofactor, pyridoxal-5'-phosphate-dependent cystathionine β-lyase, and we demonstrated that this enzyme was able to cleave the cysteinylated precursors of 3MH and 4MMP to release the free thiols. These data provide direct evidence for a yeast enzyme able to release aromatic thiols in vitro that can be applied in the development of self-cloned yeast to enhance wine flavor.

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which a self-cloning approach could be adopted to improve the sensory properties of white wine.

MATERIALS AND METHODS

Similarities to the primary protein sequence of Str3p were identified using BLAST (http://blast.ncbi.nlm.nih.gov/BLAST/).

Yeast strains were cultivated at 30°C in either a rich medium, YPD (containing 1% yeast extract, 2% peptone, and 2% glucose), or a synthetic dropout medium, SCD (containing 2% glucose and 0.67% yeast nitrogen base without amino acids (Difco, Detroit, MI)). For the selection of sulfometuron methyl (SMM)-resistant transformants, the SCD medium was supplemented with 50 µM SMM (DuPont, Wilmington, DE) dissolved in N,N-dimethylformamide. Solid medium contained 2% agar (Difco).

DNA constructs. Standard procedures for the isolation and manipulation of genomic DNA were used as described in Ausubel et al. (1). The pDLG42-LS1 plasmid (29) was served as a template to amplify the E. coli tryptophanase (tnaA) gene using Phusion High-Fidelity DNA Polymerase (Finnzymes). For E. coli expression of proteins with a C-terminal six-histidine tag, we first cloned the tnaA gene into the pET-24(+) vector (Merck Biosciences) with the primers pET24-Ta-FWD (5'-CTCTAGATCCAAAAATATGAGAAGAAATGATTAGTGAAGAAACATTTAAAAC-3') and pET24-Ta-REV (5'-GGTCTGCGAAGACTCTTTCAAGTATTGCGTGAAG-3') using BamHI and XhoI restriction sites (underlined in both primers). The subsequent construct, designated pET-T, was then sequenced and kept at -20°C.

Size exclusion chromatography. Size exclusion chromatography was carried out with a Superdex 200HR 10/30 analytical column (GE Lifesciences) using an AKTA Explorer 100 fast protein liquid chromatograph (FPLC; Pharmacia/GE Lifesciences). The flow rate was 0.3 ml/min. Prior to sample loading, columns were equilibrated with buffer A. The column was calibrated with proteins of known molecular weights (Sigma-Aldrich) to produce standard curves.

For MALDI-TOF MS, Twenty-five micrograms of purified Str3p was dissolved by SDS-PAGE and subjected to a trypsin digestion and matrix-assisted laser desorption/ionization-time of flight mass spectroscopy (MALDI-TOF MS) by the Australian Proteome Analysis Facility Ltd., Sydney, Australia. All subsequent steps were carried out in the MassLab, Sydney, Australia.

Carbon-sulfur lyase assay. Reactions were carried out in a total volume of 1 ml containing a final concentration of 2 µg/ml of Str3p, 50 mM phosphate buffer, pH 8.5, 20 µM PLP, 1 mM EDTA, and a 2 mM concentration of the sulfur-containing amino acid substrate. Reaction mixtures were incubated for 1 h at 37°C and kept frozen until assayed for CS lyase activity. For pH optimum tests, the reaction buffer contained 50 mM morpholinopropanesulfonic acid (MES), 50 mM bis-Tris-propane (BTP), and 50 mM CAPS (N-cyclohexyl-3-aminopropanesulfonic acid), and, depending on the target pH (5.5 to 11), it was titrated with HCl or NaOH.

Cleavage of Cys-4MMP and Cys-3MH. Twenty-five micrograms of purified Str3p was incubated with 2 µg/ml purified Str3p. The data from three individual experiments were pooled and fitted to the Michaelis-Menten equation using PRISM (version 5) with R² values of 0.91 and 0.94 for l-cystathionine and l-djenkolate, respectively. For calculation of the catalytic turnover and catalytic efficiency, we used the assumption that Str3p was purified to homogeneity.

Enzymatic reactions with Cys-4MMP and Cys-3MH. Cleavage of Cys-4MMP and Cys-3MH was done in a total volume of 1.3 ml E. coli tryptophanase was included as a positive control. For negative controls, reactions were performed with Str3p that was heat inactivated for 2 min at 95°C and using protein eluted from nickel affinity chromatography and extracted from induced E. coli cells transformed with empty pET-24(+) vector. Indistinguishable concentrations of free thiol were detected in both negative controls. The conditions of the
Wine fermentation.YPD medium was inoculated with strains VIN 13, VIN 13 (STR3), and VIN 13 (CSL1). Yeast starter cultures were made in autoclaved grape juice and incubated for 48 h at 28°C to stationary phase. Frozen 2007 Viognier L. cv. Sauvignon blanc clarified juice (obtained from the Adelaide Hills, Australia) was thawed, thoroughly mixed, and filter sterilized using a VacuCap 60PF filter unit (0.8/0.2-μm pore diameter; Pall Life Sciences).

The basic chemical parameters of the juice were 198 g/liter sugar, 460 mg/liter yeast-assimilable nitrogen, and a pH of 3.2. A volume of 200 ml of the juice was transferred to 250-ml fermentation flasks with air locks, and the juice was inoculated at a density of 1 x 10⁹ cells/ml from the starter cultures. The wines were fermented in triplicate at 18°C for 15 days and then cold stabilized at 4°C. The wines were then racked and kept in 100-ml glass reagent bottles at 4°C until analysis. The concentrations of sugars, ethanol, glycerol, acetic acid, malic acid, tartaric acid, and succinic acid were measured by HPLC using a Bio-Rad HPLC-87H column as described above for quantitation of α-ketocids. Low-molecular-weight carbohydrate compounds that are known off-odors were quantified by gas chromatography coupled with sulfur chemiluminescence detection (GC/SCD) (27).

Headspace GC/MS analysis. An aliquot of 1 ml of the enzymatic reaction mixture (or diluted tryptophanase reaction mixture) was assayed in a total volume of 5 ml containing approximately 20 mg EDTA and 2 g NaCl in a 20-ml solid-phase microextraction vial with a magnetic crimp cap (Gerstel, Baltimore, MD). A solution containing a mixture of deuterated standards of [2H10]4MMP (9.85 μg/ml) and [2H10]3MH (14.32 μg/ml) in ethanol was added using a glass syringe with a 0.5-μl loop. Grace Davison Discovery Sciences, Rosville, Victoria, Australia) to each of the samples. The instrumental conditions were as described in Swiegers et al. (29) with the following modification: the autosampler was fitted with an automated 2-cm divinylbenzene/carboxybetadimethylsiloxane solid-phase microextraction fiber (Supelco, Bellefonte, PA). For quantification, mass spectra were recorded in the selective ion monitoring mode. The ions monitored were m/z 81, 96, 108, 141, and 142 for [2H10]4MMP; m/z 55, 75, 89, and 99, and 132 for [2H10]3MH; m/z 60, 62, 92, 109, and 144 for [2H10]3MH; and m/z 55, 82, 100, and 134 for 3MH. Selected fragment ions were monitored for 20 ms each. The underlined ion for each compound was the ion typically used for quantitation, having the best signal-to-noise ratio and the least interference from other components. The other ions were used as qualifiers. Analysis of 3MH at wine-like concentrations was carried out using a newly developed method (6).

RNA purification. Approximately 1 x 10⁷ yeast cells were harvested from fermentations by centrifugation, prepared in RNA-Later (Ambion), and kept at −80°C until analysis. The cells were washed by resuspension in 300 μl of ice-cold nuclease-free H₂O and centrifuged at 1,500 x g at 4°C. The cells were then resuspended in 100 μl of Zymolyase buffer (50 mM Tris-HCl pH 7.5, 1 M sorbitol and 10 mM MgCl₂) containing 30 mM dithiothreitol (DTT), and incubated for 15 min at room temperature to remove any disulfide bonds. Zymolyase 20T from Arthrobacter latus (MP Biomedicals, Aurora, OH) (20 units) was added to Zymolyase buffer containing 1 mM DTT and incubated at 30°C for 40 min.

RNA was isolated using a PureLink RNA mini-kit (Invitrogen) according to the manufacturer's instructions. Briefly, lysis was carried out by adding 200 μl of lysing buffer containing 1% β-mercaptoethanol, followed by centrifugation at 16,000 x g for 2 min. An equal volume of 99% ethanol was added to the supernatant, followed by thorough vortexing. The resulting lysate (500 μl) was transferred to a PureLink RNA mini-spin column, and RNA was isolated according to the manufacturer's instructions. Purified RNA was treated with 1 unit of amplification-grade DNase I (Invitrogen) for 15 min at room temperature. The reactions were stopped by the addition of 5 mM EDTA and incubation at 65°C for 5 min. A typical yield of 500 ng of total RNA was obtained as quantified by the QUBIT quantification platform using a Quant-iT RNA assay kit and standards (Invitrogen).

Reverse transcription. cDNA was synthesized from 100 ng of total RNA using an oligo(dT)₁₂₋₁₆ primer and an Affinity Script quantitative PCR (qPCR) cDNA synthesis kit (Stratagene, Agilent Technologies). All steps in cDNA synthesis were performed according to the manufacturer's instructions.

qPCR. Quantitative PCR was performed using a Bio-Rad CFX96 real-time detection system with Brilliant II SYBR green reagent (Agilent Technologies) and cDNA made from 2.5 ng of total RNA in a volume of 25 μl. To quantify the transcript level of the STR3 gene, data were normalized using ACT1 as a reference transcript. Primers for STR3 (STR3.FWD, 5'-TCAAACTCTACCGAGAACAAACAACAG-3' ; STR3.REV, 5'-CGTCAAGCCGCTATATACCTAG-3') and ACT1 (Act.FWD, 5'-GACAAGATAGAACCACCAATCC-3' ; Act.REV, 5'-CTGATGTGATGTCGATGTCCGTAAGG-3') were validated with efficiencies of 99.4% and 100.6%, respectively. Threshold cycle (Cₜ) values were obtained from duplicate fermentations, and STR3 expression was normalized against the actin gene reference by the 2⁻⁻ΔΔCₜ method and expressed relative to the native promoter at day 5.

Nucleotide sequence accession number. The sequence of the variant STR3 gene used in this study was submitted to GenBank under accession number HQ008776.

RESULTS

Analysis of the S. cerevisiae cystathionine β-lyase STR3 sequence. The STR3 gene from the diploid commercial wine yeast, EC-1118, displayed three heterozygous allelic variants, G244A, A411G, and T633C, compared with the S288c haploid reference strain. Of these, only the G244A variant results in an amino acid change in the protein sequence (A82T). This variant is not present in the recently published whole genome shotgun sequence of the EC-1118 strain (21), assembled as a pseudo-haploid due to the low rate of heterozygosity observed in this strain. The STR3 gene used in our study contained both G244A and T633C variants (GenBank under accession number HQ008776). Based on its primary amino acid sequence, Str3p is highly conserved among other budding yeasts, with identities of 66% for Lachancea thermotolerans and 51% for Clavispora lusitaniae. It also displays 44% identity to the fission yeast S. pombe STR3 homologue, 40% identity to an A. thaliana CBL and 29% identity to the E. coli CBL encoded by metC. The Str3p amino acid sequence diverges from that of E. coli tryptophanase; both belong to the large group of aspartate aminotransferase fold type I enzymes (25).

Purification of S. cerevisiae Str3p. We expressed recombinant Str3p in E. coli and purified the protein in the presence of its cofactor, PLP, using Ni-nitritriacetic acid (NTA) chromatography to capture the C-terminal six-histidine-tagged protein (Fig. 1). Monomeric recombinant Str3p has a predicted molecular size of 53 kDa and migrated at approximately 52 kDa on an SDS-PAGE gel. A yield of 40 mg of pure protein per liter of culture was typically obtained. The buffer exchange conditions to stabilize the isolated protein and maintain its enzymatic activity included 500 mM KCl and 20 mM EDTA. By including glycerol in the buffer, less than 6% of the activity was lost in a freeze-thaw cycle, compared to the 56% loss in the
We have demonstrated that Str3p displays a broad specificity toward cysteine-S-conjugates, we asked whether Str3p would also release the aromatic thiols 3MH and 4MMP from their respective cysteinylated precursors, Cys-3MH and Cys-4MMP. *S. cerevisiae* Str3p was able to release 12.3 μM 4MMP and 2.1 μM 3MH from 2 mM concentrations of their respective precursors when reactions were conducted with 31 μg/ml purified enzyme (Fig. 3). This side activity of Str3p against Cys-4MMP and Cys-3MH corresponds to 1.3% and 0.2% of the *E. coli* tryptophanase activity, respectively, and approximately 0.6 to 0.1% of the specific activity toward its physiological substrate, t-cystathionine. In addition, a reaction at pH 7.0 released 47% of the 4MMP released by Str3p at pH 7.5 (Fig. 3B). This reduced activity at pH 7.0 is consistent with the pH rate profile of Str3p with t-cystathionine as a substrate (Fig. 2). The amount of 3MH and 4MMP formed was dependent on the concentration of precursor for both enzymes. Cys-3MH was, however, the most effective substrate at a concentration of 0.25 mM (0.54 versus 0.30 μM free 3MH and 4MMP, respectively). This led us to investigate the influence of Str3p in 3MH release under fermentation conditions.

**Volatile thiol release during fermentation of a *V. vinifera* L. cv. Sauvignon blanc grape must.** Since Str3p displayed a modest side activity toward the cysteinylated aroma precursors, we tested whether overexpression of the *STR3* gene in the wine yeast VIN 13 could increase the release of the aromatic thiol 3MH under oenological conditions. The modified strain, VIN 13 (*STR3*), was used to ferment a *V. vinifera* L. cv. Sauvignon blanc grape juice, alongside the VIN 13 (CSL1) strain previously engineered to express the *E. coli* tryptophanase gene *tnaA* (29). The level of the *STR3* mRNA transcript was monitored during fermentation by reverse transcription-quantitative PCR (RT-qPCR) at day 5 (when 60% of the sugar was consumed) and at day 15 (at the end of fermentation). In the VIN 13 control strain, the level of *STR3* transcript under the control of its native promoter increased 12.3-fold between day 5 and day 15 of fermentation (Fig. 4). Compared with the control strain, *STR3* transcript levels in the modified strain, VIN 13 (*STR3*), were 18-fold higher at day 5 but only 50% higher by the end of fermentation. The *STR3* overexpression strain displayed sustained induction during the course of fermentation, whereas *STR3* under the control of the native promoter was highly expressed toward the end of fermentation.

All fermentations proceeded at similar rates and were dry (less than 2 g/liter sugar) after 15 days. Other chemical param-

### TABLE 1. Substrate specificity of purified Str3p

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Relative activity (%)</th>
</tr>
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<tbody>
<tr>
<td>L-Cystathionine</td>
<td>100 ± 10.9</td>
</tr>
<tr>
<td>L-Djenkolate</td>
<td>154 ± 11.0</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>220 ± 2.1</td>
</tr>
<tr>
<td>S-Ethyl-L-cysteine</td>
<td>9.0 ± 0.7</td>
</tr>
<tr>
<td>S-Methyl-L-cysteine</td>
<td>7.4 ± 0.3</td>
</tr>
<tr>
<td>L-Cysteine</td>
<td>1.0 ± 0.3</td>
</tr>
<tr>
<td>L-Methionine</td>
<td>0.0</td>
</tr>
</tbody>
</table>

* The formation of pyruvate was detected by HPLC and expressed relative to the activity with t-cystathionine ± standard deviations. Data are from triplicate experiments.

* Specific activity toward L-cystathionine was 1,258 ± 138 μmol/min/mg of protein.
eters (pH, glycerol, ethanol, and organic acid production) were analyzed after fermentation with the different strains and found to be almost identical (Table 2), including concentrations of other key low-molecular-weight volatile sulfur compounds known to adversely affect wine flavor.

The VIN 13 (CSL1) strain released substantial amounts of 3MH, 9.5 times more than the control VIN 13 strain, consistent with the levels described previously in a synthetic medium spiked with the Cys-3MH precursor (29). The 3MH concentration after fermentation with the VIN 13 (STR3) strain was 27% (278 ng/liter) higher than in fermentation with the VIN 13 control strain ($P = 0.014$) (Table 3).

**DISCUSSION**

The biochemical properties of the *S. cerevisiae* STR3 gene product have not previously been characterized. Its function has been inferred by gene disruption, yielding a yeast strain that could not grow on cystathionine as the sole sulfur source (3, 12). In this study, we purified Str3p in order to determine some of its biochemical properties. We confirmed that Str3p is a CBL, with the highest catalytic efficiency for L-cystathionine.

In accordance with previously characterized CBL enzymes, Str3p forms a stable tetrameric enzyme consisting of four PLP-bound subunits.

As observed for enzymes of this class from *A. thaliana, S. oleracea,* and *E. coli,* Str3p also displayed broad substrate specificity, including some activity toward the cysteine-S-conjugate substrates S-ethyl-L-cysteine and S-methyl-L-cysteine. The latter substrate, together with S-methyl-L-cysteine sulfoxide, occurs in high concentrations in *Brassica* and *Allium* vegetables. Characteristic flavors of these vegetables are partly derived through enzymatic degradation of these amino acids by CS lyases when their tissue is disrupted (11).

The *in vitro* incubation of purified Str3p with cysteine-S-conjugates of 3MH and 4MMP confirmed our hypothesis that the enzyme has a residual cysteine-S-conjugate β-lyase activity.

**FIG. 3.** GC/MS quantification of enzymatic reactions with purified Str3p. The release of 3MH and 4MMP was quantified with headspace GC/MS in reaction mixtures incubated with 0.25 mM or 2 mM cysteine-S-conjugated precursor (29). Data shown are the means of triplicate experiments ± standard deviations. ND, not detected.

**FIG. 4.** Quantitative RT-PCR of the STR3 mRNA level at day 5 and day 15 during fermentation with the commercial wine yeast, VIN 13, and a strain modified to overexpress STR3, VIN 13 (STR3). Data shown are the means of four data points from duplicate fermentations ± standard errors of the means.

**TABLE 2.** Basic composition of the *V. vinifera* L. cv. Sauvignon blanc wines made using the modified VIN 13 (STR3), VIN 13 (CSL1), and the control VIN 13 strains.

<table>
<thead>
<tr>
<th>Detection method and component</th>
<th>Amount of component in strain†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VIN 13</td>
</tr>
<tr>
<td>Alcohol (% [vol/vol])</td>
<td>11.5 ± 0.3</td>
</tr>
<tr>
<td>Residual sugar (g/liter)</td>
<td>0.1 ± 0.0</td>
</tr>
<tr>
<td>Acetic acid (g/liter)</td>
<td>0.05 ± 0.01</td>
</tr>
<tr>
<td>Glycerol (g/liter)</td>
<td>4.6 ± 0.13</td>
</tr>
<tr>
<td>Malic acid (g/liter)</td>
<td>2.6 ± 0.02</td>
</tr>
<tr>
<td>Tartaric acid (g/liter)</td>
<td>1.8 ± 0.01</td>
</tr>
<tr>
<td>Succinic acid (g/liter)</td>
<td>2.0 ± 0.07</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Detection method and component</th>
<th>Amount of component in strain†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VIN 13</td>
</tr>
<tr>
<td>Hydrogen sulfide (μg/liter)</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>Methanethiol (μg/liter)</td>
<td>4.1 ± 1.8</td>
</tr>
<tr>
<td>Ethanethiol (μg/liter)</td>
<td>ND</td>
</tr>
<tr>
<td>Dimethyl sulfide (μg/liter)</td>
<td>11.4 ± 6.0</td>
</tr>
<tr>
<td>Carbon disulfide (μg/liter)</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>Diethyl sulfide (μg/liter)</td>
<td>ND</td>
</tr>
<tr>
<td>Dimethyl disulfide (μg/liter)</td>
<td>3.2 ± 0.8</td>
</tr>
<tr>
<td>Ethyl thioacetate (μg/liter)</td>
<td>0.3 ± 0.4</td>
</tr>
<tr>
<td>Diethyl disulfide (μg/liter)</td>
<td>ND</td>
</tr>
</tbody>
</table>

† Data are from triplicate fermentations ± standard deviations. ND, not detected.
TABLE 3. Production of 3MH in a V. vinifera L. cv. Sauvignon blanc grape must using the modified VIN 13 (STR3), VIN 13 (CSL1), and the control VIN 13 strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>3MH (ng/liter)</th>
<th>p&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIN 13</td>
<td>1,084 ± 66</td>
<td></td>
</tr>
<tr>
<td>VIN 13 (STR3)</td>
<td>1,362 ± 84</td>
<td>0.014</td>
</tr>
<tr>
<td>VIN 13 (CSL1)</td>
<td>10,268 ± 548</td>
<td>0.001</td>
</tr>
</tbody>
</table>

<sup>a</sup> Data shown are the means of triplicate fermentations ± standard deviations quantified by headspace GC/MS.
<sup>b</sup> Determined by a one-tailed student t test.

and was able to cleave these substrates to release the corresponding aromatic thiols. The reactions occurred in a concentration-dependent manner, and Str3p displayed a preference for Cys-3MH at low substrate concentrations and for Cys-4MMP at high concentrations. To our knowledge, this is the first direct evidence of a purified yeast enzyme displaying a CS β-lyase activity necessary to cleave the cysteine-S-conjugates of 4MMP and 3MH.

Previous studies based on gene disruption have identified several yeast genes (IRC7, CYS3, and BNA4) that may contribute to the release of 3MH and/or 4MMP (13, 15, 31). The contribution of each was, however, unclear since the effect of the deletions on thiol release was strongly dependent on the experimental conditions used, and some results were contradictory. Interestingly, IRC7, which was suggested to encode the main enzymatic activity involved in the release of 4MMP during fermentation (31), is annotated as a putative CBL based on sequence similarity. This activity has never been demonstrated experimentally; nonetheless, yeast CBLs, apart from their physiological function of cleaving cystathionine to yield homocysteine for methionine biosynthesis, could be harnessed to drive the formation of aromatic thiols during beverage fermentation. We therefore integrated an additional copy of the gene under the control of the constitutive promoter, PGK1<sub>p</sub>, in the commercial yeast strain VIN 13. Enhanced 3MH released by the VIN 13 (STR3) strain represents the first proof of concept that a yeast-derived gene can be used in place of the CSL1 construct (29) to harness latent flavor potential during wine fermentation. STR3 may not encode the optimal enzyme for this purpose since, in addition to its side activity against aromatic thiol precursors, the STR3 gene is among a group of genes transcriptionally upregulated during fermentation (19). Nonetheless, since the VIN 13 (STR3) strain was able to release 278 ng/liter more 3MH than a control strain and since 3MH has a sensory detection threshold of 60 ng/liter (33), this increase illustrates the potential for CBLs to modulate wine aroma. Although the results of the in vitro experiment indicate that enzymatic activity of Str3p is directly responsible for the increase in 3MH during wine fermentation, we cannot rule out that Str3p overexpression could affect expression of other genes in sulfur amino acid metabolism. However, overexpression of Str3p did not affect the concentrations of other low-molecular-weight volatile sulfur compounds known to adversely affect wine flavor.

In conclusion, we have demonstrated that a yeast enzyme, Str3p, is a CBL with side activity toward cysteine-S-conjugated thiols and that expression of STR3 can be manipulated in wine yeast to effectively alter the composition of volatile thiols in a wine fermentation. In vitro characterization of other yeast enzymes with putative cysteine-S-conjugate β-lyase activity, in conjunction with structural bioinformatics, represents a path forward to improve our understanding of volatile thiol release during fermentation and develop optimal self-cloned yeast strains for enhanced wine flavor.

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We also acknowledge Drew Sutton at Flinders University for assistance with size exclusion chromatography and Paul Chambers for critical reading of the manuscript.

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