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Screening of brewing yeast β-lyase activity and release of hop volatile thiols from precursors during fermentation

Volatile thiols derived from some hop varieties largely impact the flavour of beer even though they are found in relatively low concentrations. The described thiols are further present in slightly higher concentration as flavourless cysteinylated precursors in hops. Yeasts have been found to release these thiols via the enzyme β-lyase and therefore increase the amount of flavour-active thiols. To investigate the β-lyase activity of brewing yeasts and increase the volatile thiol content derived from hops in beer, 148 strains of three different species (*Saccharomyces pastorianus*, *S. cerevisiae*, *Torulaspora delbrueckii*) were screened. The *IRC7* genotype of each strain was investigated as this gene was described as having a big impact on the β-lyase activity of *S. cerevisiae* wine yeasts. A selective media was also used to detect yeast strains with increased β-lyase activity. Fermentations were then performed to show the impact of six yeast strains on the content of the volatile thiols 3-mercaptohexanol and 4-mercapto-4-methylpentan-2-one. A very homogeneous distribution of the *IRC7* gene was found for the investigated brewing yeasts. The applied selective medium showed low predictability for β-lyase activity but needs further investigations. Transfer rates for 3-mercaptohexanol were found to be above 1000 % in the finished beers. The rise in 4-mercapto-4-methylpentan-2-one was found to be significantly influenced by the yeast strain and indicated the presence of other precursors or a potential biotransformation.

Descriptors: 3-mercaptohexanol, 4-mercapto-4-methylpentan-2-one, *Saccharomyces cerevisiae*, *Torulaspora delbrueckii*, *Saccharomyces pastorianus*

1 Introduction

Some volatile thiols have become highly desired flavour compounds in hopped beers due to their fruity flavours and their low flavour threshold [1–4]. These thiols are present in varying concentrations in flavour-active and flavourless precursor forms in different hop varieties such as Mosaic®, Citra®, Eureka®, Nelson Sauvin® and Tomahawk® [1, 4]. The main desirable flavour-active thiols are 3-mercaptohexanol (3MH), 4-mercapto-4-methylpentan-2-one (4MMP) and 3-mercaptohexyl acetate (3MHA). The flavour threshold for 4MMP is as low as 1.5 ng/L with a blackcurrant and passion-fruit-like flavour impression in beer. 3MH has a rhubarb and grapefruit flavour, which is noticeable in beer at a threshold of 55 ng/L. 3MHA has a passion fruit and grapefruit flavour and a flavour threshold of 4 ng/L [3, 5]. The low perception thresholds of these thiols have attracted attention in recent years due to new ultra-precise analysis procedures and the revival of dry hopping following the increased popularity of craft beer [6].

The desired volatile thiols described above, along with some undesired thiols (such as 2-sulfanylethyl acetate and 3-sulfanylpropyl acetate (roasted-/burnt-like)), are well known as some of them contribute to flavour expressions of many different foods such as juices, onions, bell peppers and wine [7, 8]. For wine makers they are essential. 4MMP in particular is a major contributor to the primary flavour impression of the Muscat, Scheurebe, Sauvignon Blanc and Petite Arvine varieties [4, 9, 10]. It has been shown that yeast and, more precisely, β-lyases derived from yeast are partly responsible for the conversion of the bound flavour-inactive thiols present in grapes of special varieties to free volatile and therefore flavour-active compounds [9, 11]. The release of flavour-active thiols from their corresponding cysteinylated precursors is dependent on the yeast strain. The conversion rate in wine fermentation experiments was found to be below 5 % [12]. Keeping in mind that the concentrations and flavour thresholds of these compounds are comparably low, a conversion rate of 5 % makes a big difference to the total amount [13]. A selection process for *Saccharomyces cerevisiae* wine yeasts with increased β-lyase activity was recently published by *Belda* et al. [9]. A selective medium, S-methyl-L-cysteine (YCB-

https://doi.org/10.23763/BrSc19-26michel

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SMC), was used to differentiate between yeast strains that had varying β-lyase activity. This made it possible to select potentially high β-lyase-active strains, to increase thiol release from cysteine-bound precursors during wine fermentation. This selective medium included cysteine as the only nitrogen source that could only be set free by β-lyase. The authors reported that the growth of a culture could therefore be directly linked to the β-lyase activity and therefore to the ability to set free cysteinylated volatile thiols [9]. As it has been shown that it is also possible to enzymatically release volatile flavour-active thiols from precursors in hops with the help of β-lyase derived from *Escherichia coli* [4], the selection of industrial brewing strains for increased β-lyase activity may be one way to potentially boost hop flavour and/or potentially lower hop addition due to better exploitation.

Belda et al. also investigated the *IRC7* genotype of their applied *S. cerevisiae* and *Torulaspora delbrueckii* strains, as *IRC7* has been described as one of the genes mainly responsible for encoding carbon-sulphur β-lyase activity along with STE3 [9, 11, 14]. *IRC7* was found in two different genotypes in these yeasts. A 219 bp allele which represents a full length copy of the *IRC7* gene (homozygous for the full-length *IRC7* 7 allele), a short copy of the gene without 38 bp (homozygous for the short *IRC7* allele), and a version where the long and short copy of the gene are both present (heterozygous for the *IRC7* allele). It was found that the presence of either of these genotypes determined the ability of the particular yeast strain to release flavour-inactive cysteinylated thiols [9]. Belda et al. screened 223 wild strains of *S. cerevisiae* isolated from wine environments and 22 industrial wine strains in order to find differences for these alleles in wild and industrially used yeasts [9]. In a later study [11], Belda et al. also investigated *T. delbrueckii* for its β-lyase activity and, in contrast to other authors [12, 15, 16], found an increased ability to release volatile thiols from their precursors in wine. As *T. delbrueckii* has become of interest as a novel species for brewing [17–20], brewers will also be interested in the interaction of hops and this particular species.

As brewing yeasts (*S. cerevisiae* and *S. pastorianus*) represent a specially domesticated and diverse group [21, 22] the aim of this study was to look at the differences in brewing yeasts in the *IRC7* alleles to compare with the wild and industrial wine strains used by Belda et al. [9] and a variety of wine/distillers yeast strains from our collection. As *T. delbrueckii* has generated interest in regard to brewing and cysteine-bound thiol release, two *T. delbrueckii* brewing strains and 13 wild isolates were investigated along with the *Saccharomyces* brewing (98 in total) and wine/distillers (25 in total) -strains. Additionally, ten *S. cerevisiae* var. *diastaticus* strains were investigated as they represent a special variation of *S. cerevisiae* and are known to produce a greater variety of enzymes such as glucoamylases [23]. We looked further at the differences in utilisation of the YCB-SMC selective medium. The test was modified to be able to screen in liquid. We then tried to link the genotype to the growth in the medium and to select strains that could potentially change the hop aroma in terms of volatile flavour-active thiols. The initial approach was to manipulate the hop flavour by performing beer fermentations with promising strains.

Table 1 Yeast strains of various species used in this study

Yeast species	Application	Strain abbreviations
S. pastorianus	Bottom-fermenting beer yeast	CBS1513, CBS2440, TUM PIBA124, TUM 193, TUM 194, TUM 204, TUM 224, TUM 195, TUM 59, TUM 206, TUM 92, TUM 199, TUM 34/70, TUM 202, TUM 84, TUM 105, TUM 34/78, TUM 120, TUM 168, TUM 182, TUM 234, TUM 44, TUM 69, TUM 170, TUM 128, TUM 206, TUM 164, TUM 34/94, TUM 172, TUM 152, TUM 502, TUM 159, TUM 54, TUM 403, TUM 500, TUM 477, TUM 26, TUM 140, TUM 162, TUM 106, TUM 145, TUM 139, TUM 183, TUM 66/70, TUM 144, TUM 167, TUM 53
S. cerevisiae *wheat beer strains **ale strains ***others	Top-fermenting beer yeast	TUM 127*, TUM 306**, TUM 149*, TUM 148**, TUM 380W*, TUM 175*, TUM 192**, TUM 205*, TUM 174**, TUM 214*, TUM 338**, TUM 68*, TUM 308**, TUM 300W**, TUM 36, TUM 501*, TUM 184**, TUM 165*, TUM 341**, TUM 505*, TUM PI134***, TUM 177*, TUM 480***, TUM 378***, TUM 511**, TUM 504***, TUM 508**, TUM 476***, TUM 509***, TUM 211**, TUM 454*, TUM 506**, TUM 506 ALT**, TUM 490*, TUM 210**, TUM 335*, TUM 510**, MUC*, TUM 513**, TUM 453*, TUM 503**, TUM 435*, TUM 507*, TUM 438*, TUM 552**, TUM 220*, TUM 486*, TUM 479**, TUM 478**, TUM 381***,TUM 361N
S. cerevisiae *Distillers yeast **Sparkling wine +Banana wine ++Chicha corn wine °Sherry °°Rice wine	Wine/Distillers yeast	TUM S3**, TUM S1**, TUM S2**, TUM V2, TUM V1, TUM V15, TUM V8, TUM V12, TUM V9, TUM V6, TUM D4*, TUM D2* , TUM 545, TUM 548°, TUM 546, TUM 547°, TUM 518+, TUM 519+, BRY 465, TUM 61020, TUM 516, TUM 517, TUM V30, TUM 520++, TUM 521++
S. cerevisiae var. *diastaticus*	Spoilage yeast	TUM 376, TUM 379, TUM 71_(Cont. A), TUM 71_(Cont. B), TUM 182(PI), TUM 541, TUM 105(PI), TUM 514, TUM 512, TUM 216
T. delbrueckii *brewing strain **wild isolate	Special beer yeast	TUM T1*, TUM T90*, TUM 1-H6**, TUM 1-D6**, TUM 1-G7**, TUM 1-H1**, TUM 3-D3**, TUM 1-F5**, TUM 1-F7**, TUM 3-D7**, TUM 1-I6**, TUM 1-E2**, TUM 1-G6**, TUM 3-D4**, TUM 3-D5**

Table 2 Bound thiol precursors and free volatile thiols measured in the variety US-Mosaic® type 90 pellets determined according to Roland et al. [5]

Substance group	Substances	Abbreviation	Concentration [ng/g]
Bound thiol precursor	cysteine-3MH	C3MH	170
	cysteinyl-glycine-3MH	CG3MH	510
	γ-glutamyl-cysteine-3MH	GC3MH	n.d.*
	glutathione-3MH	G3MH	3400
	cysteine-4MMP	C4MMP	n.d.*
	cysteinyl-glycine-4MMP	CG4MMP	n.d.*
	γ-glutamyl-cysteine-4MMP	GC4MMP	n.d.*
	glutathione-4MMP	G4MMP	n.d.*
Volatile thiols	3-mercaptohexanol	3MH	24.8
	4-mercapto-4-methylpentan-2-one	4MMP	35.7

*n.d. = not detected; LOD according to [5].

2 Materials and methods

2.1 Yeast strains

Table 1 lists the yeast strains used in this study. Strains were grown on wort agar slopes for 72 hours at 28 °C and stored in a sterile environment at 2–4 °C until further use. The strains were subcultured at intervals of one month.

2.2 Hop raw material

Hop pellets type-90 of US- Mosaic® crop 2017 (α-acids: 12.1 %, total oils: 2.00 mL/100 g) were provided by Joh. Barth & Sohn GmbH & Co. (Nuremberg, Germany). Analytical data are given in table 2. The total essential oil in hops was determined according to standard ASBC method [24]. Precursors were analysed by Nyseos (Montpellier, France) according to *Roland* et al. [5].

2.3 YCB-SMC medium and application

The selective medium was created according to Belda et al. [9]. No agar was added as the test was modified to a 96-well-plate test in liquid. The medium composition was: 0.1 % (wt/vol) S-methyl-L-cysteine (Sigma-Aldrich, Taufkirchen, Germany), 0.01 % (wt/vol) pyridoxal-5′-phosphate (Sigma-Aldrich) and 1.2 % (wt/vol) Yeast Carbon Base (Difco, Detroit, MI, USA). The medium was adjusted to pH 3.5 with HCl and was filtered through a 0.2 µm sterile filter.

Each yeast strain was inoculated under sterile conditions in 5 mL of the YCB-SMC medium for two days, in a 10 ml test tube at room temperature prior to testing, as suggested by Belda et al., to eliminate the possible impact of nitrogen reserves in the yeast cells [9]. This procedure was performed twice before testing the strains in a 96-well plate to starve all the nitrogen reserves in the cells. A total of 200 µL of each of the previously enriched yeast cultures was transferred into one well of the 96-well plate, the optical density (OD_{600nm}) was measured at 600 nm in a Synergy 2 microplate reader (BioTek Instruments, Winooski, VT, USA). A blank sample of YCB-SMC medium was also measured and the blank OD_{600nm} was subtracted from the OD_{600nm} of the yeast sample. To ensure the test started each time with an OD_{600nm} of 0.1, each sample was measured and a dilution factor for each sample was calculated. To start the test, 200 µl YCB-SMC medium was pipetted into a new well and an aliquot of the yeast sample was added to a starting OD_{600nm} of 0.1. The utilisation test was performed in triplicate. Enrichment was performed at constant shaking at 28 °C for 48 hours to measure the maximum growth rate with OD_{600nm} measurement every 10 minutes inside the microplate reader.

2.4 *IRC7* PCR-Amplicon capillary electrophoresis

DNA was isolated from each investigated yeast isolate by taking a single colony from the corresponding wort agar slant, using an inoculation loop. This colony was transferred to a 1.5 mL Eppendorf tube, and mixed with an aliquot of 200 µL Insta-GeneTM Matrix solution (Biorad, Munich, Germany). The mixture was vortexed for ten seconds and incubated at 56 °C for 30 minutes, followed by another ten seconds of vortexing and incubation at 96 °C for eight minutes. The incubation steps were performed in a Thermomix 5436 (Eppendorf, Hamburg, Germany). After incubation, the Eppendorf tube was centrifuged at 13,000 × g for two minutes then a 100 µL aliquot of the DNA-containing supernatant was transferred to a new 1.5 mL tube. The DNA concentration was adjusted to 25 ng/µL after being measured by a Nanodrop 1000 spectrophotometer (Thermo Scientific, Wilmington, USA).

PCR was performed with 12.5 µL RedTaq Mastermix (2x) (Genaxxon, Ulm, Germany) and 2.5 µL template DNA with a total reaction volume of 25 µL. The Mastermix contained 12.5 µL buffer solution (RedTaq Mastermix), 7.0 µL DNA-free PCR water and 1.5 µL of each primer (Biomers, Munich, Germany). The cycling parameters were: A pre-denaturing step at 94 °C for 300 s, then 35 cycles for denaturing at 95 °C (94 °C for *T. delbrueckii*) for 15 s, for annealing and elongation at 56 °C (57 °C for *T. delbrueckii*) for 30 s and 72 °C for 60 s and for final elongation at 72 °C for 300 s. PCR was performed using a SensoQuest LabCycler48s (SensoQuest GmbH, Gottingen, Germany). Amplified PCR products were analysed using a capillary electrophoresis system (Agilent DNA 1000 Kit) following the manufacturer`s recommendations (lab on a chip, Bioanalyzer Agilent 2100, Agilent Technologies, Santa Clara, CA, USA).

Primers applied for the *IRC7* gene fingerprint of *Saccharomyces* species were PF6 5′- AGCTGGTCTGGAGAAAATGG-3′ and PR7, 5′-TCTTCTGCGAGACGTTCAAA-3′ according to *Roncoroni* et al. [25]. Primers for the corresponding region in *Torulaspora delbrueckii* were ITdF 5′- AGCTGGGCTCAAGGATATGC- 3′ and ITdR 5′-GTGACTCTTGAGACGCTCAA-3 used according to Belda et al. [11].

2.5 Statistics and bioinformatics

Statistics: One-way analysis of variance (ANOVA), Tukey test (triple determinations), Scheffé-test (unbalanced sample amount) Boxplots and linear fitting were calculated using Origin 2018b

(OriginLab Cooperation Northampton, United States). Significant levels were chosen between $p < 0.01$ and $p < 0.05$. Different letters in figures indicate significant different groups, same letters indicate significant same groups.

Bioinformatics: Based on the specific capillary electrophoresis *IRC7* patterns, a degree of relationship was calculated using the Bionumerics program 7.6 (Applied Maths, Belgium). For the calculation a curve-based cluster was analysed using a Pearson correlation with an optimisation degree of 0.5 % and a band-based cluster was analysed using a Jaccard correlation with an optimisation of 0.5 % and a tolerance set of 1 %.

2.6 Volatile thiol detection

The thiols 3MH and 4MMP were quantified in beer and hop samples using LC-MS/MS according to *Capone* et al. [26]. An Agilent 1290 Infinity II high-performance liquid chromatography system was therefore directly connected to an Agilent 6470 triple quadrupole mass spectrometer (Agilent Technologies, Waldbronn, Germany). Separation was performed using a Raptor FluoroPhenyl column (2.7 µm, 100 x 2.1 mm, Restek, Bad Homburg, Germany).

2.7 Fermentation trials

Small-scale 2 L fermentation trials were performed using stainless steel vessels as stated by *Meier-Dörnberg* et al. [23]. Fermentations were carried out with bunging pressure of 0.5 bar. An all-malt wort of 12 °P was produced by diluting unhopped wort extract (72 °P original gravity) (Weyermann®, Bamberg, Germany) with deionised water. Individual yeast strains were pitched at 20 °C at a pitching yeast count of 30 x 10^6 cells/mL propagated in unhopped wort at 20 °C. In addition, 1.5 g of US - Mosaic® type 90 hop pellets (see Table 2 for the data) was added to each 2 L fermentation in hop bags. For each yeast strain four 2 L fermentations were created, one of which did not contain any hops (blank sample). Fermentation vessels were closed and left to ferment at 20 °C without pressure for ten days. After fermentation a maturation step of three weeks was performed at 0 °C.

2.8 Beer biochemical analysis

Specific gravity, pH value and ethanol concentration were measured using an Anton Paar DMA 5000 Density Meter with Alcolyzer Plus measuring module, pH measuring module, and Xsample 122 sample changer (Anton-Paar GmbH, Ostfildern, Germany).

3 Results and discussion

3.1 *IRC7* genotypes

The release of aroma-active thiols from their cysteinylated precursors in wine fermentations was found to be influenced by the presence of two varying genotypes of the ICR7 gene of the applied yeast strain [9, 11]. We started this investigation to compare wine and beer yeasts for their *IRC7* genotype and to find potential brewing strains with high β-lyase activity. We investigated 47 bottom-fermenting (*S. pastorianus*) and 51 top-fermenting (*S. cerevisiae*) beer yeast strains along with ten *S. cerevisiae* var. *diastaticus*, 25 wine and distillers yeast strains (*S. cerevisiae*) and 15 *T. delbrueckii* strains for their *IRC7* genotype (Table 1). As already described by Roncoroni et al. [25] we found a short and a long version of the *IRC7* gene. Surprisingly, we also found that three of the wild isolates of *T. delbrueckii* (TUM 1-F7, TUM 3-D4, TUM 3-D5) did not possess an *IRC7* gene that could be detected with our primers (Table 3). In contrast to the wine yeasts investigated in this study the beer yeasts showed a very homogenous result for the *IRC7* genotype distribution as visible in table 3. One of the *S. pastorianus* strains (TUM 206) was in possession of a long and a short version of the *IRC7* gene whereas TUM 502 only had a short version. All other investigated bottom-fermenting strains were in possession of the long *IRC7* genotype. This underlines the thesis that bottom-fermenting yeasts are genetically a very homogenous group as *S. pastorianus* was found to be a hybrid of one hybridisation event of one *S. cerevisiae* and one *S. eubayanus* parent [27, 28]. Three (TUM 335, TUM 438, TUM 480) of the 51 top-fermenting beer strains possessed a long and short version of *IRC7* whereas all others possessed only the long genotype. All ale yeasts were in possession of only the long *IRC7* allele. The investigated wine yeasts showed an equal share of the long and the short and long version of the *IRC7* gene. These findings indicate that almost all yeast strains investigated in this study should be able to release cysteinylated precursors by β-lyase.

Table 3 Distribution of the *IRC7* genotype across all the investigated yeast species

Yeast species	Application	Total number of investigated strains	Genotype of *IRC7* Gene			
			Long	Short	Long/ Short	None
S. pastorianus	Bottom-fermenting beer yeast	47	45	1	1	0
S. cerevisiae	Top-fermenting beer yeast	51	48	0	3	0
S. cerevisiae var. diastaticus	Spoilage yeast	10	3	0	7	0
S. cerevisiae	Wine/Distillers yeast	25	14	0	11	0
T. delbrueckii	Special beer	15	12	0	0	3
Total (Equivalent in [%] of total strains)		148 (100 %)	122 (82.4 %)	1 (0.6 %)	22 (14.8 %)	3 (2.0 %)

3.2 YCB-SMC medium growth and *IRC7* comparison

The selective medium YCB-SMC containing S-methyl-L-cysteine as the only nitrogen source was used to identify yeast strains with differing β-lyase activity. Yeast strains able to grow in this medium could release cysteine due to the presence of β-lyase [9]. A faster release of cysteine would in theory mean faster growth in this medium and therefore a higher β-lyase activity. As we used

differing species with differing optimal growth temperatures (*S. cerevisiae*, *S. pastorianus* and *T. delbrueckii*) we only compared the strains by species as all growth experiments were performed at 28 °C. However, to get a better overview, figure 1 includes the maximal growth rate over 48 hours of all the species used.

T. delbrueckii strains showed much higher maximum growth on average than the used *Saccharomyces* species, which is expressed by the significantly higher values shown in figure 1. This can be explained as generation times are much lower for *T. delbrueckii* than for *Saccharomyces*. The optimal growth temperature for *T. delbrueckii* was determined at 27 °C by *Salvadó* et al. [29]. *S. cerevisiae* has an optimal growth temperature of 32 °C [29] whereas *S. pastorianus* has an optimal growth temperature at 30 °C [30]. In terms of maximum growth in the SMC medium, the distribution of the *S. pastorianus* species is homogenous in comparison with all other species. However, one strain, TUM 206, outperformed all other strains as visible by the outlier of the *S. pastorianus* group in figure 1. It is worth mentioning that this strain was the only one of all the *S. pastorianus* strains that possessed a long and a short allele of the *IRC7* gene. All the investigated strains of the *S. cerevisiae* species showed heterogeneous growth behaviour as some grew very well and some did not show growth at all (no growth: TUM 68, TUM 490, TUM 513, TUM 486). These findings suggest that the strains TUM 68, TUM 490, TUM 513, TUM 486 are not capable of producing the particular β-lyase for releasing cysteine from the applied precursor as they were not able to grow. The strain TUM 68 was therefore chosen for further investigations. Strains of the species *S. cerevisiae* var. *diastaticus* did not show significantly different growth patterns to the other *Saccharomyces* strains (Fig. 1).

As the group of *S. cerevisiae* wine/distillers yeast was found to be split almost in half by two differing genotypes (Long and Long/Short *IRC7* genotype) we compared the growth behaviour and the genotype. In comparison to Belda et al. [9] we did not find a significant dependency upon the genotype and the growth in the selective media as visible in figure 2 ($p < 0.01$). As for all other species, there were not enough strains that had differing genotypes to make a statistical comparison. It can be concluded that the complete *IRC7* gene is the most common across top- and bottom-fermenting brewing strains.

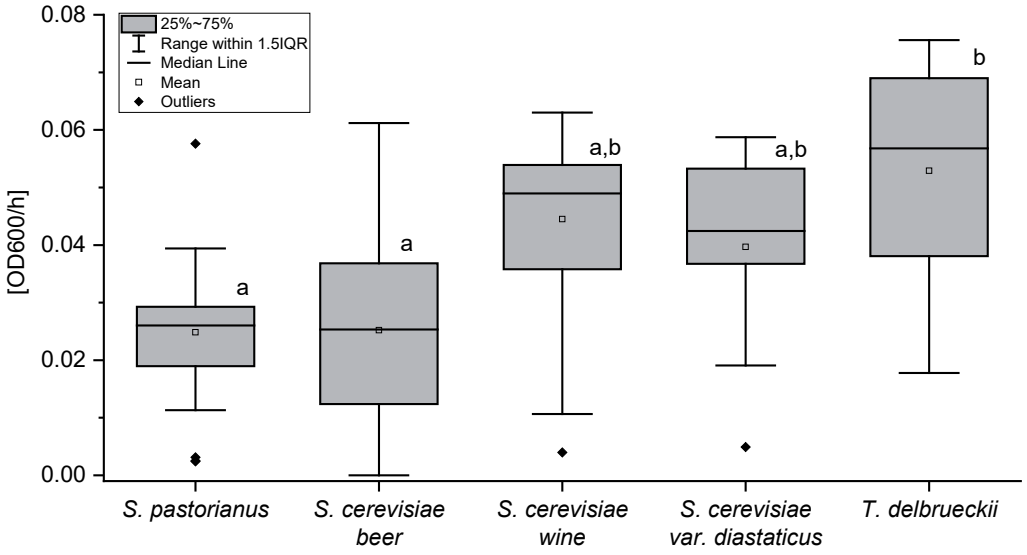

Fig. 1 **Maximum growth rate (OD_{600}/h) of all investigated yeast species measured over 48 hours incubated at 28 °C in the YCB-SMC medium. *S. pastorianus* (n = 47), *S. cerevisiae* beer (n = 51), *S. cerevisiae* wine (n = 25), *S. cerevisiae* var. *diastaticus* (n = 10) and *T. delbrueckii* (n = 15). Different letters indicate significantly different groups according to the Scheffé test ($p < 0.01$)**

3.3 Fermentation trials

After obtaining the findings explained above, six different strains were chosen for further testing on 3-MH release by fermentation of unhopped wort with an addition of 1.5 g US - Mosaic® type 90 hop pellets (Fig. 3, see page 184). Strain TUM 206 was chosen as it outperformed all other *S. pastorianus* strains by growth in the applied SMC medium and it possessed a short and long version of the *IRC7* gene. In contrast, TUM 128 was chosen because of the lowest growth of the *S. pastorianus* strains and a long *IRC7* gene. The strain TUM 545 (long *IRC7* gene) was chosen as a representative of the *S. cerevisiae* wine group as it showed the

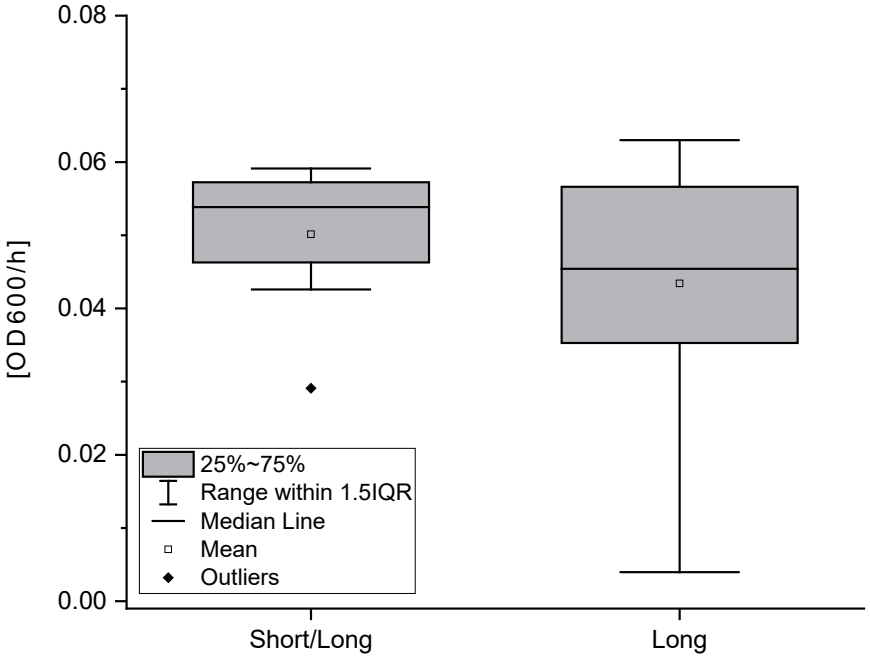

Fig. 2 **Comparison by genotype and maximal growth rate (OD_{600}/h) of the wine/distillers yeast strains after 48 hours incubated at 28 °C in the YCB-SMC medium (Long and Short IRC7 n = 11, Long IRC7 n = 12). No significant difference between the two groups according to the Scheffé test ($p < 0.01$)**

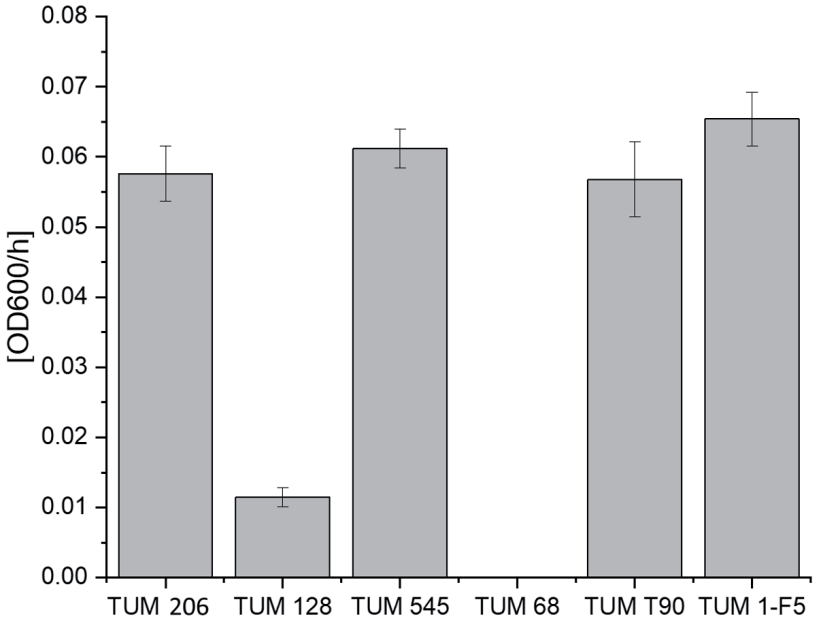

Fig. 3 Maximum growth rate (OD_{600}/h) of the strains after 48 hours incubated at 28 °C in the YCB-SMC medium which were chosen for further investigation (n = 3)

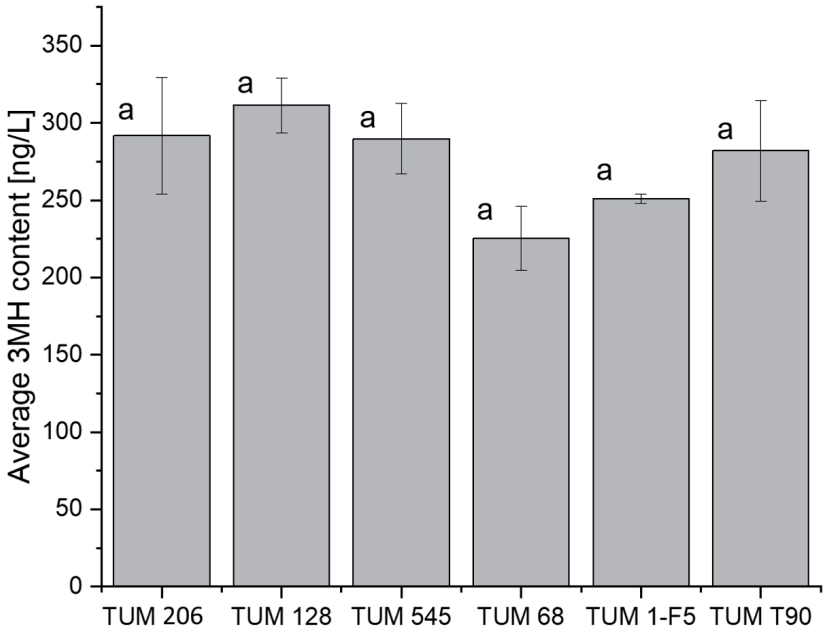

Fig. 4 Average 3MH content of the fermentations (n = 3) with the different yeast strains at 20 °C after 10 days of fermentation and three weeks of maturation at 0 °C (different letters indicate significantly different groups according to the Tukey test (p < 0.01)

highest maximal growth. TUM 68 (long *IRC7* gene) was chosen in comparison because it showed no growth at all in the SMC medium out of all *S. cerevisiae* beer strains. As all *T. delbrueckii* strains showed high growth, a wild strain, TUM 1-F5, and a brewing strain, TUM T90, were chosen (both had a long *IRC7* gene).

All strains were propagated in unhopped wort as described in the methods section to prevent an abduction of hop flavour compounds. Blank fermentation samples containing no hops were simultaneously run to investigate the potential synthesis of flavour compounds from wort by the differing yeast strains. No 4MMP was detected in the blank fermented samples of each individual yeast strain. As 3MH precursors also derive from malt [31], little amounts of free 3MH were detected in the fermented blank samples which contained no hops (TUM 206: 16.3ng/L, TUM 128: 39.3 ng/L, TUM 545: 17.0 ng/L, TUM 68: 21.9 ng/L, TUM 1-F5: 14.0 ng/L, TUM T90: 23.3 ng/L).

The amount of 1.5 g of US - Mosaic® type 90 hop pellets added contained a total of 37.2 ng of free 3MH and 53.5 ng of free 4MMP and a total of 6120 ng of 3MH precursors (Table 2). As the hop pellets were added to 2 L of unhopped wort, a total concentration of 18.8 ng/L of 3MH and 26.7 ng/L of 4MMP was expected when dissolving 100 % in the samples. The concentration of 3060 ng/L of 3MH precursors was further available for conversion to free 3MH by β-lyase derived from yeast. No 4MMP precursors were detected in the hop sample. Figure 4 shows the average concentration of free 3MH in the finished fermented beers. No significant difference could be found in the 3MH content ($p < 0.01$) between the samples fermented with differing yeast strains. However, a conversion rate of approx. 8 % was found, presumably from precursors as the beers contained an average of 270 ng/L of free 3MH. These results are interesting when compared with the findings of *Murat* et al., who reported a maximal conversion rate of about 5 % in wine [12]. This indicates a possibility to change the flavour derived by volatile thiols as their concentration and perception thresholds are relatively low [3, 5]. However, this also depends on the precursor concentration and enzyme activity as well as the time it takes for the enzymes to digest the precursors. A correlation between the growth in the YMC medium and the 3MH content in the final beer cannot be drawn as for example TUM 68 did not grow in the medium at all but showed no significant difference in releasing 3MH from its precursors.

A significant difference was found for the 4MMP content of some of the beers fermented with the different yeasts as visible in figure 5 (see page 185). This is particularly interesting as no precursors of 4MMP were detected in the used hop sample (Table 2). An average of approx. 35 ng/L was detected for the beers fermented with the *Saccharomyces* strains in contrast to approx. 15 ng/L on average for the *T. delbrueckii* strains. However, the expected concentration of free 4MMP available from hops was calculated at 26.7 ng/L. The blank samples did not contain any 4MMP. The 4MMP content of the beers fermented with the two *T. delbrueckii* strains further showed amounts of 4MMP that were much lower than expected. As table 4 (see page 185) shows, the beers produced with TUM

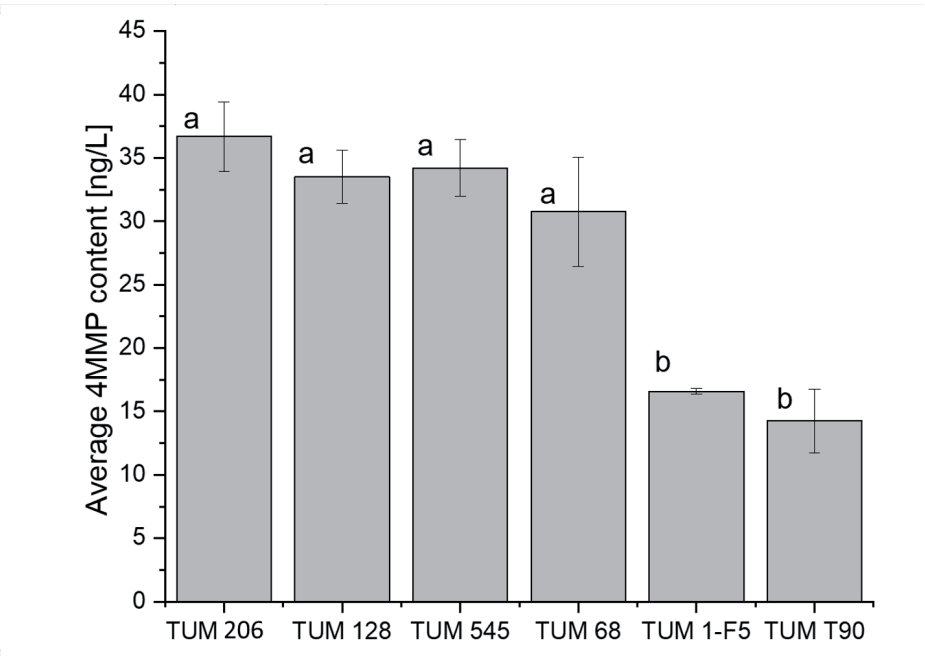

Fig. 5 **Average 4MMP content of the fermentations (n = 3) with the different yeast strains after 10 days of fermentation at 20 °C and three weeks of maturation at 0 °C (different letters indicate significantly different groups according to the applied Tukey test ($p < 0.01$)**

Table 4 **Biochemical attributes of the fermented beers with the different yeast strains (n = 3)**

Strain Abbreviation	Original Gravity (°P)	Ethanol (% v/v)	pH Value
TUM 206	12.64 ± 0.27*	4.64 ± 0.21*	4.46 ± 0.1*
TUM 128	12.71 ± 0.15*	5.41 ± 0.17*	4.45 ± 0.1*
TUM 545	12.63 ± 0.11*	5.68 ± 0.11*	4.31 ± 0.2*
TUM 68	12.74 ± 0.24*	5.47 ± 0.12*	4.41 ± 0.1*
TUM 1-F5	12.52 ± 0.14*	2.4 ± 0.28*	4.66 ± 0.2*
TUM T90	12.47 ± 0.21*	5.07 ± 0.15*	4.26 ± 0.1

**Standard deviation

1-F5 had a much lower ethanol concentration than all other beers. This could suggest lower solubility, however the beers fermented with TUM T90 had an ethanol concentration of 5 %v/v and showed almost the same amount of free 4MMP, which doesn't support the solubility hypothesis.

These results are consistent with the different findings of *Zott* et al. and *Renault* et al. who described *T. delbrueckii* as a poor producer of 4MMP [15, 32]. However, the results are contrary to Belda et al. who described a high ability of *T. delbrueckii* to release 4MMP [11]. In our study, it was striking that there was a significant difference in the 4MMP content in the absence of the four known precursors, indicating the presence of other precursors or a potential biotransformation into other substances under certain circumstances. However, this hypothesis must first be proven.

4 Conclusion

Top and bottom-fermenting brewing yeast strains as well as *Torulaspora delbrueckii* have the ability to impact volatile thiol concentrations such as 3MH and 4MMP in beer. Overall, our findings do not support the ability to screen with the SCM medium for β-lyase activity in yeast nor the investigation of the *IRC7* genotype as TUM 68 did not grow at all but showed similar abilities to release 3MH from precursors as well as TUM 128. It has to be kept in mind that the release of the investigated thiols is an enzymatic reaction which is influenced by pH value, temperature, time, concentration of the enzyme and precursors [33]. The ability to release the investigated thiols might vary in speed for each strain, which will be investigated in future and can then potentially be correlated to the growth in the SMC medium. The fermentation of hops performed in this case together with wort is also not the classical way of adding hop flavour to beer, however as an initial attempt to show the impact of yeast strains on the thiol content of beer, it seemed the most promising approach. This investigation was a first step towards an investigation into β-lyases derived from yeasts of different species that are used for brewing and shows that there is a potential for higher thiol contents.

Acknowledgements

The authors would like to thank Joh. Barth & Sohn GmbH & Co. and especially Dr. Christina Schönberger for supplying the hop samples and supporting this work with the Barth Haas Grant 2018. We would further like to thank Weyermann® for supplying the wort extract. A special thank you to Aofei Cheng for his great laboratory work.

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Received 24 September 2019, accepted 18 November 2019