Non-tandem repeat polymorphisms at microsatellite loci in wine yeast species

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Non-tandem repeat polymorphisms at microsatellite loci in wine yeast species

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Abstract
Yeasts microsatellite loci consist of short tandem-repeated DNA sequences of variable length. The high mutational rate at these loci generates a remarkable repertoire of alleles, useful for strain differentiation and population genetic studies. In this work, we analyze the DNA sequences of thirteen alleles from each of ten microsatellite loci described for the yeast Starmerella bacillaris. Our results show that polymorphic variants of some informative alleles are dependent on SNPs and indels rather than on length variation at their originally defined tandem-repeated motifs. The analysis was extended to 55 previously described hypervariable microsatellite loci from a total of 26 sequenced genomes of yeast species that dominate the microbiota of spontaneously fermenting grape musts (i.e., Hanseniaspora uvarum, Saccharomyces cerevisiae, Saccharomyces uvarum, and Torulaspora delbrueckii) or lead to wine spoilage (Brettanomyces bruxellensis and Meyerozyma guilliermondii). We found that allelic variants for some microsatellite loci of these yeast species are also dependent on SNPs and/or indels flanking their tandem-repeated motifs. For some loci, the number of units at their tandem repeats was found to be identical among the various characterized alleles, with allelic differences being dependent exclusively on flanking polymorphisms. Our results indicate that allele sizing of microsatellite loci using PCR, although valid for strain differentiation and population genetic studies, does not necessarily score the number of units at their tandem-repeated motifs. Sequence analysis of microsatellite loci alleles could provide relevant information for evolutionary and phylogeny studies of yeast species.

Keywords Yeast · Microsatellite · Tandem repeat · Polymorphism · Mutation

Introduction
The characterization of indigenous yeast populations in oenological ecosystems is of considerable interest in ecological and evolutionary studies (Capece et al. 2016; Combina et al. 2005; Vigentini et al. 2016). Several molecular methods, including RAPD-PCR fingerprinting, mtDNA-RFLP, AFLP, delta elements, and microsatellite loci analyses, are widely used to genotype wine yeasts (Masneuf-Pomarede et al. 2016a). Microsatellite loci, consisting of tandem repeats of a variable number of short DNA motifs (i.e., 1–6 bp) (Guillamón and Barrio 2017), are useful markers for yeast strain differentiation and population studies of yeast species (Albertin et al. 2014a, b, 2016; Estoup et al. 2002; Hranilovic et al. 2017; Legras et al. 2005; Masneuf-Pomarede et al. 2015, 2016a). Allele variants of microsatellite loci primarily originate on DNA polymerase slippage, during DNA replication, and/or unequal crossover events at their tandem-repeated motifs (TRM) (Ellegren 2004; Guillamón and Barrio 2017). In addition to the variable lengths of their TRM, single-nucleotide polymorphisms (SNPs) and/or insertions/deletions (indels) flanking their TRM have been documented in yeast (Sampaio et al. 2007).

Here, we report the sequence analysis of 65 hypervariable microsatellite loci from 31 sequenced genomes of selected wine yeast species. Eight Brettanomyces bruxellensis (Albertin et al. 2014b), ten Hanseniaspora uvarum (Albertin et al. 2016), four Meyerozyma guilliermondii (Wrent et al. 2016), sixteen Saccharomyces cerevisiae (Legras et al. 2005), nine
Saccharomyces uvarum (Masneuf-Pomarede et al. 2016b), ten Starmerella bacillaris (Masneuf-Pomarede et al. 2015), and eight Torulaspora delbrueckii (Albertin et al. 2014a) microsatellite loci were analyzed. Additional information was obtained by sequencing indigenous alleles of two microsatellite loci (i.e., CZ11 and CZ54) from Starm. bacillaris. Our results show that SNPs and indels at sequences flanking the TRM largely contribute to the allelic repertoire (i.e., size and/or sequence) of some wine yeast microsatellite loci. As a consequence of indels, allele sizing using PCR does not necessarily reflect the number of repeated units at the TRM of a given microsatellite locus.

Materials and methods

In silico analysis of wine yeast microsatellite loci

BLAST software (https://blast.ncbi.nlm.nih.gov) was used for sequence analyses of previously described microsatellite loci from B. bruxellensis (Albertin et al. 2014b), H. uvarum (Albertin et al. 2016), M. guillermondii (Wrent et al. 2016), S. cerevisiae (Legras et al. 2005), S. uvarum (Masneuf-Pomarede et al. 2016b), Starm. bacillaris (Masneuf-Pomarede et al. 2015), and T. delbrueckii (Albertin et al. 2014a) (Table 1; Table S1). Microsatellite sequences were obtained, using forward (FS) and reverse (RS) primer sequences (Fig. 1; Table S1) as queries, from five sequenced genomes each of B. bruxellensis (AWR11499, LAMAP2480, CBS 2796, CBS 2499, UMY321), H. uvarum (DSM 2768, AWR13581, 34-9, AWR13580, CBA6001), S. cerevisiae (AWR11631, JAY291, W303, M22, YPS163), S. uvarum (MCYC 623, U1, U2, U3, U4) and Starm. bacillaris (FR175I, PAS13, PYCC 3044, NP2, CBS 9494), as well as four and two sequenced genomes of M. guillermondii (RP-YS-11, SO, W2, ATCC6260) and T. delbrueckii (CBS 1146, SRCM101298) (Table 2). Missing alleles for some microsatellite loci correspond to BLAST searches where a single contig including both FS and RS sequences, was not found. For each microsatellite locus, the analyzed sequence included five additional nucleotides upstream and downstream of the genomic sequence for FS and RS (Fig. 1) (Albertin et al. 2014a, b, 2016; Legras et al. 2005; Masneuf-Pomarede et al. 2015, 2016b; Wrent et al. 2016). Sequence alignments were performed using the Clustal Omega Multiple Sequence Alignment tool (https://www.ebi.ac.uk/Tools/msa/clustalo).

Microsatellites CZ11 and CZ54 from Starm. bacillaris

PCR amplification of microsatellite loci CZ11 and CZ54 from Starm. bacillaris was performed using the originally described forward and reverse primers (Masneuf-Pomarede et al. 2015) as well as primers CZ11-FL/CZ11-RL (5’CAA CAAAGAGGTCTTCAGCG-3’/5’AAGAGCTTCTGCT CCTTC3’) and CZ54-FL/CZ54-RL (5’AATGGAA-TTG

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Table 1 Yeast species and microsatellite loci

| Yeast species          | Microsatellite loci                                                                 | References                        |
|------------------------|------------------------------------------------------------------------------------|-----------------------------------|
| B. bruxellensis        | B101, B122, B135, B174, B22, B224, B273, B301                                     | Albertin et al. (2014b)           |
| H. uvarum              | HU292, HU440, HU508, HU593, HU467, HU620, HU409, HU853, HU68, HU594               | Albertin et al. (2016)            |
| M. guillermondii       | sc15, sc22, sc32, sc72                                                            | Wrent et al. (2016)               |
| S. cerevisiae          | C3, C4, C5, C6, C8, C9, C11, SCAAT1, SCAAT2, SCAAT3, SCAAT5, SCAAT6, YKR072CS, SCYOR267C, YKL172, YPL009 | Legras et al. (2005)              |
| S. uvarum              | SuARS409, SuYBR049C, SuYKL017C, SuYKR045C, SuYGC170W, SuYHR042-043, SuHTZ1PLB3, SuYHR102W, SuYIL130W | Masneuf-Pomarede et al. (2016b)   |
| Starm. bacillaris      | CZ1, CZ4, CZ11, CZ13, CZ15, CZ20, CZ23, CZ45, CZ54, CZ59                           | Masneuf-Pomarede et al. (2015)    |
| T. delbrueckii         | TD1A, TD1B, TD1C, TD2A, TD6A, TD7A, TD5A, TD8A                                     | Albertin et al. (2014a)           |
Table 2 Yeast strains and yeast sequenced genomes used for microsatellite loci sequence analyses

| Species                | Strain               | Description                                      | Accession #             | References                        |
|------------------------|----------------------|--------------------------------------------------|-------------------------|-----------------------------------|
| B. bruxellensis        | AWR11499             | Australian wine isolate                         | PRJNA78661              | Curtin et al. (2012)              |
| LAMAP2480              | Colombian wine isolate | PRJNA231184                                     | Valorlos et al. (2014)  |
| CBS 2796               | Sparkling wine       | PRJNA335438                                     | Cheng et al. (2017)     |
| CBS 2499               | French wine isolate  | PRJNA76499                                       | Pişkur et al. 2012      |
| UMY321                 | Italian red wine isolate | PRJEB21262                                     | Fournier et al. (2017)  |
| H. uvarum              | DSM 2768             | Type strain                                      | PRJNA178141             | Langenberg et al. (2017)          |
| AWR13581               | Australian Chardonnay grape isolate | PRJNA305659                                   | Sternees et al. (2017)  |
| 34–9                  | Chinese isolate from epiphytes of citrus roots | PRJNA254213                                    | Liu et al. (2016)        |
| AWR13580               | Australian Chardonnay fermenting grape must isolate | PRJNA325557                                | Sternees et al. (2016)  |
| CBA6001                | South Korean “kimchi” isolate | PRJNA434537                                    | Kim et al. (2019)       |
| M. guilliermondii      | RP-YS-11             | Soil isolate                                     | PRJNA559974             | Singh et al. (2019)               |
| SO                     | Spoilage orange isolate | PRJNA57962                                    | Zainudin et al. (2018)  |
| W2                     | Retrieved activated sludge | PRJEB27464                                   | Yang et al. (2019)      |
| ATCC6260              | Culture from type material of Candida guilliermondii | PRJNA12729                                | Butler G et al. (2009)   |
| S. cerevisiae          | AWR11631             | Haploid derivative of South African commercial wine strain N96 | PRJNA30553                 | Borneman et al. (2008)          |
| JAY291                 | Haploid derivative of Brazilian industrial bioethanol strain PE-2 | PRJNA32809                  | Argueso et al. (2007)    |
| W303                   | Laboratory strain    | PRJNA83445                                       | Ralsker et al. (2012)   |
| M22                    | Italian vineyard isolate | PRJNA28815                                    | Doniger et al. (2008)   |
| YPS163                | Pennsylvania woodland isolate | PRJNA260311                          | Fay et al. (2004)       |
| S. uvarum              | MCYC 623             | Microbiology Collection of Yeasts Cultures, Spain | PRJNA1441                | Kelis et al. (2003)              |
| U1                     | Monosporic clone of grape must isolate PM12 | PRJNA388544                       | Albertin et al. (2018)   |
| U2                     | Monosporic clone of PIP3 | PRJNA388544                    | Albertin et al. (2018)   |
| U3                     | Monosporic clone of apple juice fermentation isolate BR6 | PRJNA388544                | Albertin et al. (2018)    |
| U4                     | Monosporic clone of grape must isolate RC4-15 | PRJNA388544                  | Albertin et al. (2018)   |
| S. cerevisiae          | CBS 9494             | C. zemplinina Type strain                  | PRJNA476215             | Rosa et al. (2018)               |
| FRI751                 | Italian dried Raboso grapes isolate from fermenting must | PRJNA376556                | Lemos Junior et al. (2017b)  |
| PAS13                  | Italian isolate from destemmed dried grapes | PRJNA376556              | Lemos Junior et al. (2017a)  |
| PYCC 3044              | Portuguese yeast culture collection | PRJNA416493             | Gonçalves et al. (2018)    |
| NP2                    | Isolate from peach peels | PRJNA397469                | Ko et al. (2017)         |
| MT017-011              | Isolate from Malbec fermenting must | MK733726/MK733731         | Raymond Eder et al. (2018)  |
| MT017-020              | Isolate from Malbec fermenting must | MK733720                   | Raymond Eder et al. (2018)  |
| MT017-021              | Isolate from Malbec fermenting must | MK733723                   | Raymond Eder et al. (2018)  |
| MT017-022              | Isolate from Malbec fermenting must | MK733729                   | Raymond Eder et al. (2018)  |
| MT017-029              | Isolate from Malbec fermenting must | MK733727/MK733732       | Raymond Eder et al. (2018)  |
| MT117-003              | Isolate from Malbec fermenting must | MK733728                  | Raymond Eder et al. (2018)  |
| L13                    | Isolate from V. labrusca grapes | MK733724                    | Drumonde-Neves et al. (2016) |
| L14                    | Isolate from V. labrusca grapes | MK733725                    | Drumonde-Neves et al. (2016) |
| T. delbrueckii         | CBS 1146             | Type strain                                      | PRJNA79345              | Gordon et al. 2011               |
| SRCM101298             | Isolate from food    | PRJNA388014                                     | M.I.F.I. (South Korea)   |

TGCACGCAAG3’5’ACAAGTGAGGAATGGTCAG3’), designed on the basis of the genome sequence of C. zemplinina (syn. Starm. bacillaris) strain CBS 9494 (Rosa et al. 2018). Primers CZ11-FL/CZ11-RL and CZ54-FL/CZ54-RL recognize genome sequences ~100 bp upstream and downstream (Fig. 1) of the loci CZ11 (GenBank #LN864678.1) and CZ54 (GenBank #LN864684.1), respectively (Manseuf-Pomarede et al. 2015). For sequencing purposes, universal
M13 primer sequences (i.e., F: 5´GCAA-AACGACGCC CAGT3´; R: 5´CAGGAAACAGCTGATGAC3´) were added at the 5´ end of the CZ11 and CZ54 FL/RL primers. PCR mixtures contained 100 ng DNA, 1.5 mM MgCl2, Taq polymerase buffer 1X (Invitrogen, USA), 200 µM dNTPs, 10 pmol of each forward, and reverse primer and 1.25 units of Taq polymerase (Invitrogen, USA). Amplification reactions were performed in a MJ Mini Bio-Rad thermocycler (Bio-Rad, USA) using an initial denaturation step at 93 ºC for 3 min, followed by 35 cycles of 93 ºC for 30 s, annealing at 53 ºC for 45 s, extension at 72 ºC for 60 s followed by a final extension at 75 ºC for 5 min.

Results

Apparent microsatellite heterozygosity in rare Starm. bacillaris strains

Initial studies of microsatellites CZ15 and CZ59 in indigenous isolates of Starm. bacillaris suggested allele heterozygosis for a single rare strain (i.e., strain 11-6) (Masneuf-Pomarède et al. 2015). Additional apparent heterozygosis for microsatellites CZ11, CZ13, and CZ54 was recently recognized (Raymond Eder et al. 2019).

To study if these findings reveal rare diploid Starm. bacillaris strains or represent technical artifacts, we first analyzed in silico the sequences of loci CZ11, CZ13, CZ15, CZ54, and CZ59 using the available draft genome sequence of C. zemplinina (syn. Starm. bacillaris) type strain CBS 9494 (Rosa et al. 2018). Although no explanation was found for the apparent heterozygosity previously observed for microsatellites CZ15, CZ54 and CZ59, analyses of loci CZ11 and CZ13 showed the presence of potential alternative genomic binding sites for the forward and reverse primers, respectively, originally described for PCR amplification (Fig. S1 and S2). A potential alternative genomic site for the binding of the reverse primer was also recognized in microsatellite CZ20 (Fig. S2). These alternative primer binding sites at loci CZ11, CZ13 and CZ20 were also found in the genomes of Starm. bacillaris strains FR1751, PAS13, PYCC 3044 and NP2 (Fig. S1 and S2), indicating that they do not represent rare polymorphisms present in strain CBS 9494. Based on these observations, we hypothesized that alternative genomic sites for annealing of the primers used for PCR may generate more than one PCR product, resulting in apparent heterozygosity at these loci.

To test this hypothesis, we analyzed locus CZ11 using PCR and primers CZ11-FL/CZ11-RL, designed to bind genome sequences 100-bp upstream and downstream of the originally described CZ11-FS/CZ11-RS primers (Fig. 1; Table S1). Single PCR amplification products were obtained when primers CZ11-FL/CZ11-RL were used to genotype either strain CBS 9494 or indigenous Starm. bacillaris strains L13, L14, MT017-011, MT017-029, and MT117-003 (not shown). However, at least two major CZ11-derived PCR fragments were obtained when the same strains were genotyped using primers CZ11-FS/CZ11-RS (Table 3). Sequence analysis of PCR products (Fig. S1) obtained with primers CZ11-FL/CZ11-RL confirmed that all the analyzed strains have the alternative annealing site for the originally described forward primer.

A detailed analysis of the various CZ11 alleles characterized (i.e., five alleles from indigenous strains and five alleles from the available Starm. bacillaris sequenced genomes—Fig. S1) showed that primer CZ11-FS overlaps a highly polymorphic A+T rich, TRM of 16-nt (Fig. 2; Fig. S1). The number of units of this 16-nt TRM, among the analyzed CZ11 alleles, was: 3 (strain L13), 4 (strain L14), 5 (strains MT017-011, MT017-029 and PYCC 3044), 6 (strains CBS 9494, PAS13 and MT117-003) and 8 (strain NP2) (Fig. S1). Interestingly, size differences among CZ11 alleles genotyped using primer CZ11-FS are consistent with their different number of 16-nt repeated units, as determined by DNA sequencing (Table 3). Thus, annealing of primer CZ11-FS at alternative linked genomic sites may generate more than one PCR product, resulting in apparent heterozygosity at locus CZ11.

Multiple sequence alignment of the CZ11 alleles characterized (Fig. S1) showed that the recognized polymorphic 16-nt TRM, rather than the various lengths of its originally recognized GT/TA/GA TRM, is a major determinant of CZ11 informativity (Masneuf-Pomarède et al. 2015). Thus, we concluded that the apparent heterozygosity previously observed in Starm. bacillaris strains for microsatellite CZ11 may result from more than one PCR product, originated from alternative annealing of the forward primer to the recognized 16-nt TRM (Fig. 2; Fig. S1).

Table 3 Apparent heterozygosity of CZ11 and CZ54 microsatellite loci

| Starm. bacillaris strain | Microsatellite locus<sup>a</sup> |
|--------------------------|---------------------------------|
|                          | CZ11   | CZ54   |
| MT017-011                | 276/325| 265/277|
| MT017-029                | 275/310| 265/280|
| MT017-003                | 276/325| 265    |
| L13                      | 273/289| 289    |
| L14                      | 287/319| 292    |
| MT017-020                | 310    | 265/286|
| MT017-021                | 273    | 271/277|
| MT017-022                | 275    | 277/280|

<sup>a</sup> Alleles for the CZ11 and CZ54 loci correspond to the size of the PCR products obtained with primers CZ11-FS/CZ11-RS and CZ54-FS/CZ54-RS, respectively (see Fig. 1)
Sequence analyses of *Starm. bacillaris* microsatellite CZ54

The lack of explanation for the apparent heterozygosity observed in some *Starm. bacillaris* strains for microsatellite CZ54 prompted us to study this locus using PCR with primers CZ54-FL/CZ54-RL (Fig. 1), followed by sequence analysis. A remarkable repertoire of CZ54 alleles, dependent on its TRM (i.e., 6 to 20 AGA units), was observed in *Starm. bacillaris* strains MT017-011, MT017-020, MT017-021, MT017-022 and MT017-029, as well as CZ54 sequences corresponding to the four *Starm. bacillaris* draft genome sequences analyzed (Fig. S3). In addition, two different indel polymorphisms were recognized upstream of the CZ54 TRM: (i) a variable number of units (i.e., 2 to 3) of the sequence AGACCAAGA (Fig. S3), and (ii) a deletion of 18 nt in strain PYCC 3044 (Fig. S3). Thus, non-TRM polymorphisms also contribute to the repertoire of allele size variations at locus CZ54. Interestingly, SNPs observed among CZ54 alleles were all located in the region upstream of its TRM (i.e., 6 SNPs in a region of 94 nt), while no SNPs were present downstream of the TRM (i.e., no SNPs in a region of 96 nt).

**Tandem and non-tandem repeat polymorphisms at other *Starm. bacillaris* loci**

The finding that non-TRM polymorphisms largely contribute to allele size variations of loci CZ11 and CZ54 prompted us to explore the molecular bases of allele differences at other *Starm. bacillaris* microsatellite loci. Six loci (i.e., CZ1, CZ4, CZ15, CZ33, CZ45, and CZ59) were characterized (Table 1). The in silico analysis of these loci was performed using the draft genome sequences from *Starm. bacillaris* strains CBS 9494, FRI751, PAS13, PYCC 3044, and NP2. Taken together the results showed that, in addition to the expected size variations associated with the polymorphic TRM at these loci, allele differences among the various loci analyzed were also dependent on indels and/or SNPs, both at their TRM as well as in upstream (5′-FR) and downstream (3′-FR) regions (Fig. 1) flanking the TRM (Table 4; Fig. S2).

**Microsatellite loci from *B. bruxellensis*, *H. uvarum*, *M. guilliermondii*, *S. cerevisiae*, *S. uvarum*, and *T. delbrueckii***

The analysis of microsatellite loci sequences was extended to the wine yeast species *H. uvarum*, *S. cerevisiae*, *S. uvarum*, and *T. delbrueckii* as well as the wine spoilage yeasts *B. bruxellensis* and *M. guilliermondii* (Table 1). These species were selected based on the availability of their previously characterized microsatellite loci as well as their relevance in oenological ecosystems. For this purpose, the available genome sequences of five strains each of *B. bruxellensis*, *H. uvarum*, *S. cerevisiae*, and *S. uvarum* as well as four strains of *M. guilliermondii* and two strains of *T. delbrueckii* (Table 2), were analyzed.

In silico study of DNA sequences corresponding to microsatellite loci HU292, HU409, HU467, HU508, HU593, HU594, HU620, and HU853 from *H. uvarum* (Table 1) did not show allele size variants dependent on their repeated units at the TRM (Fig. S4). Moreover, as it was observed for some alleles of *Starm. bacillaris* microsatellite loci, allele size variants for some *H. uvarum* loci (i.e., HU440, HU593, and HU594) were mostly dependent on indels at the 5′-FR or 3′-FR (Fig. S4). Interestingly, most of the alleles of *H. uvarum* microsatellite loci contain a remarkable repertoire of SNPs and/or indels at their TRMs (Fig. S4). With the exception of loci HU68 and HU292, SNPs and/or indels were also recognized at the 5′-FR and/or 3′-FR of all the *H. uvarum* analyzed loci (Fig. S4). Thus, allelic variants of *H. uvarum* microsatellite loci, at least for the analyzed genomes, may be better recognized by sequence analyses than by allele sizing using PCR.

Sixteen *S. cerevisiae* microsatellite loci (i.e., C3, C4, C5, C6, C8, C9, C11, SCAAT1, SCAAT2, SCAAT3, SCAAT5, SCAAT6, YKR072CS, SCYOR267C, YKL172,
Table 4: Non-tandem repeat polymorphisms in yeast microsatellite loci

| Yeast species | SNP | indels |
|---------------|-----|--------|
|               | TRM | 5'-FR/3'-FR | TRM | 5'-FR/3'-FR |
| H. uvarum     | HU68, HU292, HU594 | HU409, HU440, HU467, HU508, HU593, HU594, HU620, HU853 | HU68, HU292, HU409, HU440, HU467, HU508, HU593, HU853 |
| S. cerevisiae | C4, C6, C8, C11, SCAAT1, SCAAT2, SCAAT3, YPL009, YKR072CS, SCYOR267C | C3, C4, C6, C8, C11, SCAAT1, SCAAT2, SCAAT3, SCAAT5, SCAAT6, YKR072CS, SCYOR267C | C6, C8, SCAAT2, SCAAT3, SCAAT6, YKL172C | C3, C4, C8, C9, C11, SCAAT5, SCAAT6 |
| Starm. bacillaris | CZ1, CZ4, CZ11, CZ15, CZ33, CZ45, CZ59 | CZ1, CZ4, CZ11, CZ13, CZ15, CZ45, CZ54, CZ59 | CZ1, CZ11, CZ45, CZ59 | CZ11, CZ45, CZ54 |
| T. delbrueckii | TD1A, TD1B, TD5A, TD6A, TD8A | TD1A, TD1C | TD7A | TD1A |
| S. uvarum     | SuARS409, SuHTZ1PLB3, SuYHR042-043, SuYHR102W, SuYIL130W, SuYKR045C | SuYHR042-043, SuYHR102W, SuYIL130W, SuYKR045C | – | SuYHR042-043, SuYK017C, SuYKR045C |
| M. guilliermondii | SC15, SC22, SC32 | SC15, SC32, SC72 | SC15, SC22 | – |
| B. bruxellensis | B22, B101, B122, B224, B273, B301 | B22, B122, B135, B174, B273, B301 | – | B273 |

Single-nucleotide polymorphisms (SNP) and/or insertion/deletions (indels) are present in the tandem-repeated motifs (TRM) and/or the TRM-flanking regions (5'-FR and 3'-FR) of the indicated wine yeast microsatellite loci.
Discussion

Wine yeast microsatellite loci are widely used for strain genotyping and analysis of yeast populations (Albertin et al. 2014a, b, 2016; Hranilovic et al. 2017; Legras et al. 2005; Masneuf-Pomarede et al. 2015, 2016a; Raymond Eder et al. 2019). Recent studies of informative Starm. bacillaris microsatellite loci suggested heterozygosis for loci CZ15 and CZ59 in a single European isolate (Masneuf-Pomarede et al. 2015), as well as for loci CZ11 and CZ13 and/or CZ54 in some isolates from Argentina and Portugal (Raymond Eder et al. 2019). In this work, we propose that alternative genomic annealing sites for the primers used for PCR may explain the apparent heterozygosity observed for loci CZ11 and CZ13. No explanation was obtained from in silico analysis of genomic DNA sequences for the previously apparent heterozygosity of loci CZ15, CZ59, and CZ54.

Following an extensive sequence analysis of all the currently used Starm. bacillaris microsatellite loci (Masneuf-Pomarede et al. 2015; Raymond Eder et al. 2019), we show in this work that SNPs and/or indels upstream and downstream of the microsatellite TRM largely contribute to the sequence and size polymorphisms, respectively, at some of these loci.

Our DNA sequence studies of microsatellite loci were extended to the wine yeast species B. bruxellensis, H. uvarum, M. guilliermondii, S. cerevisiae, S. uvarum and T. delbrueckii. In these studies, we found that 3 out of 8 loci of B. bruxellensis, 3 out of 10 loci of H. uvarum, 1 out of 4 loci of M. guilliermondii, 6 out of 16 loci of S. cerevisiae, 3 out of 9 loci of S. uvarum and 1 out of 8 loci of T. delbrueckii have SNPs and/or indels that markedly contribute to both sequence and size differences between alleles. For some microsatellite loci, multiple SNPs and/or indels were recognized within the same allele (e.g., HU440, HU467, HU508, HU593, and HU594 of H. uvarum; sc32 of M. guilliermondii; C4, C11, SCAAT3, and SCAAT6 of S. cerevisiae; TD1A of T. delbrueckii). SNPs were also found at the TRM of loci SCAAT1, C6 and SCYOR267C of S. cerevisiae (Table 3; Fig. S5), which were previously considered “perfect” (i.e., pure motifs at their TRM) microsatellite loci (Legras et al. 2005). Finally, although there were some examples of a bias in the distribution of SNPs among regions upstream and downstream of the TRMs, most of the SNPs at the microsatellite loci analyzed were evenly distributed along their sequences.

Studies conducted in other organisms, such as salmonid fishes (Angers and Bernatchez 1997), insect species (Behura and Severson 2015), and clinical isolates from the yeast species Candida albicans (Sampaio et al. 2007), revealed that indels and SNPs also contribute to the allelic variation of microsatellite loci. To our knowledge, there are no previous studies addressing the structural complexity of non-tandem repeat polymorphisms at microsatellite loci in wine yeast species. Taken together, our results show that SNPs and indels, located upstream and downstream of TRM sequences, largely contribute to allele sequence and allele size variations of wine yeast microsatellite loci. The sequence analyses of these allelic variants could provide useful information for the evolutionary and phylogenetic analyses of wine yeast populations.

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Author contributions MLRE and ALR contributed to the study conception and design, material preparation, data collection, analysis, and writing of the manuscript. Both authors read and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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