ROLE OF TANDEM REPEAT-CONTAINING GENES IN *SACCHAROMYCES CEREVISIAE*

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Abstract

Genetic variation is essential for species to evolve and adapt to dynamic environments. Mutations are a primary source of variation, but they typically occur slowly, limiting immediate adaptation. Organisms, therefore, require faster mechanisms to cope with environmental changes. Sexual reproduction facilitates rapid adaptation through genetic recombination, generating diverse and robust individuals. However, asexual organisms lack this mechanism and must rely on other strategies for adaptation. It has been proposed that genes containing tandem repeats (TR-ORFs) may undergo mutations faster than non-repetitive DNA, which could help organisms adapt more quickly to environmental shifts. These TR-ORFs might contribute to both short- and long-term adaptation. Saccharomyces cerevisiae S288C, a predominantly asexual organism, was chosen as a model to investigate this. A wide range of *S. cerevisiae* strains, isolated from various niches, have been sequenced, and their genomic data is publicly available. The reference strain S288c, with manually annotated genes, was used as a baseline for analysis. Tandem repeats were identified in this reference strain using the Tandem Repeat Finder (TRF) tool, revealing 48 mega-satellite-TR in 35 genes, many associated with cell surface proteins. These genes were then compared to the genomes of 20 other S. cerevisiae strains from different ecological niches using BLAST searches. The results showed no significant correlation between the repeat patterns and the strains' ecological niches or genetic backgrounds. Additionally, most BLAST hits were missed, and no matches were found in other strains. This suggests that these TRs may be specific to certain environments or that gaps in genome assemblies, which often occur in repetitive regions, hindered detection. Further sequencing of selected TR-ORFs across multiple strains could provide valuable insights into their potential role in adaptive evolution.

Key words: TRs, Non-repetitive DNA, ORFs, NCBI, BLAST.

Introduction

Variation within organisms refers to changes in their lifestyle or genetic makeup compared to their ancestors, a phenomenon essential for evolutionary success (Barton *et al.*, 2021). These variations allow species to adapt to dynamic environments and compete with other organisms for survival. While phenotype alterations resulting from these genetic changes are often the primary focus, the genetic shifts themselves-whether subtle or latent-can have significant evolutionary implications (O'Neill *et al.*, 2023). Mutations, which are fundamental sources of genetic variation, occur spontaneously and are often a result of DNA damage or errors during replication (Xue *et al.*, 2022). Mobile genetic elements further contribute to mutations, altering the genetic landscape (Venkataraman *et al.*, 2022). Although mutations are crucial for providing the raw material for evolution, most mutations can be harmful, leading to cancer or genetic disorders (Davis *et al.*, 2023).

Mutation rates, typically measured at around 10^3 to 10^6 per locus per generation in eukaryotes, vary across species, adding complexity to their measurement and comparison (Simons *et al.*, 2024). Interestingly, selective pressures often influence the mutation rate, with laboratory mutants sometimes exhibiting lower mutation rates than their natural counterparts (Schaaper, 2021). Despite these complexities, mutations are an essential means by which populations adapt to new environments. However, their rate of occurrence is often too slow to immediately respond to rapid environmental changes (Fraser *et al.*, 2022). Consequently, organisms must have other adaptive strategies at their disposal.

Sexual reproduction is vital in accelerating adaptation by recombining genetic material, allowing beneficial mutations to spread faster than in asexual populations (Panchal, 2022). This recombination process reduces genetic linkage disequilibrium and enhances selection efficiency by bringing together advantageous mutations (Linder *et al.*, 2023). Over a century of research has shown that sex facilitates the rapid accumulation of beneficial mutations and plays a key role in adaptation (Gray & Goddard, 2022). However, the precise mechanisms underlying these benefits remain an area of ongoing investigation (Smith *et al.*, 2023). Sexual reproduction, despite being more energetically costly than asexual reproduction, is maintained in many species due to its adaptive advantages, including the ability to rapidly combine beneficial mutations (Fisher & Muller, 2021). Studies in various organisms, including *Saccharomyces cerevisiae*, have demonstrated that sexual populations can adapt more quickly than asexual ones by more effectively combining advantageous mutations (Morran *et al.*, 2022). In contrast, asexual populations rely on the slower accumulation of mutations through clonal reproduction, a process subject to "clonal interference," where beneficial mutations within different clones compete (Gerrish & Lenski, 2023). This slower process can limit the rate of adaptation in asexual populations, especially in fluctuating environments (Zhou *et al.*, 2024). Nevertheless, mutations fuel adaptation, mainly when small beneficial mutations accumulate over time (Lipton & Longhurst, 2023).

Tandem repeats (TRs) are regions of the genome where sequences of DNA are repeated consecutively, and they exhibit higher mutation rates compared to non-repetitive DNA (Wang *et al.*, 2021). These TRs are often found in protein-coding regions and can lead to the production of proteins with repetitive amino acid sequences (Wilkins *et al.*, 2023). In many eukaryotes, up to 20% of open reading frames (ORFs) contain these tandem repeats, and they may play a significant role in genetic variation and adaptation (Gemayel *et al.*, 2022). Interestingly, in microorganisms, these rapidly mutating repeat-containing genes, also referred to as contingency genes, might provide short-term adaptability by altering protein function or expression in response to environmental changes (Moxon *et al.*, 2023). Such genes are crucial in adapting pathogens to new hosts (Caporale, 2022).

This study focuses on *Saccharomyces cerevisiae*, a well-established model organism, to explore the role of TR-containing genes in adaptation. *S. cerevisiae* is a haploid yeast that typically reproduces asexually, and its fully sequenced genome allows for in-depth comparisons across various strains (Engel & Cherry, 2023). The availability of annotated genomic data makes it an ideal organism for investigating the dynamics of tandem repeat-containing genes and their role in evolutionary processes (Matheson *et al.*, 2023). In summary, while mutation rates and the process of adaptation through sexual recombination are well understood in some contexts, the role of tandem repeat-containing genes in rapid adaptation, especially in asexual organisms like *S. cerevisiae*, remains an area ripe for exploration. By studying these genomic features, we hope to uncover insights into the mechanisms driving evolutionary change in rapidly fluctuating environments.

Material and Methods

Obtaining genome of reference strain S288c: *S. cerevisiae* S288c was used as a reference strain. The genome of this strain was downloaded From the NCBI genome database http://www.ncbi.nlm.gov/genom/. The genome of reference strain S288c downloaded in " orf_genomic_fasta" is a complete genome with a total length of 11.8906 Mb, approximately equal to 12 Mb, with a total chromosome number 16 in October 2023. *Saccharomyces* genome database (SGD) also has a genome sequence file for this strain along with coding sequence and protein sequences (http://www.yeastgenome.org) (Zhang *et al.*, 2023). All genomic sequences were downloaded in the FASTA format, a format introduced for ubiquitous exchanging for single-letter alphabets of most biological molecules, especially DNA, RNA, and even protein (Pearson *et al.*, 2023).

Finding tandem repeats in a genome of reference strain S288c: To identify tandem repeats in the reference strain S288c, the complete genome was analyzed using Tandem Repeat Finder (TRF) version 4.09, a software developed by G. Benson at Boston University (Benson *et al.*, 2023). TRF was applied with the following parameters: match = 2, mismatch = 5, indel = 7, minimum alignment score = 50, and a maximum period size (repeat unit length) of 500. The output was processed using Excel to organize the results into columns representing the gene accession number, repeat start and end positions, period size, repeat number, total repeat length, consensus sequence, and flanking sequence. A total of 1,258 repeats were identified, with multiple repeats within the same ORF treated as separate entities for analysis (Gelfand *et al.*, 2022).

Identification and arrangement of various groups of repeats in reference strain S288c: Following identification, the tandem repeats were categorized into three main groups based on repeat unit size: (1) trinucleotides repeat clusters, (2) multiples of trinucleotides repeats, and (3) repeats with non-multiple trinucleotides units. Microsatellites and minisatellites were excluded from further analysis (Yang *et al.*, 2023).

Selection of megasatellites TRs in reference strain S288c: A total of 48 megasatellite TRs were selected for further analysis based on repeat unit size. These repeats were extracted from the TRF output file by searching for genes containing them using their gene names or accession numbers (Zordan & Cormack, 2023). The selected megasatellite repeats were compiled into a separate file for further analysis.

Extraction of the gene with complete sequences containing megasatellites in S288c: All 48 genes containing megasatellites of interest were extracted from the whole genome sequence file and arranged in a separate file. Also, repeat regions from these 48 genes were extracted and added to a separate file. Twenty *S. cerevisiae* strains were selected from seven different ecological niches for comparison: seven strains from baker fermentations, three from wine fermentation, three from tree barks, and the remaining strains from clinical, tomato, grape orchard, food fermentation, and rice brewing environments (Fig. 1). These strains were selected based on their genomic size, comparable to the reference strain (approximately 12 Mb). The origin of each strain was carefully considered to ensure a diverse representation of different niches (Hernandez *et al.*, 2023) (Fig. 1).

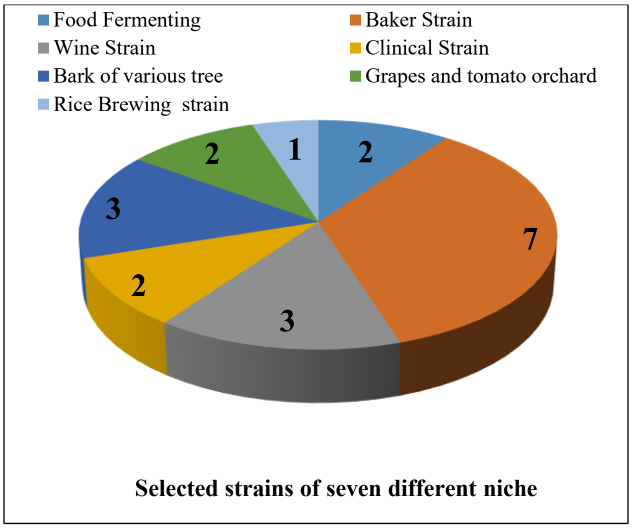

Fig. 1. Total number of strains with prescribed niche.

Obtaining the genome of selected strains used in the study: The genomes of the selected strains were downloaded from the Saccharomyces Genome Database (SGD) and NCBI datasets (https://www.ncbi.nlm.nih. gov/genome). A total of 21 genome sequences were collected: the reference strain S288c and 20 other strains from various ecological niches. All genomes were downloaded in FASTA format (Gronchi *et al*., 2023) (Fig. 2).

Comparisons of S288c megasatellite TR-containing ORFs with genomes of 20 other yeast strains: To compare the megasatellite TRs of the reference strain S288c with those of the selected 20 strains, the Basic Local Alignment Search Tool (BLAST) from NCBI was employed (Altschul *et al*., 2023). The BLASTn program was used to compare nucleotide sequences against the nucleotide database using default parameters. The best hits were selected based on the e-value, percentage identity, and repeat coverage. Reciprocal BLAST was then performed to verify the accuracy of these hits (Suzek *et al*., 2023).

Extraction of ITS sequences: A BLAST comparative analysis of the reference strain S288c with the 20 other strains revealed no significant matches based on whole genome sequences. As a result, the Internal Transcribed Spacer (ITS) sequences of the reference strain S288c were extracted from NCBI (https://www.ncbi.nlm.nih.gov). The ITS sequence was found to be 752 bp in length (Leaw *et al*., 2023). Since no ITS sequences were available for the remaining strains in the NCBI database, four additional ITS sequences were extracted from the SGD database to make a total of five ITS sequences, including the reference strain (Cherry *et al*., 2023).

Extraction of SRA reads: For strains lacking ITS sequences in NCBI, sequence reads were retrieved from the Sequence Read Archive (SRA) (https://www.ncbi.nlm.nih.gov/sra). A maximum of 100 reads were extracted for each strain, and these reads were processed using Geneious Prime version 2021.1.1.0 for further analysis (Biomatters, 2023).

Consensus sequence building and phylogenetic tree: Consensus sequences were built using Geneious Prime version 2021.1.1.0 (https://www.geneious.com). The sequence reads for each strain were mapped to the reference strain ITS sequence, and consensus sequences were generated for each strain. All consensus sequences were aligned using the CLUSTAL-W algorithm available in Geneious Prime with default parameters (Forth *et al*., 2023). A phylogenetic tree was constructed based on the multiple sequence alignment using the Neighbor-Joining method with 1,000 bootstrap replicates. The reference strain ITS sequence was used as an outgroup for phylogenetic analysis (Gultepe *et al*., 2023).

Results

A Significant number of ORFs contain tandem repeat sequences (TRs): *Saccharomyces cerevisiae* S288c was chosen as a reference strain to compare TRs against 20 other yeast strains. Both coding and non-coding sequences of S288c were downloaded from NCBI and analyzed for the presence of TRs using Tandem Repeat Finder (TRF). Many ORFs and non-ORF sequences contained tandem repeat (TR) sequences. Both regions contained a total of 33.13% TRs (3023 in number). Approximately 10.2% (614/6000) of the ORFs containing genes included TR sequences. These 614 genes of reference strain had a total of 1258 tandem repeats. The number of TRs per gene ranged from 1 to more than 5. Genes YEL033W, MTC7, and YNL134C of chromosomes V and XIV had only one TR per gene, while genes YKR102W, FLO10, and YFL067W of chromosomes XI and VI had 22 and 15 TRs per gene, respectively.

Minisatellites were the most abundant type of repeats: As discussed in the literature review, repeats with a unit size of <10 were classified as microsatellites, repeats with a unit size between 10 and 100 were classified as minisatellites, and repeats with a unit size >100 were classified as megasatellites or simply satellites. Analysis of micro-, mini-, and megasatellites indicated that minisatellites were the most abundant type of TRs in the S288c genome, constituting approximately 67.7% (853/1258) of all TRs. Microsatellites comprised 27.8% (357/1258), while only 3.8% (48/1258) of TRs were megasatellites. The variability among repeats was observed based on period size (Fig. 3). Most repeats had a unit size between 25-50 bp, with around 536 TRs, followed by 335 repeats with a unit size ranging from 50-75 bp (Fig. 4). Only 9 repeats had unit sizes in the 1-25 bp range.

Only seventy-two (72) repeat unit or period sizes were identified among all 614 TRs: A total of seventy-two repeat units (period sizes) were recognized in the ORFs of S. cerevisiae strain S288c through TRF (Fig. 5). The total length of these repeat units was 127,259 bp. Some of these repeat units were repeated in different genes with different motif sequences. Fifty-one of these TR sequences were multiples of three. The remaining 21 TR sequences were non-multiples of three (Figs. 6 and 7). Among the multiples of three, TRs with a unit size of 15 were repeated 147 times, while TRs with unit sizes of 3, 12, 18, and 21 were repeated 139, 135, 129, and 109 times, respectively. TRs with unit sizes multiples of three constituted 1161 repeats out of the total of 1258 (Fig. 6). For repeats that were non-multiples of three, the highest number of repeat units was observed for TRs with repeat units of 22, 14, and 17. These repeat units were repeated 10 times each, while repeat units 16 and 19 were repeated 7 and 6 times, respectively (Fig. 7). The maximum repeat unit size was 460 bp. It should be noted that TRF was pre-set to find repeat units with a maximum size of 500 bp. The most abundant repeat unit was a mononucleotide (1) repeated 63 times in the gene YNL112W DBP2 of chromosome XIV. The second most abundant repeat unit was trinucleotide (3), repeated 3 times in the gene YIL130W ASG1 of chromosome IX. Repeat units 26 and 35 in the genes YER075C PTP3 and YIL011W TIR3 on chromosomes V and IX were less abundant, with copy numbers of 2.3 and 1.9, respectively.

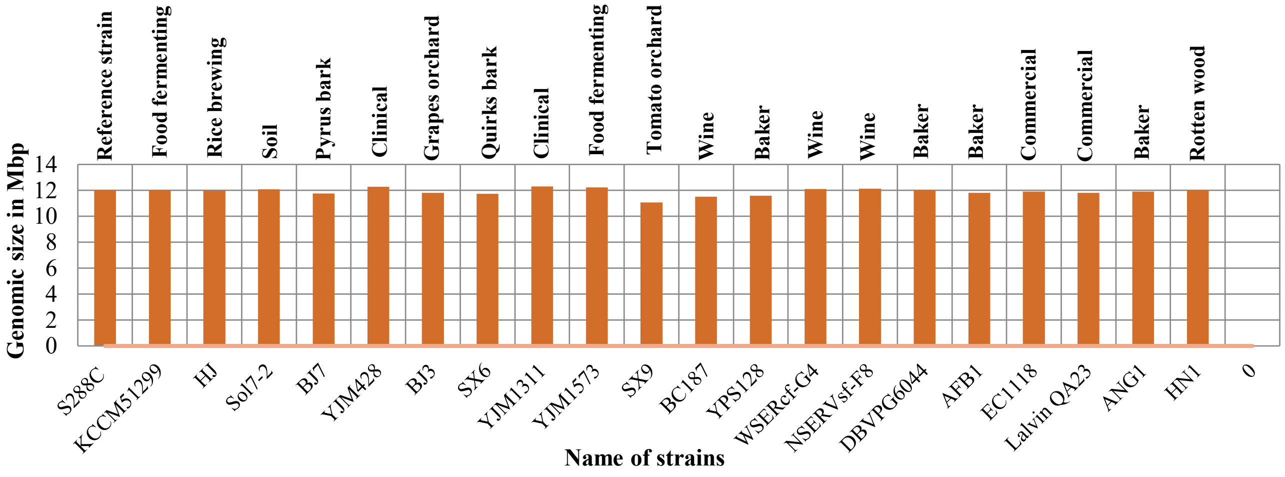

Fig. 2. The genome size and niche of each strain were considered for comparison.

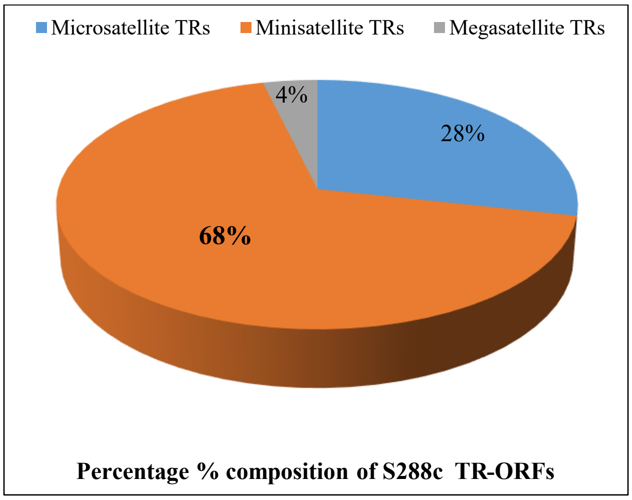

Fig. 3. Minisatellites were the most abundant type of TR-ORFs.

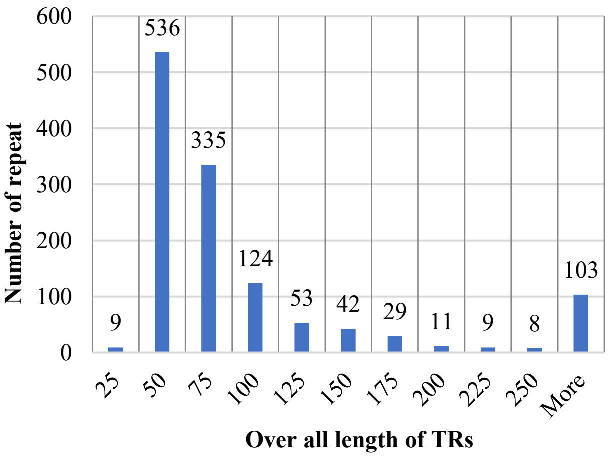

Fig. 4. Frequency distribution of TRs in reference strain S288c of *S. cerevisiae.*

TR regions range in length from 40 to 2446.5 bp: The smallest repeat consisted of a repeat unit (period size) 10, repeated 4 times, resulting in a total length of 40 bp. The largest repeat had a repeat unit of 135 bp, repeated 17.9 times in different genes, making the entire repeat region approximately 2446.5 bp in length. The second-longest repeat had a unit size of 192, repeated 12.2 times, resulting in a total length of 2342.2 bp. Four repeats had lengths ranging from 1500 to 2450 bp, while five repeats had maximum sizes around 1000 bp. The remaining repeat units had sizes ranging from 100-500 bp (Fig. 8).

All megasatellites repeat-containing genes were randomly distributed in strain S288c of *S. cerevisiae*: All 48 megasatellite repeat-containing genes were randomly distributed across chromosomes in S. cerevisiae S288c. Five chromosomes contained more than seven megasatellite repeats, while the remaining seven each had 2-3 megasatellite repeats. No megasatellite repeats were identified on chromosome II or in the mitochondrial genome. However, the repeat motif sizes and the number of copies of repeat-containing genes varied across chromosomes (Fig. 9). The gene with the longest megasatellite repeat, with a 456 bp motif size, is located on chromosome XI. In contrast, the smallest megasatellite repeat, with a 102 bp motif size, is located on chromosome XV. Seventeen of the 48 selected megasatellite repeats were found in genes associated with the cell periphery. This may aid in cell-to-cell attachment, cell wall stability, cell wall permeability, and resistance to various stresses such as heat and environmental hazards.

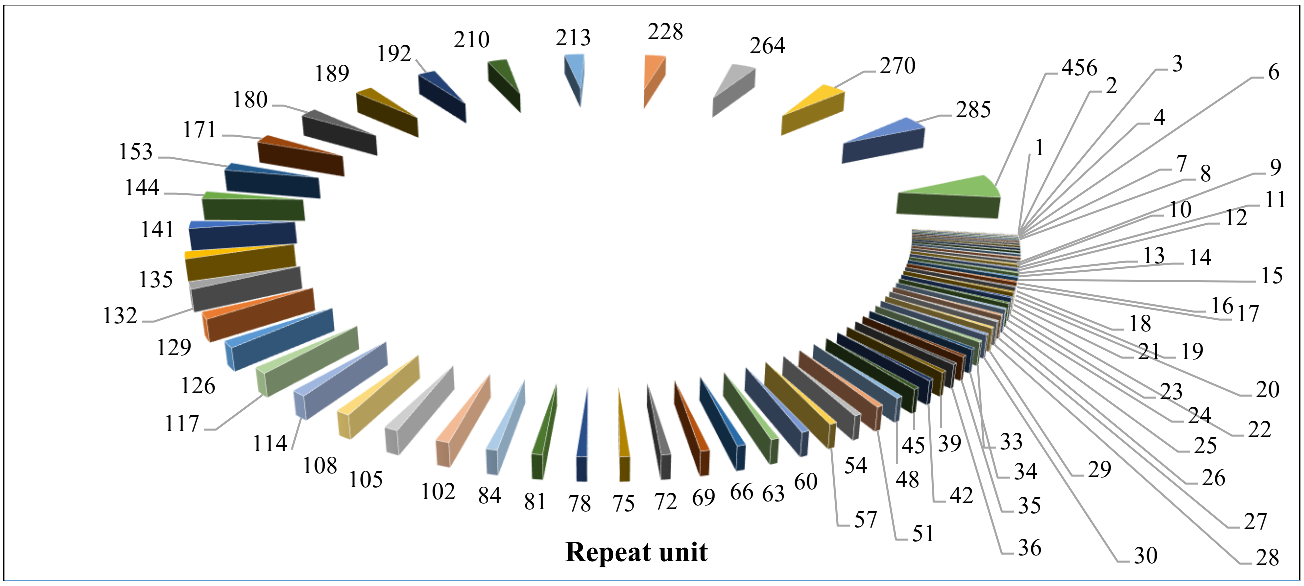

Fig. 5. Total repeat units (period size).

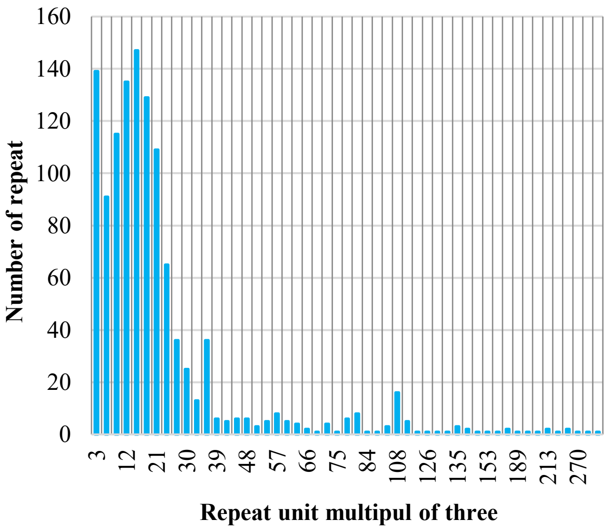

Fig. 6. Repeat unit multiple of three.

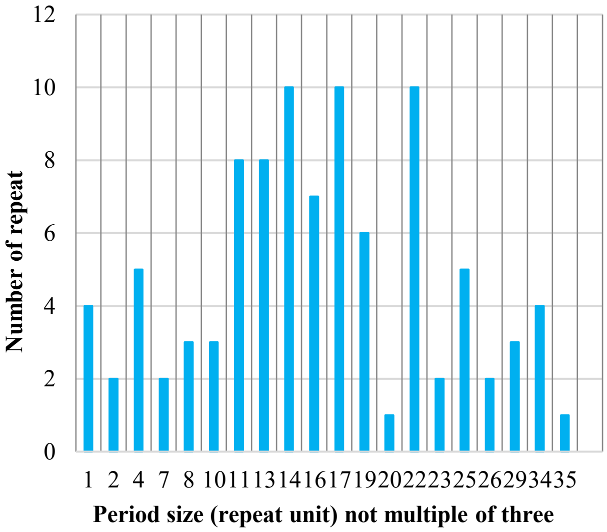

Fig. 7. Repeat unit not multiple of three with number of repeats.

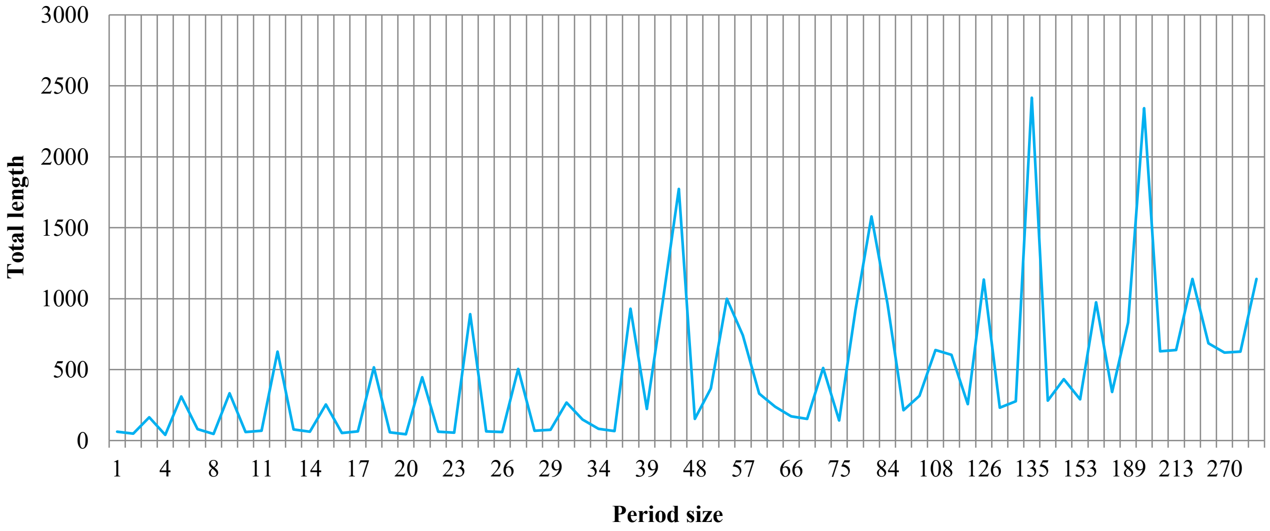

Fig. 8. Number of repeat or copy numbers with total length.

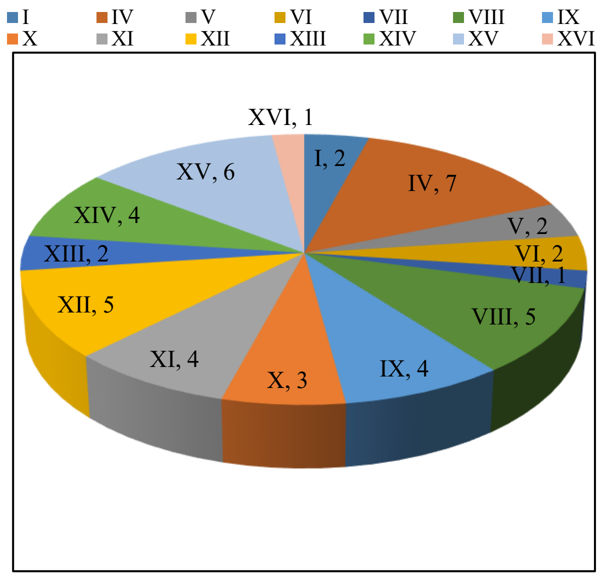

Fig. 9. Random distribution of megasatellite TRs in S288c chromosomes.

All 48 Megasatellite TRs in reference strain S288c are depicted below: All 35 genes containing 48 megasatellites TRs are represented with a bar without any coordination number. The representation was based on the blast hits representative bar but not the actual blast hits. The bar with blue color showed ORFs containing the gene, while the red bar showed repeat region in the gene based on Tandem Repeat Finder (TRF) output file analysis (Figs 10).

Description of Selected Strains: All strains used in the study were selected from different niches with variable genomic sizes sequenced to date. Seven (7) out of twenty (20) strains are baker fermenting and have genomic sequence 12Mb (median). All clinical strains have a maximum size of 12Mb (median) assembly, higher than the remaining strains. The remaining eleven (11) strains were collected from the bark of trees, soil, and food with a maximum genomic size of 12.2Mb (median). Most, if not all, of the strains are available everywhere in the world. Very few strains are specific in origin (KCCM51299), while few others are unknown.

Genes containing these 48 megasatellites from S288c were compared against 20 yeast strains using BLAST: The 48 megasatellite TRs of reference strain S288c were compared against 20 other yeast strains using BLASTN. The results showed that megasatellite repeats were distributed randomly across different strains. Nine of the 20 strains selected from various niches showed some similarity or a match in different genes containing TRs with the reference strain. In comparison, the remaining 11 strains differed in TR regions (Fig. 11). Interestingly, most strains that showed no match in TR regions with the reference strain had genome sizes greater than 12 Mb (median). The strain NSERVsf_F8 showed the highest number of TRs (23 out of 48) containing megasatellite TRs. The second-highest number of megasatellite TRs (20 out of 48) was found in strain Sol7-2, while the remaining strains showed variable numbers of megasatellite TRs.

Most of the Genes from S288c Had No Blast Hit in Other Yeast Strains: A comparison of the 48 megasatellite TR-containing genes of S288c against 20 other yeast strains yielded no BLAST hits for 11 of the selected strains (Fig. 11). Half of these strains had genome sizes of 12 Mb (median), while the remaining half had genome sizes of 11.9 Mb (median) comparable to the reference strain. This suggests that the assemblies were of reasonable quality, but megasatellite TRs may be absent in these strains. To confirm this, five megasatellite TR-containing genes were randomly selected and searched against the Sequence Read Archive (SRA) database. None of the searches yielded any significant matches.

Blast results of best hits for megasatellites repeat across nine (9) strains out of 20 selected strains: The sequences of various strains included in the study were analyzed through BLAST (Basic Local Alignment Search Tool), which is used to study sequence similarity. The BLAST results showed that some of the hits for query sequences matched with subject sequences in the repeat region. Some of the better hits also showed similar flanking sequences (Fig. 12). Many BLAST hits showed flanking sequences on one side, and a few showed flanking regions on both sides. Some query sequences showed deletions in the middle, while others exhibited deletions on alternate sides. Some hits also showed high variability in the repeat regions across various strains, with most hits showing flanking sequences on both sides. However, there were instances of insertion and deletion within the sequence (Fig. 12).

A significant number of megasatellites TR-containing genes encode for cell surface proteins: To evaluate the distribution of megasatellite TR-containing genes in the reference strain S288c across various cellular components, we performed Gene Ontology (GO) analysis using the GO Slim Mapper tool. The GO annotations of all the genes in the reference strain were used as a reference. The results showed that many megasatellite TRs were predominantly associated with specific cellular components, processes, and functions. In contrast, the remaining megasatellite TR-containing genes exhibited similar patterns to the reference strain. TRs were significantly over-represented in genes encoding for membranes, extracellular regions, the cell wall, cellular buds, sites of polarized bodies, and the plasma membrane. Other cellular components, such as the cytoplasm and various types of membranes, also showed some enrichment, though not as pronounced. Cellular components like the mitochondrion, vacuole, and endoplasmic reticulum showed an under-representation of TRs compared to the reference strain (Fig. 13).

GO analysis for processes indicated that megasatellite TR-containing genes were involved in 29 different processes. Around 62% (18 out of 29) of the processes, including DNA recombination, cell wall organization, organelle inheritance, invasive growth, pseudohyphal growth, response to chemical and oxidative stress, and cytoskeleton organization, showed megasatellite TR enrichment. The most prominent processes were DNA recombination, telomeric organization, and pseudohyphal growth. Meanwhile, other processes, such as the cell cycle, organelle organization, ion transport, proteolytic activity, and organelle fission, were under-represented in our dataset (Fig. 14). The remaining 20% (6 out of 29) of processes showed no significant differences between the frequency of selected genes and overall gene frequencies involved in cellular processes. These processes included response to chemicals, the mitotic cell cycle, cellular amino acid metabolic processes, protein modification, nucleobase-containing compound processes, and oxidative stress.

Analysis of GO "Function" revealed a similar trend to that of "Component" and "Process." Some categories were over-represented, some were under-represented, and others remained the same as in the reference. Over-represented functions included ion-binding capability, hydrolase activity associated with the breakdown of various molecules, helicase activity for cutting double-stranded DNA during replication, and lyase activity. Genes involved in processes like nucleic acid (DNA and RNA) binding and transferase enzymes (acting as carriers of various groups, such as alkyl transferase enzymes) were under-represented in our dataset (Fig. 15).

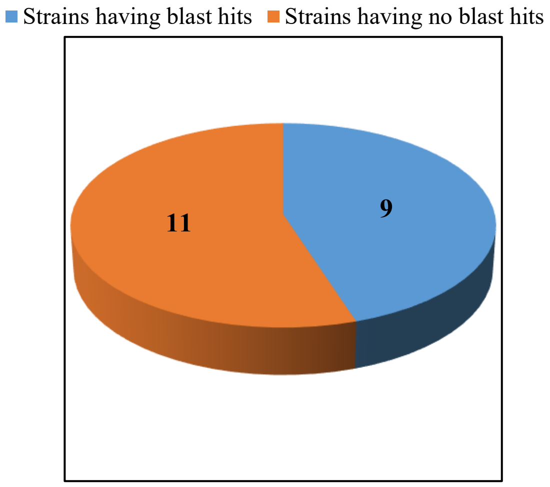

Fig. 11. Nine strains had the match in a few genes containing megasatellites TRs.

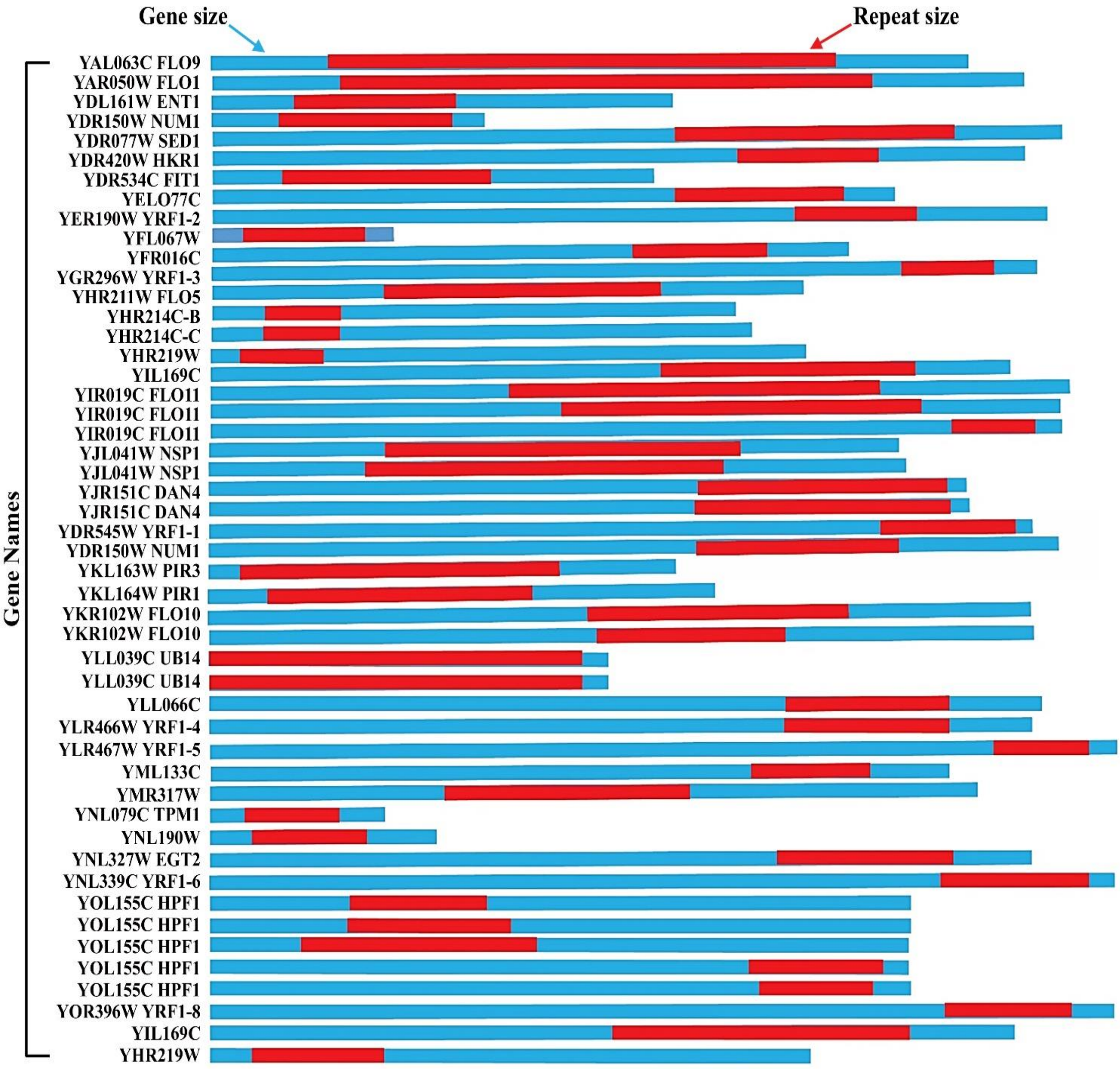

Fig. 10. General representation of 35 genes containing 48 Megasatellite TRs in reference strain S288c of *S. cerevisiae*.

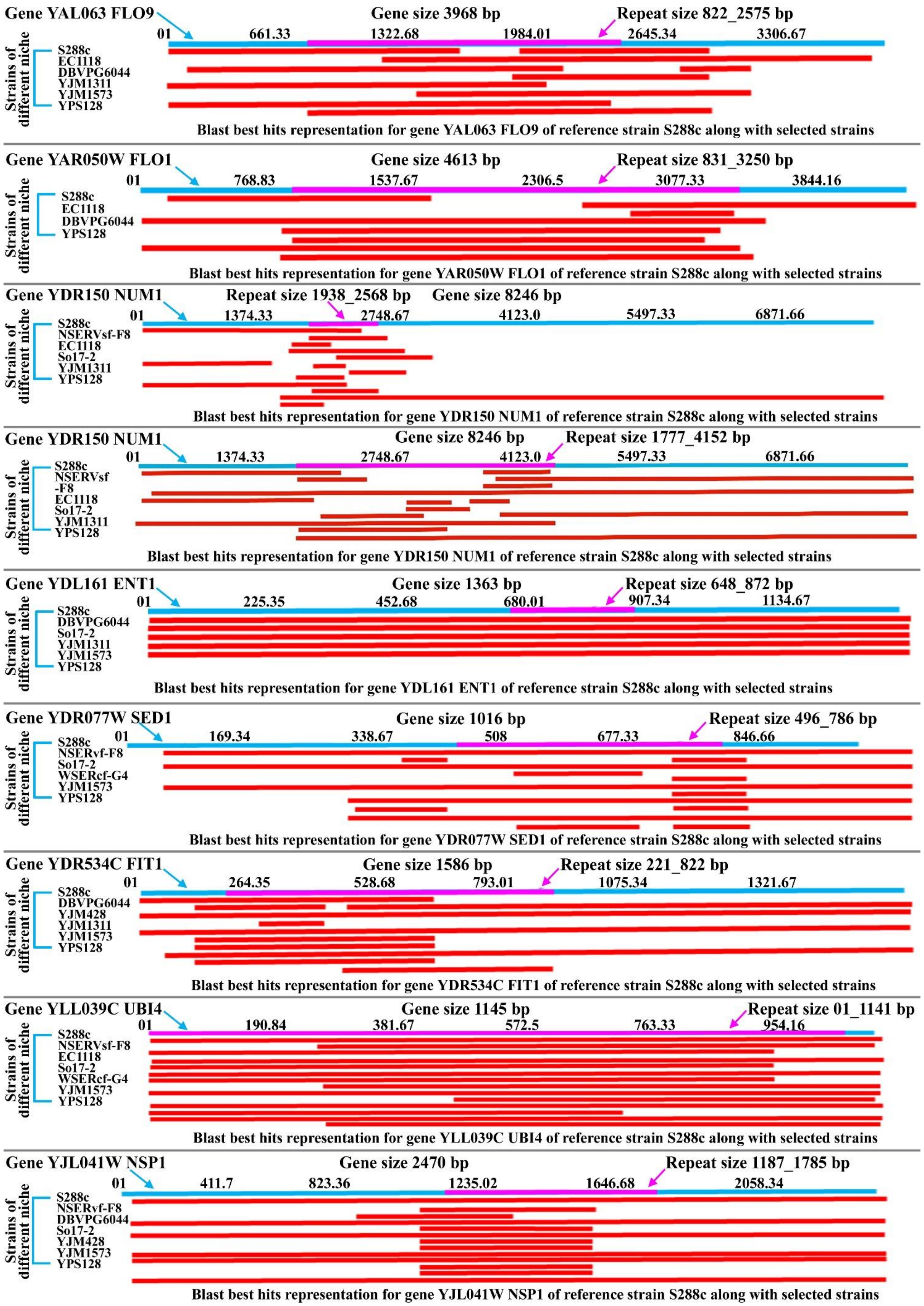

Fig. 12. Blast best hits representation of various gene-containing megasatellite repeats of reference strain S288c along with selected strain.

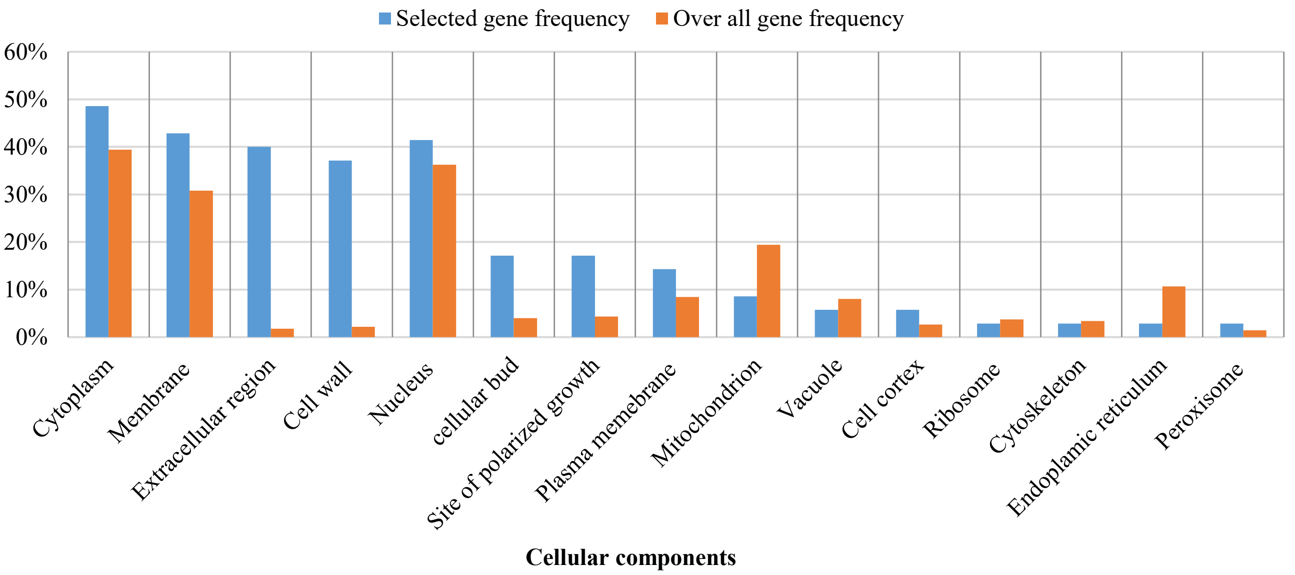

Fig. 13. Representation of cellular components using GO slim mapper. The blue bar represents megasatellite TRs enrichment, while the red bar shows overall genes involved in cellular components (underrepresentation).

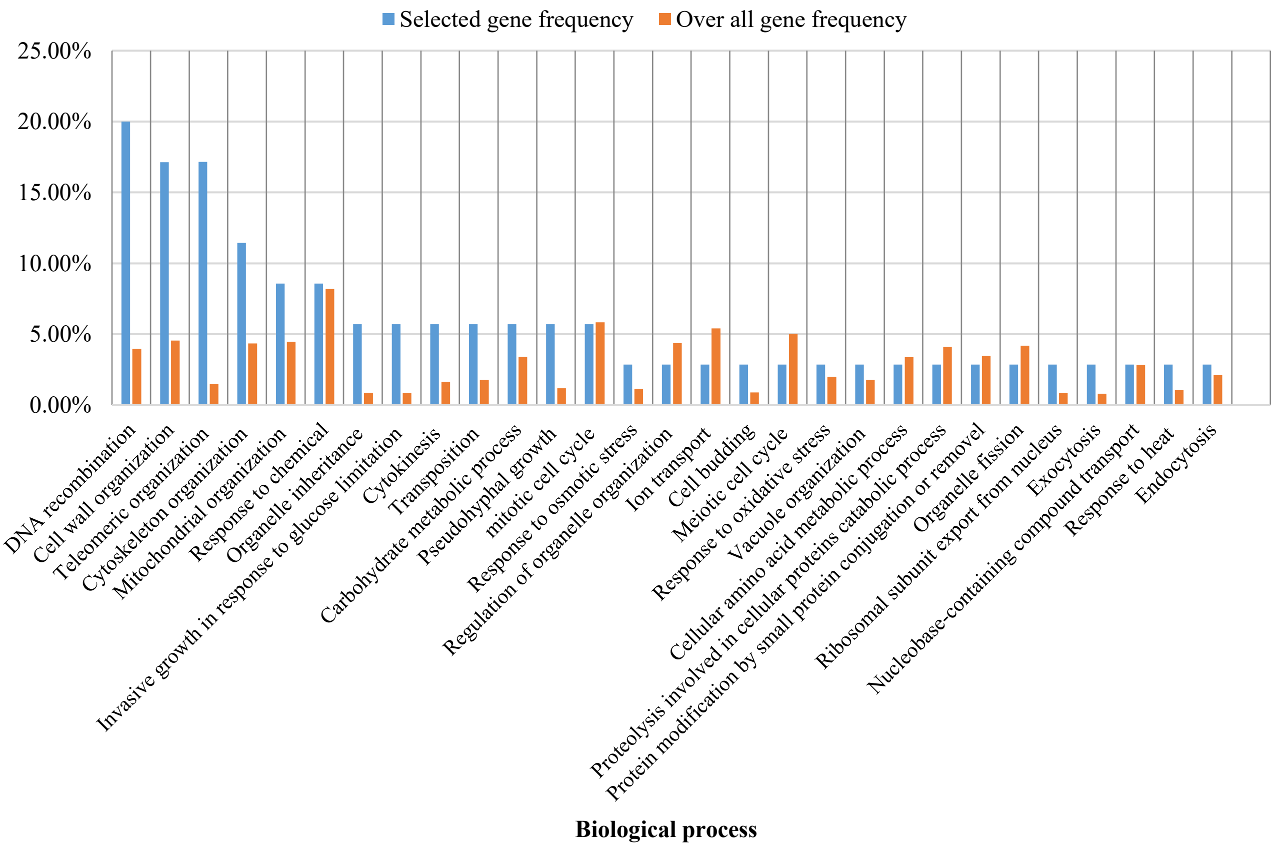

Fig. 14. Representation of biological processes using GO analysis. Around 62% of biological processes are controlled by megasatellite TRs containing genes that showed enrichment processes, represented in the blue bar. Underrepresenting processes are described in the red bar.

The distribution of megasatellites in different strains was not influenced by the niche of the strain: As mentioned earlier, all selected strains had genomic sizes between 11.5 Mb and 12.04 Mb. These strains were isolated from various environments, including bakeries, wine fermentation, clinical settings, brewing, food fermentation, soil, and the bark of trees. BLAST comparisons of the 48 megasatellite TRs in these strains revealed that the niche did not influence the distribution of megasatellite TRs. Seven of the 48 megasatellite TRs from the reference strain had no match in any other strains. No match was found for any of the selected TRs in 11 strains. None of the TRs were found in all strains. Out of 960 possible hits, only 162 (16.8%) were found. These few matches were randomly distributed across strains from various niches.

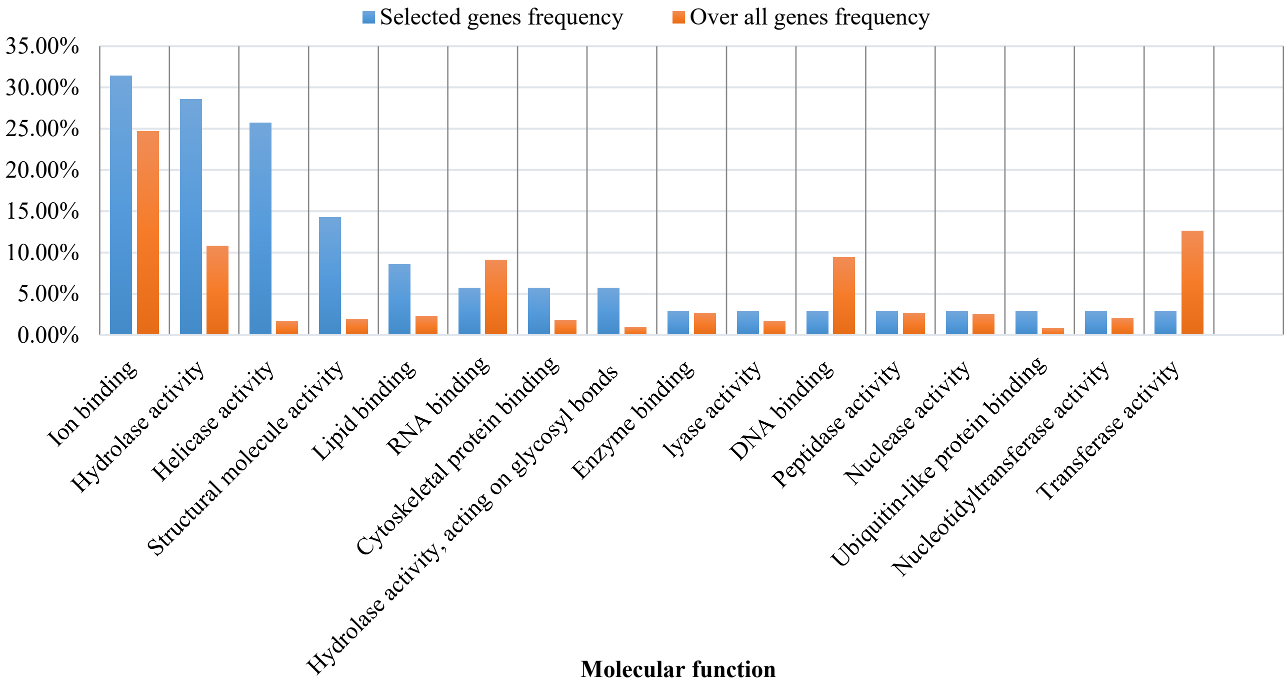

Fig. 15. Using GO slime analysis to represent molecular function under the control of gene-containing megasatellite TRs.

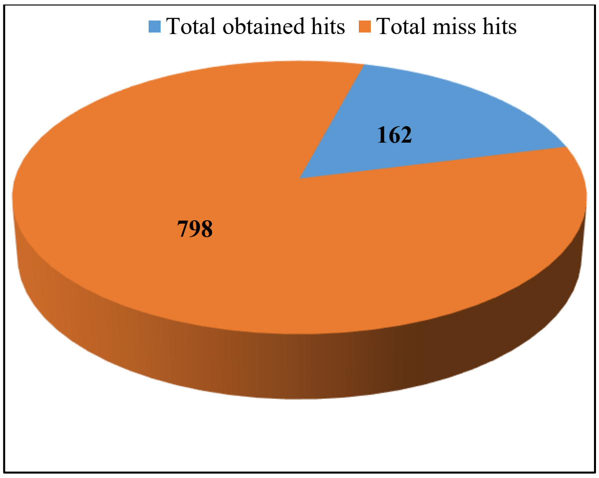

Fig. 16. Majority of Blast hits were missed.

Discussion

Many tandem repeats (TRs) are associated with the genomes of yeast species like *Saccharomyces cerevisiae*. In our analysis of open reading frames (ORFs) for TRs, we identified that approximately 33.13% of the genome of *S. cerevisiae* reference strain S288c was covered by TRs. This finding aligns with recent studies showing that TRs are abundant in yeast and other eukaryotic organisms (Kondo *et al*., 2023). However, it contrasts with the findings by Toth *et al*. (2000), who reported that nearly 50% of *S. cerevisiae*' s genome is covered by TRs, including both coding and non-coding regions. Our results indicate a lower percentage of TRs within the coding regions (10.2%), which is consistent with more recent insights suggesting that TRs in coding regions are often linked with gene variability and functional implications, especially for genes involved in stress responses and phenotypic adaptability (Liu *et al*., 2024).

Our investigation also highlights the variability of TRs based on their motif sizes, which play a crucial role in the gene functions of *S. cerevisiae* and other organisms. For instance, recent studies have revealed that the distribution of TRs in species like *Arabidopsis thaliana* and *Citrus* shows notable differences in both frequency and distribution, where up to 25% of the *Arabidopsis* genome and around 20% of the *Citrus* genome are covered by TRs (Zhang *et al*., 2023). Similarly, recent reports have highlighted that about 30% of human genes also contain TRs, underlining the importance of TRs in coding sequences across various species, particularly for their roles in neurological diseases and genetic disorders (Vidal *et al*., 2023).

When focusing on specific types of TRs, such as microsatellites, our results align with recent findings that microsatellite repeats in *S. cerevisiae* are highly variable, with tri- and hexanucleotide repeats being the most abundant (Johnson *et al*., 2023). Previous research has shown that microsatellites are prone to polymorphism and instability, often leading to gene expression changes and the phenotypic variability seen in various organisms (Liu *et al*., 2024). Our data also reflect the scarcity of mono- and dinucleotide TRs in the coding sequences of *S. cerevisiae*; however, they are frequently found in non-coding regions, a trend that mirrors findings in other eukaryotes (Smith *et al*., 2023).

Minisatellite TRs, as identified in *S. cerevisiae*, constitute a significant proportion (67.8%) of the TRs within the genome, with notable diversity in motif size. This observation is consistent with recent research, showing that minisatellites are critical in genome evolution and adaptive processes (Verstrepen *et al*., 2024). Minisatellite repeats are commonly linked to genomic variability and functional diversity, contributing to phenotypic plasticity and adaptation to environmental stresses (Johnson *et al*., 2023). Similar results have been noted in other organisms, where minisatellites are often associated with cell adhesion and stress resistance genes, further supporting the hypothesis that these repeats are integral to organismal adaptability (Hernandez *et al*., 2023).

Regarding megasatellite TRs, our analysis reveals that *S. cerevisiae* strains carry specific megasatellite repeats within ORFs associated with cell adhesion and stress resistance, particularly in subtelomeric regions. Recent research supports this finding, suggesting that megasatellite repeats involve telomeric organization and cellular stress responses (Jiang *et al*., 2024). We observed that megasatellite TRs are often found in genes like *FLO1, which are* responsible for cell flocculation and adhesion. This aligns with studies suggesting that such TRs contribute to pathogenicity and adaptation in fungal species (Polakova *et al*., 2024). Interestingly, despite the similarity of megasatellite repeats across certain *S. cerevisiae* strains, some strains lacked these repeats, possibly due to genomic divergence or specific environmental conditions (Jiang *et al*., 2024).

The variability of megasatellite TRs among strains, especially within specific niches, mirrors recent work demonstrating that such repeats are often specific to certain fungal lineages and strain backgrounds (Kaur *et al*., 2024). For instance, *Candida albicans* and *C. glabrata* have shown niche-specific megasatellite TR distributions, which could be linked to their ability to adapt to distinct host environments (Rolland *et al*., 2024). Our results similarly indicate that no specific pattern of megasatellite TRs is directly linked to the niche of a strain, suggesting that while some strains share common megasatellite TRs, others may lack them due to environmental pressures or strain-specific genetic backgrounds.

Moreover, our findings indicate that megasatellite TRs are highly involved in genes related to the extracellular region and cell wall structures, consistent with their role in adaptation to environmental changes and stress resistance. This aligns with recent studies suggesting that TRs regulate fungal adhesion and pathogenicity, particularly those found in genes encoding for cell wall proteins (Teunissen *et al*., 2023; Liu *et al*., 2024).

Conclusion

In conclusion, our study sheds light on the significant presence and functional roles of tandem repeats (TRs) within *Saccharomyces cerevisiae*, especially in the context of genetic variability and adaptability. We observed that approximately 33.31% of the *S. cerevisiae* reference strain S288c genome is covered by TRs, with 10.2% of genes containing TRs within their open reading frames (ORFs). Among the different types of TRs, minisatellites were the most abundant, followed by microsatellites and megasatellites. Notably, most of these TRs exhibited multiple bases, suggesting they may act as a selection against frameshift mutations, highlighting their evolutionary significance. Furthermore, our analysis of megasatellite TRs, particularly in genes associated with cell adhesion and flocculation (e.g., *FLO1* and *FLO9*), underscores their potential role in stress responses and environmental adaptation. Despite observing high variability in the distribution of these megasatellite repeats across strains, the data emphasizes that their presence is strain- and niche-dependent, further demonstrating the complexity of TR functions across different environments.

Recommendation

Considering these findings, we recommend several avenues for future research to deepen our understanding of the role of TRs in yeast genetics and evolution. Expanding the scope of the study to include a broader range of strains, particularly those isolated from diverse environmental niches, could provide valuable insights into the functional diversity of TRs across species and their adaptive significance. Additionally, exploring the regulatory mechanisms by which TRs influence gene expression and phenotypic variation-particularly about stress resistance and pathogenicity-would contribute to a more comprehensive understanding of how TRs shape yeast genome evolution. Further investigation into the potential of TRs as biomarkers for strain identification or stress response could have practical applications in biotechnology. Ultimately, more in-depth analyses of TRs, especially megasatellites, could reveal new insights into their contribution to the genetic and phenotypic diversity of fungal species.

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