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Characterization of SSR Markers for Genome-Wide *Staphylococcus aureus*

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ABSTRACT

Objective: *The significant genetic diversity of Staphylococcus aureus, a significant human pathogen, supports its virulence, adaptability, and drug resistance. High-resolution molecular markers are essential for epidemiological surveillance and efficient strain classification. Simple sequence repeats (SSRs) are a potent but little-used class of genetic markers in bacterial systems.* ***Method:*** *An extensive genome-wide survey of SSRs in Staphylococcus aureus was conducted utilizing the Crate v1.5.1 platform to identify, categorize, and assess microsatellite sites for possible primer production.* ***Results:*** *25,727 accurate SSRs were discovered out of 159,187 genomic sequences totaling 465.4 Mb with a GC level of 34.11%. SSRs have an average length of 43.94 bp and make up 1.13 Mb, or 0.25% of the genome. 2428.49 bp/Mb and 55.28 loci/Mb were determined to represent the overall density and relative abundance, respectively. Dinucleotide duplication (54.34%) and mononucleotide duplication (27.89%) were the most prevalent classes. Repeats of tri-, tetra-, pent-, and hexanucleotides made up progressively decreasing percentages. AC, AG, and AT were the most prevalent dinucleotide motifs, and a substantial bias towards A/T-rich repeats was revealed by motif structure analysis. A trade-off between polymorphism and stability was suggested by the majority of SSRs, which displayed intermediate repeat numbers, primarily between 5 and 13 units.* ***Novelty:*** *The AT-rich composition of the S. aureus genome is compatible with the reported SSR distribution patterns, which show selective restrictions on repetitive evolution. Crucially, dinucleotide repeats' high frequency and beneficial repeat characteristics highlight how well-suited they are for SSR primer production. These locations are excellent choices for producing dependable, reasonably priced, and incredibly educational content..*

INTRODUCTION

The bacteria of *Staphylococcus aureus* is one of the clinically relevant bacterial diseases, causing simple skin infections to severe systemic diseases such as endocarditis and pneumonia [1], [2], [3]. The clinical misuse of antibiotics and natural selection of *S. aureus* caused the appearing of methicillin-resistant *Staphylococcus aureus* (MRSA). The MRSA needs sophisticated molecular studies to understand more of the MRSA genetic variation [5]. The understanding of population studies of bacteria has significantly advanced the path to molecular applications, including whole-genome sequencing (WGS) and multilocus array. Yet these techniques can be expensive, take more time, and require complex infrastructure, therefore greatly limiting their laboratory practice. Finding replacement molecular markers that are affordable, especially polymorphic, and suitable for intensified studies has thus become important [5], [7], [8].

The simple sequence repeats, or microsatellites (SSRs), are the most widely used molecular markers. SSRs are short, repetitive DNA motifs found abundantly in both coding and non-coding genes of the genome [9], [10]. The SSRs contain 1 to 6

nucleotides. SSRs are particularly polymorphic due to their variation; therefore, they are particularly used for genetic diversity, population studies, and a molecular approach. SSRs have been confirmed to be important for gene regulation and genome evolution in bacterial genomes research [11],[12]. The study of SSRs in *S. aureus* has not been studied as thoroughly as in eukaryotes. The discovery of SSR loci throughout entire bacterial genomes has become possible due to advances in NGS technology, which has made large-scale genome research more accessible [13], [14].

Traditionally, the SSRs are isolated from whole genomic sequences. This method yields a representation of the microsatellites in the genome. With the revolution in NGS technology, it has now become feasible to identify microsatellites in organisms [15], [16]. The methods are extremely successful in molecular marker development for the purpose of population studies, molecular mapping, etc. However, the selection of SSR mining criteria and algorithms used for the purpose of checking sequences for identifying microsatellites therein offers a lot of diversity. The SSR repeats differed in their stability and quantity. The monorepeats were common but may be less stable due to sequencing errors, while dinucleotide and trinucleotide SSRs are usually abundant and stable [8], [17], [18]. In addition, the sequences of the SSR repeat motif are often affected by nucleotide composition, including the concentration of GC, which can affect SSR distribution and frequency [8], [17], [18], [13], [19]. Increased polymorphism is generally associated with longer repeats and larger repeat numbers, making them more useful for genetic research. On the other hand, repeats that are too long may be less stable and more prone to mutations. Therefore, it is important to find SSR loci with the best repeatability characteristics to create reliable molecular markers [10], [20].

The markers can be used to create primers for amplification using the polymerase chain reaction (PCR), enabling rapid and economical genotyping. Construction of SSR-based markers. There is much promise in advancing molecular typing techniques for *S. aureus*. These marketplaces can improve existing methods by offering more flexibility and solutions[21], the SSR markers can be used to follow transmission routes, distinguish between closely related strains and investigate population structure in environmental and clinical contexts. In addition, SSR based methods are available and somewhat easier to use, making them suitable for laboratories with low funding[15].

In this study, *S. aureus* provides an increasing amount of genomic data. The researchers can use this data to select useful SSR loci to find conserved and variable regions of the bacterial genome. In addition, the patterns of genetic diversity and adaptation can be found by studying the SSR patterns. This is necessary to understand the epidemiology of *S. aureus* infection. Thus, the current study aimed to identify and characterize SSR patterns in the *S. aureus* genome and to assess their potential for constructing specific SSR primers for use in future genetic studies.

RESAERCH METHODS

Genome Data Preparing

The high-throughput sequencing of *S. aureus* (SRX32667246) provided the genomic dataset used in this study. A total number of sequences, about 159,187, and a genome length of 465,404,851 bp. FASTA format was used in this study. Before analysis, we evaluated the sequence of integrity and quality. We also ensured sequence length was equal to the genome size by confirming that all sequences had only standard nucleotide bases (A, T, C, and G) and no ambiguous nucleotides (Ns). The total sequence number, genome size, and GC content were computed.

Simple Sequence Repeats (SSRs) Identification

Krait v1.5.1, a high-performance program for quick and precise microsatellite detection and primer creation, was used to identify SSRs across the whole genome [22]. Perfect SSRs, which are tandem repetitions with continuous repeat motifs, were the only subject of the analysis. Minimum repeat limits were established based on motif length as mononucleotide repeats to guarantee strict detection and prevent bogus repeats: ≥12 repeat units, ≥7 repeat units for dinucleotides, ≥5 repeat units for trinucleotides, ≥4 repeat units for tetranucleotides, ≥4 repeat units for pentanucleotides, and ≥4 repeat units for hexanucleotides [23]. At Level 3, motif standardization was implemented, which reduces redundancy and permits biologically significant comparisons by combining complimentary and reverse-complement motifs into a single representative class. The efficiency of the Krait platform for large-scale genomic datasets was demonstrated by the SSR mining procedure, which was carried out under default computational conditions. The overall runtime for analysis was about 52 seconds [24].

Estimating SSR Density and Genome Coverage

Two crucial factors were computed to evaluate the genomic distribution of SSRs: The number of SSR loci per megabase of genomic sequence is known as relative abundance (loci/Mb), while the total length of SSRs per megabase is known as relative density (bp/Mb). These data enable comparison with other bacterial genomes, which indicates the amount of SSR frequency. SSR-based genome coverage was computed as [25]:

$$\text{SSR coverage (\%)} = \frac{\text{Total SSR length}}{\text{Total genome length}} \times 100$$

Data Visualization and Statistical

Krait used integrated visualization tools based on Data Tables, jQuery, and Plotly.js libraries to automatically generate all statistical summaries, tables, and graphical representations[22].

RESULTS AND DISCUSSION

Results

Overview of SSR Discovery and the Genome Dataset

Numerous SSR loci were found throughout the examined dataset because of the genome-wide scan of microsatellites in *Staphylococcus aureus*. Out of 159,187 sequences,

25,727 perfect SSRs were found, indicating a total genome size of 465.4 Mb. High confidence in SSR detection was guaranteed by the lack of ambiguous nucleotides, which also removed any potential bias brought on by sequence ambiguity. The overall length of SSR sections was 1,130,230 bp, or roughly 0.25% of the entire genome. This suggests that microsatellites are a minor but important part of genomic architecture. The existence of large repeat tracts was indicated by the average SSR length of 43.94 bp. SSRs are prevalent throughout the *S. aureus* genome, as further evidenced by the computed relative abundance (55.28 loci/Mb) and relative density (2428.49 bp/Mb).

Table 1. Summary information of sequences (SRX32667246).

Description	Value
Total number of sequences	159187
Sequences' total length	465404851
Total valid length of sequences	465404851
Unkown bases (Ns) in sequences	0
Percentage of unkown bases	0
GC content	34.11

SSR Type Distribution

The dinucleotide SSRs were the most prevalent, about 13,979 loci (54.34%). While mononucleotide repeats were 7,175 loci (27.89%). Pentanucleotide SSRs with 0.44% percentage and hexanucleotide repeats (0.14%), trinucleotide repeats (9.14%), and tetranucleotide repeats (8.05%). This motif frequency decrease is gradual as the length increases, a pattern seen in bacterial systems. The different repeat units of SSRs can be classified into many types. According to the length of the SSRs, they are classified into mono-, di-, tri-, tetra-, penta-, and hexanucleotide SSRs.

Table 2. Show the profile of perfect microsatellites.

Item	Value
Total number of perfect SSRs	25727
Perfect SSRs' total length (bp)	1130230
The average length of SSRs(bp)	43.94
SSRs per sequence	0
The sequence covered by SSRs (%)	0.25
Relative abundance(loci/Mb)	55.28
Relative density(bp/Mb)	2428.49

Relative Abundance and Density of SSR

The dinucleotide repeats have high distribution compared to all other repeat types (30.04 loci/Mb) and relative density (1586.48 bp/Mb). Tri- and tetranucleotide repeats had lower but similar density, whereas mononucleotide repeats had moderate rates (15.42 loci/Mb and 477.12 bp/Mb). With relative abundance values less than 0.25

loci/Mb, pentanucleotide and hexanucleotide repeats made very little contribution to the total SSR landscape. These results show that the *S. aureus* genome's SSR composition is significantly skewed toward shorter repeat units, which are probably the main source of polymorphic variation.

Table 3. The perfect microsatellite counts and length

Type	Counts	Length (bp)	Percent (%)	Average Length (bp)	Relative Abundance (loci/Mb)	Relative Density (bp/Mb)
Mono	7175	222056	27.89	30.95	15.42	477.12
Di	13979	738356	54.34	52.82	30.04	1586.48
Tri	2352	56766	9.14	24.14	5.05	121.97
Tetra	2070	109356	8.05	52.83	4.45	234.97
Penta	114	2580	0.44	22.63	0.24	5.54
Hexa	37	1116	0.14	30.16	0.08	2.4

Motif Composition and Sequence Bias

The comparatively low GC content (34.11%) of the genome was reflected in the strong bias toward A/T-rich repeat motifs seen in detailed motif analysis. Poly-A motifs made up 25.58% of all SSRs among mononucleotide repeats, but poly-C repeats were relatively uncommon. The most common motifs within the dinucleotide group were AC (25.99%), AG (13.03%), AT (10.35%), and CG (4.96%). The comparatively low frequency of CG repeats is in line with the known underrepresentation of CpG dinucleotides in bacterial genomes, which could be impacted by processes associated to methylation and mutational biases. The trinucleotide repeats were the most prevalent patterns, such as AAT, AAG, and AAC, while the contribution of dinucleotide repeats was smaller. The motif distribution pattern highlights base composition influences on SSR generation and stability, such as A/T-rich sequences.

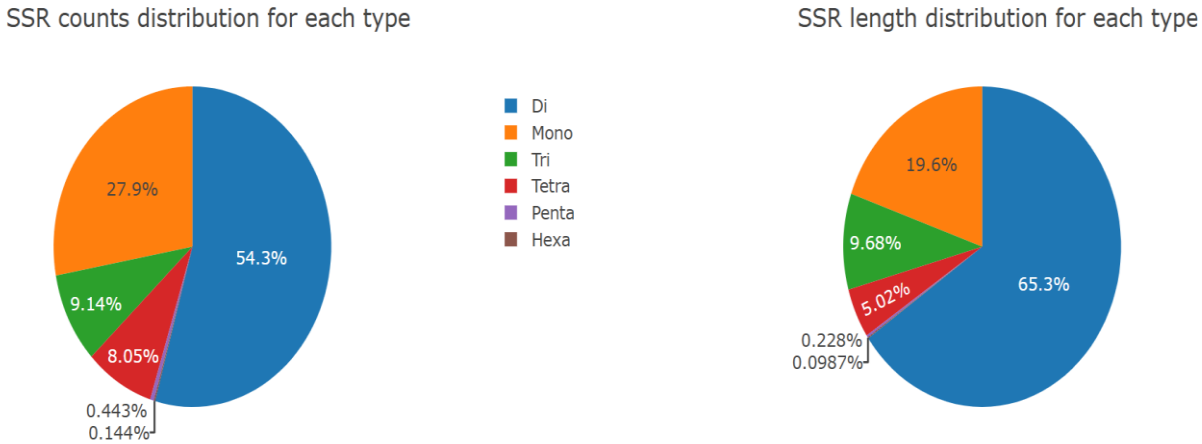

Figure 1. shown distribution of microsatellite account and length.

Repeat Numbers and Length Distribution

The SSR loci showed variable repeat numbers, predominantly 5-13, with repeats at 7-8, indicating a preference for intermediate lengths; that is, reflecting stability and polymorphic, informative marker development despite length variability. Due to their high prevalence and comparatively longer average repeat lengths, dinucleotide and tetranucleotide repeats constituted the greatest percentage of the total SSR length. On the other hand, because of shorter repeat arrays, trinucleotide repeats, despite being present in significant amounts, contributed less to the overall length. The distribution pattern shows that repeat frequency and motif size have an impact on SSR length, with shorter motifs typically accumulating longer repeat stretches.

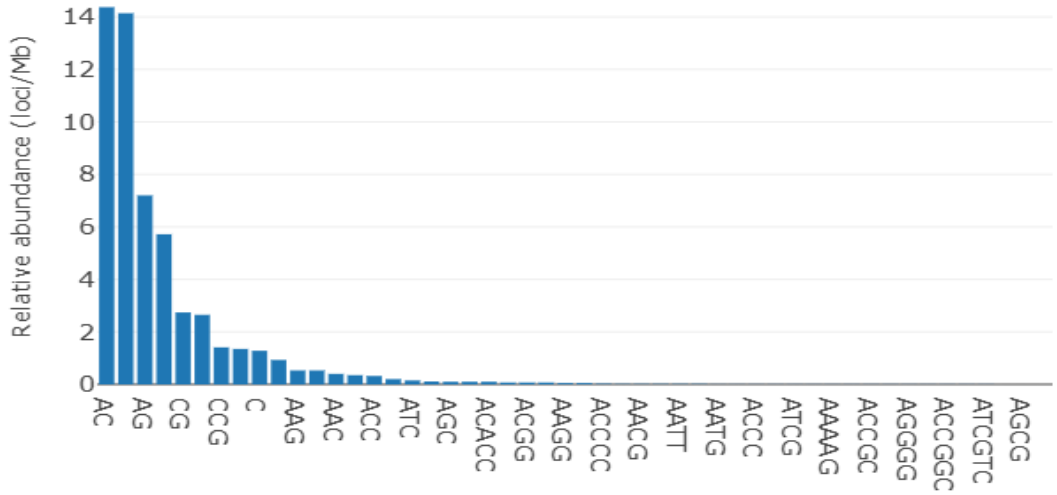

Figure 2. Shown the most abundance motif categories.

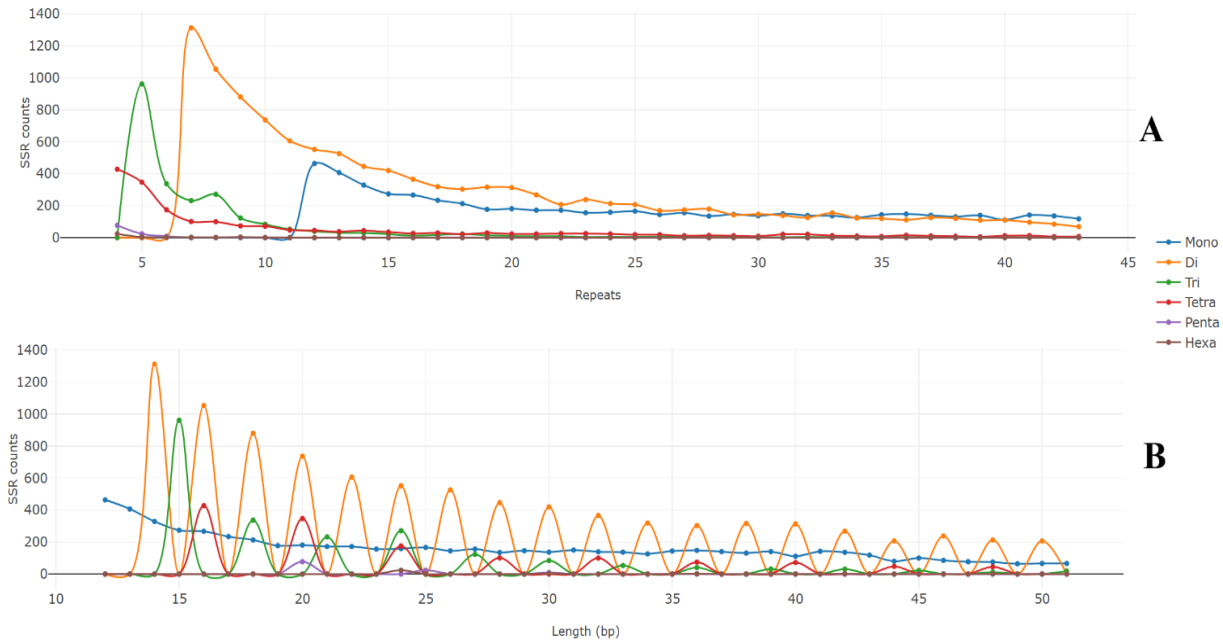

Figure 3. The repeats and length of SSR for each type: A) SSR repeats and B) SSR length

Discussion

The *Staphylococcus* aureus genome has a modest density of SSRs (55.28 loci/Mb), with a substantial prevalence of din- and mono- repeats, according to the current genome-wide research. Although significant variations in SSR abundance, motif composition, and repeat structure reflect species-specific genomic characteristics and evolutionary forces, this pattern seems to be generally constant when compared with other bacterial species. SSR density in many bacterial genomes varies significantly based on ecological habitat, GC content, and genome size. It is noted that research on Escherichia coli has shown comparatively lower SSR density than those found in *S. aureus*, with a somewhat higher prevalence of trinucleotide repeats in coding areas but a similar dominance of short repeat motifs [26]. On the other hand, the genomes of low-GC Gram-positive bacteria like Bacillus subtilis frequently show SSR patterns like those of *S. aureus*, such as a higher frequency of mononucleotide repeats and an enrichment of A/T-rich motifs [27], [28].

The bacteria often exhibit a comparatively high frequency of tri and tetranucleotide repeats, similar to the SSR pattern available in *S. aureus*, which is also such as that reported in other pathogenic bacteria, such as *Salmonella enterica* and *Pseudomonas aeruginosa*. This high GC content causes a more complicated genome and can affect the stability and development of microsatellite markers [29], [30], [31], [32].

The A/T-rich motifs in the *S. aureus* SSR were significant results. This note is in line with studies from bacterial species and is consistent with low GC concentration (34.11%) in the *S. aureus* genome. Moreover, mono-nucleotide type A/T repeats were less widespread, while G/C motifs were common in high-GC bacteria sequences, such as *Streptomyces* species and *Mycobacterium tuberculosis* [33]. Comparison studies indicated that SSR patterns were significantly affected by the sequences of genomic bases, and since these sequences are more vulnerable to slippage, A/T-rich genomic sequences accumulate more in mono and dinucleotide repeats [27], [34], [35]. Our study found the CG dinucleotide repeats are present in *S. aureus* and were also in line with other studies found in the genomes of bacteria. The mechanisms of DNA methylation and mutation bases lower the frequency of CpG loci over evolutionary time. Dinucleotide repeats dominate in *S. aureus* with a percentage of 54.34%, which is similar to findings in bacterial genomes, where short repeat regions are more prevalent because of their high rate of mutation. The *S. aureus* has a low percentage of trinucleotide repeats when compared to other bacteria [34].

A comprehensive *S. aureus* adaptation is foundational for facing the challenges presented by these pathogenic bacteria. Moreover, the phenomenon is widespread, with diverse human microbiome samples harboring *S. aureus* with distinct SSR sub-populations, indicative of niche-specific adaptations. Therefore, SSRs are strong reservoirs of previously no recognized *S. aureus* genetic heterogeneity that confer fast adaptation, including in antigens selected by bygone vaccines. Deeper knowledge of SSR's evolutionary way has the possibility to make better treatments and to predict *S. aureus* responses to new therapies[13],[36]. The SSR patterns found in our work

indicated a high moderate level of microsatellite variability, though in *S. aureus.* The prevalence of SSRs with 5–13 units in *S. aureus* is consistent with the genomes of other bacteria. Determine whether the frequencies of SSRs of the given motif length and repeat number occurred as expected by chance; ten simulated genomes were constructed by randomly choosing nucleotides at the frequencies characterizing the *E. coli* genome [26], [37]. According to comparative studies among bacterial strains, the widespread occurrence of SSRs does not correlate with genome size. Larger genome sequences may include high SSR loci, but their relative abundance (loci/Mb) can change.

The SSR density identification in *S. aureus* is like other bacteria with genome sizes, which suggests that evolutionary plasticity and sequence stability have a higher impact on SSR widespread than genome size. *Pseudomonas aeruginosa,* for example, SSR frequency showed high motif complexity but lower relative SSR abundance due to its bigger and more GC-rich genome. The SSRs of numerous types are found in the genomes of prokaryotes. These SSRs were presented in functional role and implications in mutation, giving the organism adaptation to its environment [32], [39], [40], [41].

CONCLUSION

Fundamental Finding : The genome analysis of S. aureus reveals a high abundance of microsatellites (SSRs), suggesting that these repetitive elements are systematically distributed and distinct from general nucleotide composition patterns. **Implication :** The substantial presence of microsatellites indicates a strong potential for generating both genomic variability and phenotypic diversity, which may contribute to the organism's adaptability and pathogenic characteristics. Limitation: Although microsatellites are known to play major roles in eukaryotic genome dynamics, their occurrence in prokaryotic bacteria remains limited and is mostly associated with pathogenic species, which may restrict broader generalization. **Future Research :** Further studies are needed to explore how SSR variability influences bacterial evolution and pathogenicity, and to investigate their functional roles across different prokaryotic organisms.

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