
Araştırma Makalesi / Research Article

Molecular Typing with BOX-PCR of *Staphylococcus aureus* Isolated from Milk Samples

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Abstract

The aim of this study is to characterize 98 *Staphylococcus aureus* isolated from bovine subclinical mastitis in Konya region with BOX primer. A total of 28 band profiles ranging from ~ 950-300 bp were generated by the BOX primer. This band profile was evaluated according to Nei homology and a dendrogram (UPGMA) was obtained. According to the data obtained, 95 isolates with genetic similarity varying between 50-100% were in the same group. This study demonstrates that the BOX-PCR technique provides a fast and reliable tool to investigate epidemiology in *S. aureus*.

Keywords: Molecular typing, bovine mastitis, repetitive DNA sequences, *Staphylococcus aureus*.

Süt Örneklerinden İzole Edilen *Staphylococcus aureus*'un BOX-PCR ile Moleküler Tiplendirilmesi

Öz

Bu çalışmanın amacı Konya bölgesindeki sığır subklinik mastitinden izole edilen 98 *Staphylococcus aureus*'u BOX primeri ile karakterize etmektir. BOX primeri tarafından ~ 950-300 bp arasında değişen toplam 28 bant profili oluştu. Bu bant profili Nei homolojisine göre değerlendirildi ve bir dendrogram (UPGMA) elde edildi. Elde edilen verilere göre % 50-100 arasında değişen oranlarda genetik benzerlik gösteren 95 izolat aynı grupta yer aldı. Bu çalışma, BOX-PCR tekniğinin *S. aureus*'ta epidemiyolojiyi araştırmak için hızlı ve güvenilir bir araç sağladığını göstermektedir.

Anahtar kelimeler: Moleküler tiplendirme, sığır mastitis, tekrar eden DNA dizileri, *Staphylococcus aureus*.

1. Introduction

Bovine mastitis caused by *Staphylococcus aureus* is an infectious disease that causing significant economic losses in the dairy industry [1, 2]. Therefore, *S. aureus* plays an important role in the fight against mastitis. Mastitis is treated and prevented by the use of antibiotics to cure diseases in dairy cattle herds [3-6]. However, the random use of antibiotics threatens public health due to drug multiresistance and risk of residual sprains in milk [7]. Furthermore, the source of this infection must be correctly identified for good treatment and protection against this infection [6]. DNA-based molecular typing methods have become more important because they are more efficient and have high sensitivity in uncovering the source of infection that concerns public health [8]. BOX-PCR methods have been found to be reliable in the identification of *Staphylococcus* strains [2, 9-11]. In these methods, which are based on the replication of repeating sequences placed at intergenic positions in the genome with specific primers, DNA fingerprints are extracted using 154 bp length BOX element [12, 13]. These genomic fingerprints from bacterial isolates distinguish species, subspecies and strain levels and are also

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successfully applied in medical, agricultural, industrial and environmental studies to determine microbial diversity [2, 6, 13].

In the study, advanced typing was aimed in 98 *S. aureus* isolates isolated from mastitis cows in Konya region by BOX-PCR methods.

2. Materials and Methods

2.1. Bacterial strains

98 *S. aureus* strains were isolated from milk samples with mastitis in Konya Region. These strains were recognized as *S. aureus* by a standard procedure [14]. After, these strains confirmed with the VITEK 2 system (bioMerieux).

2.2. Bacteria Cultivation and DNA Extraction

All strains were planted in brain heart infusion (BHI) broth and cultivation for 18 h at 37 °C. Later, bacterial DNA was isolated as described Ausubel et al. [15] with some modifications.

2.3. PCR methods for Genotyping

Isolated DNA was amplified using the repetitive element sequence. The nucleotide sequence of primer used in this study is as follow; BOXA1R 5'- TACGGCAAGGCGACCCTGACG-3' [16].

Each 25µL of PCR mix contained 2.5mM dNTP mix, 10X PCR reaction buffer (50mM KCl, 10mM Tris-HCl, pH=9, %0.1 TritonX-100), 3 mM MgCl₂, 75 pmol of primer, 2.5 U of Taq polymerase (Fermentas), and 50ng of template DNA. PCR reactions was performed in a Mastercycler Gradient thermal cycler (Eppendorf, Hamburg, Almanya). Amplifications of mixtures were carried out following cycling conditions: an initial denaturation at 95 °C for 7 min was followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 53°C for 1 min, an extension at 72 °C for 1 min, and ending with a final extension at 65 °C for 16 min.

Amplified products were separated by electrophoresis in 2% agarose gel, stained with ethidium bromide (0.5 µg/mL), and visualized by UV-transilluminator. Gel image was recorded with DNA imaging system (Vilber Lourmat, Lamirault, Fransa).

2.4. Data Analysis

The genotypes of *S. aureus* isolates were assessed using Bio1D++ computer program. Band profiles were recored visibly as (1) or (0). Genetic similarity between strains was evaluated according to Nei homology. And then Cluster analysis was carried out with the UPGMA.

3. Results and Discussion

With BOXA1R primer, 98 *S. aureus* isolate totally generated 28 DNA fragments in lengths between ~950-300 bp. Although monomorphic bands are not observed in all isolates, the common bands in most isolates are the band patterns in sizes 900 bp, 800 bp, 500 bp, 475 bp, 410 bp, 350 bp and ~300 bp. Although monomorphic bands are not observed in all isolates, the common bands in most isolates are the band patterns in sizes 900 bp, 800 bp, 500 bp, 475 bp, 410 bp, 350 bp and ~300 bp (Figure 1).

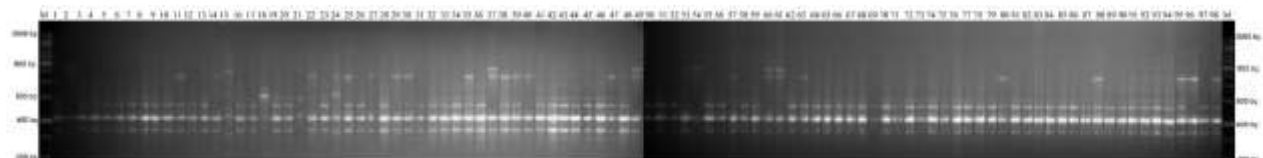


Figure 1. Agarose gel electrophoresis of BOX-PCR products (Line M; marker)

A dendrogram was obtained evaluating the presence or absence of total of 28 bands between ~950-300 bp in Figure 4. According to the dendrogram, while 95 isolates showing genetic similarity by rates ranging between 50-100% are located in the first group, three isolates (15,18,21) forming the second group were separated by 77% from the first group. In addition, the presence of many isolates which were 100% similar in the first group was noteworthy.



Figure 2. Dendrogram of genetic relationships of *S. aureus* strains by BOX-PCR

In recent years, molecular genotyping methods have become the gold standard for the definition of mastitis because they provide specific, rapid, qualitative, quantitative, economic and large-scale diagnosis [17]. Some of these may be exemplified by methods such as Restriction Fragment Length Polymorphism (RFLP) [18], PFGE [19, 20], AFLP [20], plasmid profiles [21-23], RAPD-PCR [24], REP-PCR [25] and ERIC-PCR [26].

REP-PCR typing was determined to be better at distinguishing compared to PCR methods based on repetitive elements such as amplifications of Tn916-16S rRNA gene spacer region [27] and inter 16S-23S rRNA gene spacer region [28]. It was showed that REP-PCR is a rapid screening procedure in classification of a large number of bacteria isolates and provides high distinction [29]. The repeatability of REP-PCR, which is extremely important when used for epidemiological purposes is discovered to be excellent contrary to repeatability of PCR (AP-PCR; Arbitrary Primers-Polymerase Chain Reaction) done using randomly selected PCR primers [30]. In addition, when compared with PFGE it was advocated that REP-PCR is as good as PFGE besides having easy and fast performance as an important advantage [8, 31].

REP- and ERIC- primers have been strongly used in identification of gram (-) [12, 32, 33] and gram (+) [26, 34-36] bacteria strains.

This study is the first study aimed to determine the genetic relationship between *S. aureus* obtained from the milk with mastitis by BOX primers. Although amplification of *S. aureus* strains by REP-PCR could not be performed by REP1R-I and REP2-I primers by some researchers [11], Wood et al [37], successful amplification of gram (+) bacteria such as *S. aureus* could be performed by primers REP1R-I and REP2-I. However very good products have been obtained which allow the identification of *S. aureus* strains as a result of amplification with another REP primer (RW3A) [2, 9, 30, 38, 39, 40]. In contrast to this study, BOX elements were screened in 10 *S. aureus* isolates, but no amplification was obtained [10]. Another study report confirms that BOXA1 primer was not hybridized in *S. aureus* genome.

Most isolates exhibit 100% similarity profiles, but few of them were determined to show similarity under 60% in all two methods in this study. While Alam et al [41] reported that only 2 *Streptococci* strains showed similarity more than 90%, most strains showed less than 90% (down to 40%) similarity; *Staphylococcus epidermidis* strains were extremely similar according to the findings REP, ERIC and BOX [11].

Wieser and Busse [11] obtained fragments of *S. epidermidis* strains > 1100-350 bp in BOX-PCR. BOX-PCR was not encountered in *S. aureus* strains and BOX-PCR (BOXA1) was applied to the *Streptococcus pneumoniae* [10] and *Burkholderia pseudomallei* strains [42] obtaining fragments between 300-1500 bp. In contrast to the previous study, up to 950 bp fragments were obtained in this study. The dendrogram obtained as a result of the combination was indicated by its similarity to the dendrogram as a result of the BOX-PCR. BOX-PCR (36 different profiles) showed that many strains had the same band profiles showing highly similar band patterns. We can explain so much similarity by the probability that the pathogen could be contaminated during milking in the herd and so the origin of most of the isolates are the same. Wieser and Busse [11] advocated that complementary sequences of REP primers are only in some *Staphylococcus* species and so the combination of REP- and BOX-PCR are not sufficient in the distinction of *Staphylococcus* species, but these methods provide an identification among *S. epidermidis* strains by forming specific bands to the strain.

4. Conclusion

It is possible to generate a fingerprint database with these fast and good molecular methods such as BOX PCR. Thus, it can be useful in finding the source of the disease outbreak, routine identification of the microorganism, and vaccine production studies. Thus, the duration of treatment against mastitis may be shortened by rapid prognosticate.

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Authors' Contributions

E.G.M. and E.A. conceived the original idea and build. E.A. supervised the project. The experiments were performed by E.G.M. and E.A. All authors contributed to the writing of the manuscript. Some of the bacteria samples that Dr. Emine ARSLAN isolated in her doctoral thesis were used in this study.

Statement of Conflicts of Interest

There is no conflict of interest between the authors.

Statement of Research and Publication Ethics

The author declares that this study complies with Research and Publication Ethics

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