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DEVELOPMENT OF A RAPID DETECTION METHOD FOR THE MECA GENE IN *Staphylococcus aureus* USING THE PCR METHOD

RUM IA AND MUHSININ S*

Faculty of Pharmacy, Bhakti Kencana University, Bandung, West Java, Indonesia

***Corresponding Author: Soni Muhsinin: E Mail: soni.muhsinin@bku.ac.id**

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ABSTRACT

Staphylococcus aureus infection is characterized by tissue damage accompanied by a purulent abscess. Some of the other infectious diseases caused by *S. aureus* are acne, boils, impetigo and wound infections. More severe infections include pneumonia, mastitis, phlebitis, meningitis, urinary tract infections, osteomyelitis, and endocarditis. *S. aureus* can also cause nosocomial infections, food poisoning, and toxic shock syndrome. The detection of *S. aureus* bacteria by conventional means can use the culture method. However, the culture method has several disadvantages including: it requires laboratory infrastructure for biosafty lavel 2, requires experts, takes a long time (up to 1 week) to get positive results. Therefore, the proposed research aims to develop a fast and accurate detection for *S.aureus* detection using the Polymerase Chain Reaction (PCR) method. This method has been developed in line with the development of the discovery of genes contained in *S. aureus* bacteria such as mecA. The stages of the research method consisted of *S. aureus* subcultures, primary analysis, DNA isolation, DNA amplification, electrophoresis, and data analysis. The results showed that a pair of primers could amplify the mecA gene with an amplicon length of 161 bp. The results of the optimization of the PCR components get optimal results by obtaining the appropriate DNA bands. The PCR method developed can be used to detect the mecA gene.

Keywords: DNA, agarose gel electrophoresis, mecA gene, PCR, *S. aureus*

INTRODUCTION

S. aureus, including Methicillin-Resistant *Staphylococcus aureus* (MRSA) is an important bacterial pathogen related to society and health. *Staphylococcus* infects

humans worldwide by producing a variety of virulence factors including Staphylococcal enterotoxin (SES) and Toxic Shock Syndrome Toxin (TSST) which is responsible for the occurrence of food poisoning **[1]**.

S. aureus, also known as superantigens, mediates various diseases including toxic shock syndrome, and causes skin, lung and systemic infections caused by these organisms **[2]**. When present in food sources, they can cause enteric effects commonly known as food poisoning. Rapid sensitive detection of toxins will enable clinical testing of samples and increase surveillance of food sources **[3]**.

The detection of *S. aureus* bacteria by conventional means can use the culture method. However, the culture method has several disadvantages including: requires laboratory infrastructure for biosafty level 2, requires experts, takes a long time (up to 1 week) to get positive results **[4]**. Therefore, a fast and accurate detection is needed, namely the Polymerase Chain Reaction (PCR) method. This method has been developed in line with the development of the discovery of genes contained in *S. aureus* bacteria such as mecA **[5]**. Based on the above background, a study aimed at developing detection of the mecA gene in *S. aureus* by PCR was carried out.

MATERIALS ANDMETHODS

The research was started by culturing *S.aureus* using common media, namely Nutrient Agar (NA) with incubation at 37 ^{0}C as the optimum temperature for bacterial growth for 48 H **[6]**. After that, the *S.aureus* DNA was isolated using KIT wizard genome purification (Promega). The isolated *S.aureus* DNA was then used for DNA amplification using the PCR gel base method.

The PCR stage begins with primary analysis. The primers that have been used are based on Khan *et al* **[7]** references in the following primary order:mecA-F: 5 'TCCAGATTACAACTTCACCAGG 3' and mecA-R: 5 'CAATTCATA TCTTGTAACG 3'. The primary sequences were analyzed for the primary consisting of BLAST nucleotides on the NCBI website (https://blast.ncbi.nlm.nih.gov/Blast.cgi) **[8]**; Primary projection using SnapGene Viewer **[9]** software; and calculating the primer length, melting temperature,% GC, dimer primer using the Oligo Evaluator program (https://www.sigmaaldrich.com/technical-documents/articles/biology/oligo-evaluator.html).

The next step was amplification of DNA using a Thermocycler PCR device. The isolated *S.aureus* DNA was then mixed with PCR reagents such as *Taq* Polymerase, dNTPs, Primer, Buffer, and

Nuclease Free water. The mixture was inserted into the PCR tube and DNA amplification was carried out. DNA amplification conditions consisted of denaturation at 94 ^{0}C for 1 minute, annealing 50 ^{0}C for 0.5 minutes, and extension at 72 ^{0}C for 1 minute. The cycle used is 35 cycles. At this stage, the PCR component is optimized to obtain the optimal DNA band **[10]**. Optimization of PCR components consisted of optimizing the number of DNA templates, Taq Polymerase concentrations, $MgCl_2$ concentrations, and dNTPs concentrations.

After the amplification process was complete, DNA characterization was carried out using the agarose gel electrophoresis method. The PCR-generated DNA was mixed with Loading dye as a weight and inserted into the agarose gel well which was added with 1X TBE buffer. Electrophoresis was carried out with a voltage of 100 volts for 30 minutes. The amplicon size was compared with the 100 bp marker ladder. DNA is documented by UV transiluminator, DNA with the appropriate band size will glow because it uses diamond nucleid acid dye **[11]**.

RESULTS AND DISCUSSION

The initial stage of this research was carried out by subculturing the *S.aureus* bacteria. The purpose of this subculture is to rejuvenate *S.aureus* bacteria cells with the age of the bacteria obtained, not too old or young. The results of the subcutures can be seen in **Figure 1**.

The next step was to analyze the mecA gene primer. Forward and Reverse primers that have been obtained based on reference, then the nucleotide BLAST is performed (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

The aim of this stage is to match the GenBank database at NCBI that the primers used can recognize the mecA gene. The BLAST results showed that Primer-F and Primer-R matched the nucleotide sequence of *S.aureus* bacteria.

Primary projection then performed primary projection to determine the area of attachment of the primer to the mecA gene and to obtain the amplicon size of the PCR results. The results of this stage obtained the size of the DNA amplification product (amplicon) = 161 bp and the primer stuck at 1184 .. 1344 bp. Total 2129 bp mecA gene **(Figure 2)**.

In the last primary analysis stage, the primary length was calculated using the OligoEvaluator program from sigmaaldrich ((https://www.sigmaaldrich.com/technical-documents/articles/biology/oligo-evaluator.html),% GC, Tm temperature and dimer primer The PCR primary criteria used were primers with a GC percentage of 40-60%, melting point (Tm^{o}C) 55-72^{o}C, dimers formed at the 3 'end less than three pairs, other dimers formed less than seven

bases, 5' tip stability. with 3 '(5' to 3 ') greater than or equal to 2.0 kcal, 55°C annealing, the number of bases in sequence (run of base) less than four bases, and nucleotide bases that repeat (repeats) not more than three nucleotides **[12]**. The results of this stage obtained % GC primer-F = 45.5% and primer-R = 31.6%; melting point (Tm°C) primer-F = 62.8 ^{0}C and Primer-R = 50.3 ^{0}C; while for dimer primers does not exist.

In the DNA amplification stage using the PCR method, the PCR components were optimized to obtain the optimal DNA band **[13]**. Optimization of PCR components consisted of optimizing the number of DNA templates, Taq Polymerase concentrations, $MgCl_2$ concentrations, and dNTPs concentrations. The optimization results can be seen in **Table 1**, and one of the results of DNA characterization using the agarose gel electrophoresis method shows that the amplicon size is in accordance with the results of the primary analysis **(Figure 3)**.

Figure 1: *S.aureus* grown in slant agar

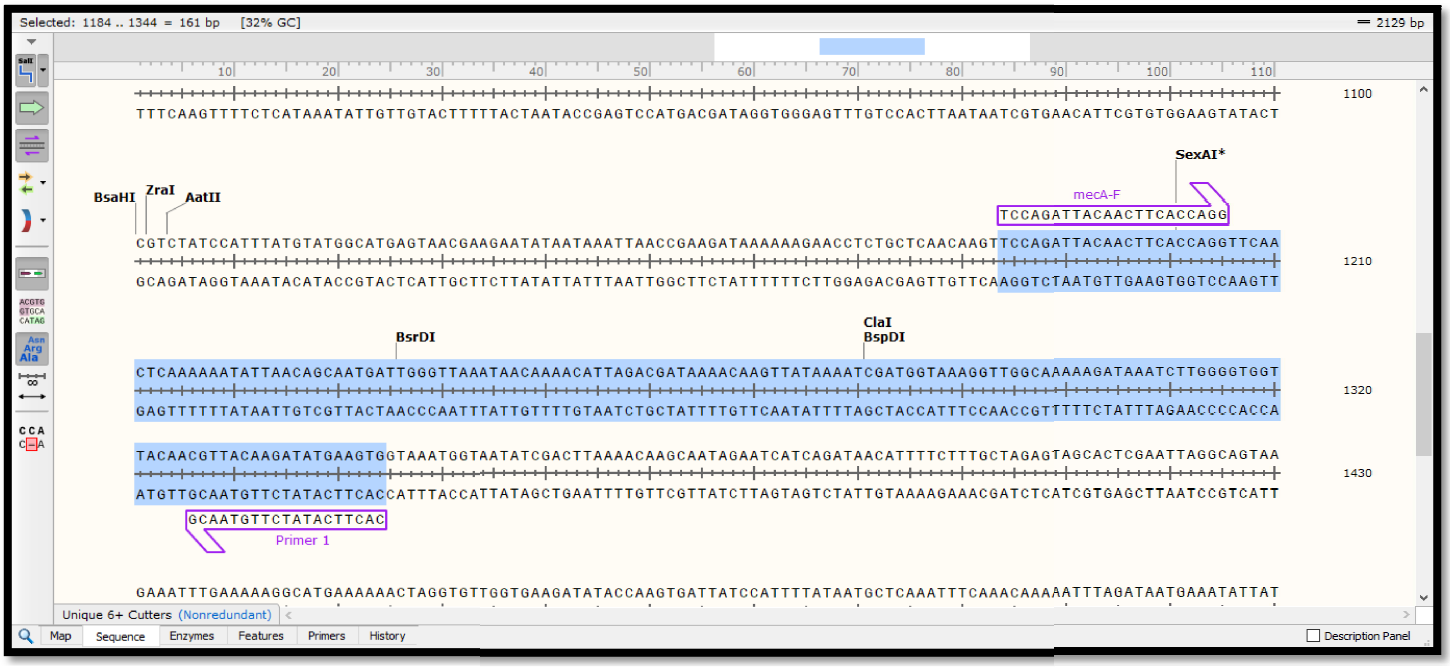

Figure 2: Result of primer proyection

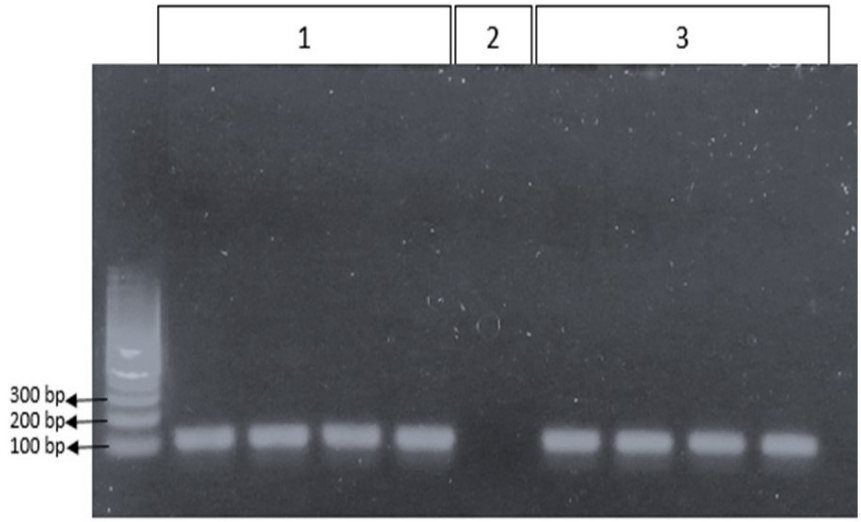

Figure 3: Electroforegram PCR optimization results taq polymerase (1= 1 U; 2= 0 U; 3= 0.5 U)

Table 1: Optimal concentration for PCR components

S. No.	Component	Initial concentration	PCR concentration	Volume
1.	*Taq* Polymerase	5 U	1U	0.2 µl
2.	Buffer	5 X	1 X	10 µl
3.	$MgCl_2$	25 µM	1.5 µM	3 µl
4.	dNTPs	10 µM	150 µM	0.75 µl
5.	Forward Primer	100 µM	100 nM	5 µl
6.	Reverse Primer	100 µM	100 nM	5 µl
7.	DNA template	4.5 ng/µl	4.5 ng/µl	1 µl
8.	NFW			Add 50 µl

CONCLUSION

The PCR method developed can be used to detect the mecA gene in *S.aureus* bacteria under optimal PCR conditions and an amplicon length of 161 bp.

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