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Cloning, Recombinant Production and Functional Analysis of Staphylococcal Phage Endolysins

Stafilokkal Faj Endolizinlerinin Klonlanması, Rekombinant Olarak Üretilmesi ve Fonksiyonel Analizleri

Serap Pektas[1], Osman Birol Ozgumus[2], İnci Durukan[3], Umit Uzun[4], Ersin Karatas[5], Ali Osman Kilic[3*]

[1]*Recep Tayyip Erdogan University, Faculty of Art and Sciences, Department of Biochemistry, 53100 Rize, Türkiye.*
[2]*Recep Tayyip Erdogan University, Faculty of Medicine, Department of Medical Microbiology, 53100 Rize, Türkiye.*
[3]*Karadeniz Technical University, Faculty of Medicine, Department of Medical Microbiology, 61080 Trabzon, Türkiye.*
[4]*Karadeniz Technical University, Graduate School of Health Sciences, Department of Medical Biology, 61080 Trabzon, Türkiye.*
[5]*Agrı İbrahım Çeçen University, Patnos Vocational School, 04500 Patnos, Türkiye.*

**Corresponding author e-mail: akilic@ktu.edu.tr*

[1]*https://orcid.org/ 0000-0003-0497-6257*
[2]*https://orcid.org/0000-0002-3665-6584*
[3]*https://orcid.org/0000-0002-9789-4738*
[4]*https://orcid.org/0000-0002-5506-0866*
[5]*https://orcid.org/0000-0002-0585-2865*
[6]*https://orcid.org/0000-0001-6848-7618*

ABSTRACT

Staphylococci are pathogens that cause serious infections in humans and animals. Nosocomial infections caused by staphylococci, particularly methicillin-resistant *Staphylococcus aureus* (MRSA) strains, are mostly transmitted through healthcare workers, patients, or contaminated materials and food. In recent years, studies have been carried out to develop alternative antimicrobial strategies due to the inadequacy of existing antibiotics in the prevention of systemic, skin and implant-related biofilm infections caused by these multi-antibiotic resistant strains. One of these new approaches is the development of products containing the bacteriophage endolysin, which is particularly effective against multi-antibiotic-resistant bacteria. In this study, endolysin genes of bacteriophages (prophages) integrated into the chromosomes of *Staphylococcus* strains were amplified by polymerase chain reaction (PCR) and cloned into pET SUMO and pET-30b(+) vectors and produced recombinantly in *E. coli*. Anti-staphylococcal and antibiofilm activity of recombinant endolysins against *S. aureus*, *S. epidermidis*, and *S. haemolyticus* strains isolated from clinical specimens, were demonstrated using turbidity reduction, biofilm removal in microwell plates by crystal violet method, and capacity of endolysins to kill biofilm-forming bacteria by confocal microscopy imaging by live-dead staining. The combination of endolysin was shown to reduce bacterial culture turbidity by at least 50% at 60 minutes and biofilms by approximately 70% at 12 hours. These results show that endolysins have the potential to be used in the prevention of staphylococcal infections.

Keywords: *Staphylococcus*, Bacteriophage, Anti-biofilm, Endolysin, Confocal Microscopy

ÖZET

Stafilokoklar insanlarda ve hayvanlarda ciddi enfeksiyonlara neden olan patojenlerdir. Stafilokokların, özellikle metisiline dirençli *Staphylococcus aureus* (MRSA) suşlarının neden olduğu nozokomiyal enfeksiyonlar çoğunlukla sağlık çalışanları, hastalar veya kontamine olmuş maddeler ve yiyecekler yoluyla bulaşır. Son yıllarda bu çoklu antibiyotik dirençli suşların neden olduğu sistemik, deri ve implant ilişkili biyofilm enfeksiyonlarının önlenmesinde mevcut antibiyotiklerin yetersizliği nedeniyle alternatif antimikrobiyal stratejiler geliştirmeye yönelik çalışmalar yapılmaktadır. Bu yeni yaklaşımlardan biri, çoklu antibiyotiğe dirençli bakterilere karşı özellikle etkili olan bakteriyofaj endolizin enzimini içeren ürünlerin geliştirilmesidir. Bu çalışmada, *Staphylococcus* suşlarının kromozomlarına entegre olmuş, bakteriyofajların (profaj) endolizin genleri polimeraz zincir reaksiyonu (PCR) ile çoğaltılarak pET SUMO ve pET-30b(+) vektörlerine klonlandı ve *E. coli*'de rekombinant olarak üretildi. Rekombinant endolizinlerin, klinik örneklerden izole edilen *S. aureus*, *S. epidermidis* ve *S. haemolyticus* suşlarına karşı anti-stafilokokal ve antibiyofilm etkileri bulanıklılık azaltma, mikrokuyucuklu plaklarda kristal viyole yöntemi ile biyofilm uzaklaştırma ve konfokal mikroskopi yöntemi ile endolizinlerin biyofilmi oluşturan bakterileri öldürme kapasiteleri canlı ve ölü bakteri görüntüleme yöntemi ile saptandı. Endolizin kombinasyonunun bakteri kültür bulanıklılığını 60 dakikada en az %50 ve biyofilmleri 12 saatte yaklaşık %70 oranında azalttığı gösterildi. Bu sonuçlar endolizin enzimlerinin stafilokokal enfeksiyonların önlenmesinde kullanılma potansiyeline sahip olduğunu göstermektedir.

Anahtar Kelimeler: *Staphylococcus*, Bakteriyofaj, Biyofilm, Endolizin, Konfokal Mikroskopi.

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INTRODUCTION

The genus *Staphylococcus* includes more than 30 different species responsible for various infections. Most of these infections are caused by *Staphylococcus aureus* (*S. aureus*), and many are found in the human microbiota.[1] Nosocomial infections often associated with S. *aureus*, including methicillin-resistant *S. aureus* (MRSA), is transmitted primarily through contaminated medical devices or direct contact with colonized healthcare workers or patients. *S. aureus*, *S. epidermidis*, and other coagulase-negative staphylococci are responsible for 30% of nosocomial infections.[2] They cause complications, especially in people with suppressed immune systems. They can lead to a prolonged hospital stay and even death due to other complications. Therefore, alternative approaches are needed to treat these infections. One such approach involves the use of lytic bacteriophages alone or in combination with conventional antimicrobial agents.[3] There has been increased interest in studies to investigate the antibiotic or enzyme-based antibacterial potentials of *Streptococcus pneumoniae*, [4] *S. aureus*,[5, 6] *Enterococcus faecalis*,[7] *Bacillus anthracis*,[8] and *Pseudomonas aeruginosa*,[9] phages. There many studies about the prevention of the formation of biofilm or removing the biofilm structure by the endolysin enzymes. A broad-spectrum phage endolysin (LysCP28) showed both antibacterial, biofilm removal and biofilm formation inhibition activity against *Clostridium perfringens*.[10] Simalarly, *S. aureus*,[11,12] *Streptococcus suis*,[13] *Streptococcus pyogenes*,[14] *Gardnerella vaginalis*[15] biofilms are eliminated by endolysins. Endolysins are enzymes encoded and released by phages in the final stage of their life cycle to release newly produced phage particles by disrupting the host bacterial cell wall.[16] When recombinant endolysin is added exogenously in a culture of gram-positive bacteria, unlike regular life cycles, the bacteria result in a short breakdown without the aid of holin proteins.[6,17,18] These properties make them attractive antibacterial agents. There are several advantages to using phage endolysins over phages and conventional antimicrobial agents because they can kill susceptible bacteria with which they come into contact. Since phage endolysins inactivate target bacteria specifically with a lytic effect without damaging the normal microflora, it is very rare for bacteria to develop resistance against them.[19] These properties of phage endolysins have enabled them to be used in various fields, such as medicine, veterinary medicine, agriculture, the food industry, and biotechnology.[16,20] In another study, an endolysin enzyme was produced recombinantly and called PlyC, was used as a disinfectant on inanimate solid surfaces and showed a disinfection effect by breaking down the peptidoglycan layer of the bacteria and showed an antibacterial effect against many Streptococcus species, including *Streptococcus equi*. They report it to be 1000 times more effective when compared to commercial disinfectants. It was determined that 1 mg of PlyC showed an antibacterial effect within 30 minutes against *S. equi* cells at a concentration of 10^8 CFU/mL. Based on these findings, the researchers put PlyC through a standardized series of tests, including the dilution methods used for disinfectants and germicide sprays prescribed by the Association of Official Analytical Chemists (AOAC).[21] In this present study, six endolysin genes belonging to bacteriophages that lysogenize *S. aureus* strains isolated from food and food workers were amplified by PCR and cloned into pET SUMO and pET-30b(+) expression vectors and recombinantly produced in *E. coli*. A mixture containing recombinant endolysins was observed to be anti-staphylococcal and antibiofilm effective against *S. aureus*, *S. epidermidis*, and *S. haemolyticus* strains isolated from clinical specimens.

METHODS

Bacterial Strains and Culture Conditions

A total of 173 *S. aureus* strains obtained from the Samsun Veterinary Control Institute were included in this study. Tryptic Soy Broth (TSB) and Tryptic Soy Agar (TSA) media were used for culturing *S. aureus* strains and phage endolysin activity tests. The broth and agar media were prepared following the manufacturer's instructions, and sterilized by autoclaving before use. Both broth and agar cultures were incubated under aerobic conditions at 37°C, and the broth cultures were incubated by shaking at 220 rpm. The *E.coli* strains were routinely cultured in Luria-Bertani (LB) broth and agar (ThermoFisher Scientific, USA).

Phage Endolysin Gene Amplification

The chromosomal DNA from *S. aureus* strains was isolated by the standard lysostaphin-phenol/chloroform method using 3 mL overnight broth cultures. Following the chromosomal DNA isolation, the presence of the endolysin gene was screened by PCR. The pET SUMO (Invitrogen, USA) and pET-30b(+) vectors (Merck, Germany). The following two sets of primers designed

based on the published endolysin genes of *S. aureus* DW2 phage (Acc. No. KJ140076) stored in the gene bank were used: Lys-F1: 5'-ATG CGT AGA ATA AGA AGA CCT AAG -3' and Lys-R1: 5'-TTA TTT CTT ATC GTA AAT GAA TTG -3' for pET SUMO cloning and expression; ELys-F: 5'- TAC TTA CAT ATG ATG CAA GCA AAA CTA ACT AAA AAA G-3' with *NdeI* restriction site, and ELys-R: 5'-GTG CTC GAG TTA ACT GAT TTC TCC CCA TAA GTC-3' with *XhoI* restriction site, and cloned into *NdeI*/*XhoI* digested pET-30b(+) vector. The following reaction conditions were applied for the PCR reaction containing 100 pg template DNA. Initial template DNA denaturation at 94°C for 5 min, 30 cycles at 94°C for 1 min, at 52°C for annealing, at 72°C for 1 min (synthesis), and at 72°C for 7 min of final extension.

Cloning and Expression of Bacteriophage Endolysin Genes

The PCR products were cloned into two different protein expression vectors using T4 DNA ligase. i) the endolysin gene fragment with a -T overhang at both ends was directly into pET SUMO expression vector (Invitrogen, USA) following manufacturer's recommandations. ii) the PCR product carrying the endolysin gene was first cloned into pJET 1.2 PCR cloning vector (Thermo Scientific, USA), and endolysin gene was removed from this vector with *NdeI*/*XhoI* double digest then cloned into pET-30b(+) expression vector (Thermo Scientific, USA), digested with the same enzymes. The recombinant plasmids carrying the endolysin genes were first transformed into *E. coli* One Shot MachlTM-T1R (Invitrogen, USA) competent cells. The recombinant plasmids were confirmed by DNA sequence analysis using a Genetic Analyzer 3130 (Applied Biosystems, USA). The recombinant plasmids were transferred into *E. coli* BL21 (DE3) competent cells (Invitrogen, USA) and induced with isopropyl-β-D-thiogalactopyranoside (IPTG) for expression at 20°C. Briefly, the transformed *E. coli* BL21 were grown in 5 mL of LB broth containing 50 µg/mL kanamycin at 250 rpm shaking overnight. The flowing morning 4 mL from overnight culture was seeded into 400 mL LB broth medium containing 50 µg/mL kanamycin, and induced with 1 mM IPTG when the culture OD_{600nm} reached 0.5-0.6.

Purification of Recombinant Endolysins

Purification processes of the endolysin protein were performed with the proBond Purification system kit (ThermoFisher, USA) in line with the manufacturer's recommendation. Briefly, cells were collected by centrifugation and were resuspended in 4-8 ml of lysis buffer (50 mM K_2HPO_4 pH 7.8, 400 mM NaCl, 100 mM KCl, 10 % glycerol, 0.5 % Triton X-100, 10 mM imidazole), followed by three cycles of freeze-thawing with alternating -20°C and 42°C. The cells were further disrupted by sonication for 10-sec sonication followed by 10-sec rest over 3 min (Vibra Cell™ Sonics, USA). The lysate was centrifuged at a maximum of 12,000 rpm for 10 min at 4°C to remove the cell debris. The supernatant was transferred to a sterile tube. The cell lysate was analyzed for the presence of expressed endolysins by SDS-PAGE analysis following the standard procedures. The endolysin-containing cell lysate was transferred to a purification column containing the nickel resin and further purified. Following the wash steps the endolysins were eluted with 2-8 mL elution buffer (50 mM Tris-HCl pH 8.00). The eluted endolysins were further concentrated using Pierce™ Protein Concentrators, 30K molecular weight cutoff (MWCO) (ThermoFisher, USA). The purity of the recombinant endolysin was checked by SDS-PAGE analysis, and the concentration of the endolysins was determined with the Qubit TM Protein Assay Kit (Invitrogen, USA) following the manufacturer's recommendations. The endolysins were sterilized using 0.22 µm pore-sized filters, and stored at -20°C until use.

Functional Analysis of Recombinant Endolysins

The lytic activities of the recombinant endolysins were tested by drop assay on the agar plate seeded with susceptible staphylococci. After 24 hours of incubation at 37°C, the formation of inhibition zones in the areas where the enzyme was dropped indicated the lytic activity. A turbidity reduction assay was performed in a 96-well microplate. Briefly, 200 µL of overnight cultures were transferred to 96-well microplate wells in triplicate. Then, the endolysin mix was added to the wells at various concentrations and incubated at 37°C for 60 minutes. After incubation, the bacterial concentration in the wells was determined by measuring the decrease in OD_{600nm}.

Anti-biofilm Activities of Endolysins

Crystal Violet Assay for Biofilm Quantification: Three test strains (*S. aureus*, *S. epidermidis*, and *S. haemolyticus*) strains were grown overnight in TSB medium containing 1% glucose (TSBG) and diluted 1/100, 200 µL was transferred to the wells of a 96-well microplate. The cell was allowed to form biofilm for 24-48 hours. Following the biofilm formation, the microwells were washed twice with PBS, followed by the addition of an equal amount of enzymes with varying concentrations, and incubated at 37 °C for 12 hours. The wells were washed twice with PBS and stained with 200 µL of 0.1% crystal violet for 5-10 min at room temperature. The wells were washed twice with distilled water, and the biofilm was dissolved in 200 µL of 95% ethanol and read in a microplate reader at OD_{595nm}. The amount of biofilm removed by endolysin was determined by comparison with the wells treated with PBS. Experiments were repeated at least three times.

Confocal Microscope Demonstration of Anti-biofilm Activities of Endolysins: Biofilms formed on an eight-chamber coverglass (Thermo Fisher Scientific, USA) were treated with endolysin for 12 hours. Then living and dead bacteria were stained with BacLight LIVE/DEAD (Invitrogen/Thermo Scientific, USA) according to the manufacturer's instructions and examined in three dimensions with Leica DMI8 Confocal Laser Scanning Microscopy with 63x objective (CLSM, Leica Microsystems, Germany). For imaging, the wavelength was set at 485/498 nm for SYTO 9 (fluorescent green) and at 535/617 nm for propidium iodide (fluorescent red) for the observation of live and dead bacteria, respectively.

RESULTS

PCR Screening of Phage Endolysin Genes in the S. aureus Genome

The chromosomal DNA from 173 *S. aureus* strains was included in the study as a template (Figure 1) for PCR amplification of endolysin genes of prophages, which are integrated into the chromosomes. The endolysin encoding gene about 1.5 kb in size, was amplified by PCR from the chromosome of six strains, TSA 2, TSA 4, TSA 5, TSA 6, TSA 22, and TSA 23 (Figure 2). The six endolysin genes, lys 2, lys 4, lys 5, lys 6, lys 22, and lys 23 were gel purified using Macherey-Nagel™ NucleoSpin™ Gel and PCR Clean-up Kit (Macherey-Nagel, Germany) and cloned into plasmid vectors.

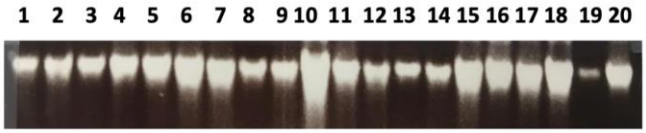

Figure 1. Agarose gel electrophoresis of chromosomal DNA from some *S. aureus* strains.

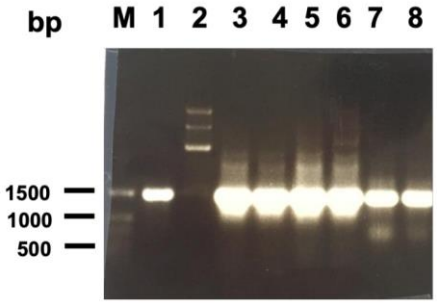

Figure 2. Amplification of phage endolysin genes from the chromosome of *S. aureus* strains by PCR. M; molecular weight standard, 1; pozitif control, 2; negative control, 3; lys 2, 4; lys 4, 5; lys 5, 6; lys 6, 7; lys 22, 8; lys 23.

Cloning of Phage Endolysin Genes

The gel prified PCR products were cloned into pET SUMO vector for expression without pre-cloning, and the pJET 1.2 PCR cloning vector before being cloned into the the pET-30b(+) vector for expression. Among six purified endolysin genes only endolysin gene 6 (lys 6) was successfully cloned into the pET SUMO vector for expression, and five endolysin genes (lys 2, lys 4, lys 5, lys 22, and lys 23) were cloned into pJET 1.2 vector (Figure 3), which were confirmed by restriction endonuclease digestion (Figure 4) before subcloned into pET-30b(+) expression vector.

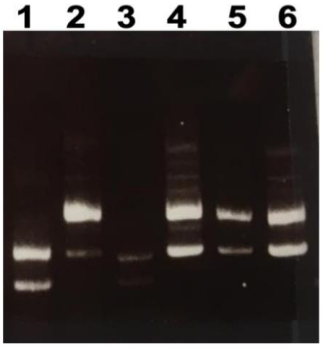

Figure 3. Undigested recombinant pJET 1.2 plasmids containing the endolysin genes. 1; control (pJET 1.2 vector without insert), 2; pJET 1.2_lys 2, 3; pJET 1.2_lys 4, 4; pJET 1.2_lys 5, 5; pJET 1.2_lys 22, 6; pJET 1.2_lys 23.

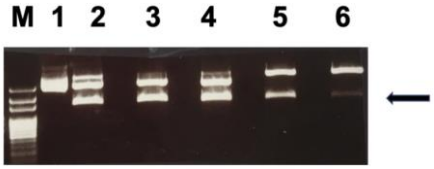

Figure 4. Confirmation the presence of endolysin genes cloned into pJET 1.2 vector by cleavage with *NdeI* and *XhoI* restriction enzymes. M; molecular weight standard, 1; pJET 1.2_lys 2 uncut, 2; pJET 1.2_lys 2 cut with *Nde*I and *Xho*I, 3; pJET 1.2_lys 4 cut with *Nde*I and *Xho*I, 4; pJET 1.2_lys 5 cut with *Nde*I and *Xho*I, 5; pJET 1.2_lys 22 cut with *Nde*I and *Xho*I, 6; pJET 1.2_lys 23 cut with *Nde*I and *Xho*I. The arrow indicates 1.5 kb endolysin gene.

The endolysin genes from pJET 1.2 vector were excised with NdeI and XhoI restriction enzymes and cloned into the pET-30b(+) vector, which was cut with the same enzymes. Except lys 22, lys 2, lys 4, lys 5, and lys 23 genes were successfully subcloned into pET-30b(+) plasmid vector, and confirmed by restriction endonuclease analysis and sequencing.

Recombinant Production of Endolysins

The expression vectors containing the endolysin genes were expressed in *E. coli* BL21(DE3) and obtained in soluble form from the cell lysates of 250-1000 mL cultures. The high enzyme yield with activity was obtained if the cells were induced at a cell density of 0.5-0.6 at OD_{600nm}, with 1 mM IPTG addition, and at 22-24°C incubation for 18-22 hours. The presence of dissolved endolysins was detected by SDS-PAGE analysis, and those with lytic activity from these crude lysates were purified by passing them through nickel columns (Figure 5).

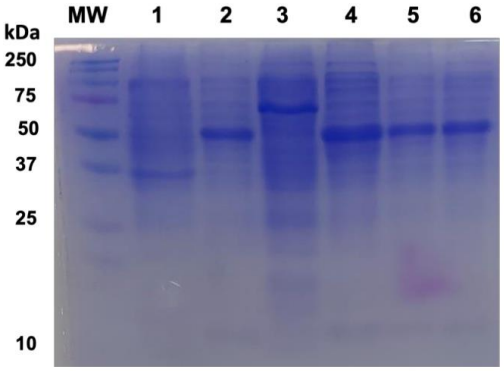

Figure 5. SDS-PAGE analysis of recombinant endolysins. MW; molecular weight standard, 1; pET SUMO_lys 6 uninduced culture lysate, 2; pET-30b(+)_lys 2, 3; pET SUMO_lys 6, 4; pET-30b(+)_lys 4, 5; pET-30b(+)_lys 5, 6; pET-30b(+)_lys 23.

Functional Analysis of Endolysins

Anti-staphylococcal activities of recombinant endolysins were tested against three different clinical *Staphylococcus* species (*S. aureus, S. epidermidis* and *S. haemolyticus*) in our collection using the agar-drop assay (Figure 6).

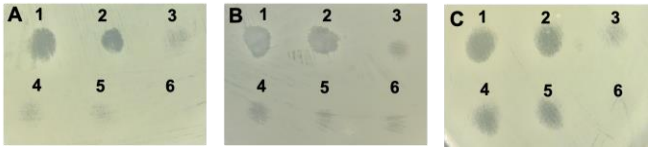

Figure 6. Demonstration of antibacterial activities of recombinant endolysins by agar-drop assay. A; *S. aureus* TRSA 2, B; *S. epidermidis* TRSE 6, C; *S. haemolyticus* TRSH 1, 1; Control (pET SUMO_lys10), 2; pET SUMO_lys 6, 3; pET-30b(+)_lys 2, 4; pET-30b(+)_lys 5, 9; pET-30b(+)_lys 23.

It was determined that both vectors (pET-30b(+) and pET SUMO) functionally produced recombinant phage endolysins in *E. coli*. However, these recombinantly produced endolysins were obtained relatively low concentrations ranging from 0.4 to 1 mg/L using shake flask cultures. Therefore, turbidity reduction, anti-biofilm, and biofilm reduction tests were carried out using a mixture five endolysins at 1-5 µg/mL after further concentration.

Turbidity Reduction

The turbidity reduction test was performed to demonstrate the antibacterial activity of endolysins (33). The reduction in bacterial cell concentration was measured at OD_{600nm} and plotted against time (Figure 7). The growth of three different species of staphylococci was inhibited approximately 50% indicated by the less cell density after 60 minutes compared to the controls.

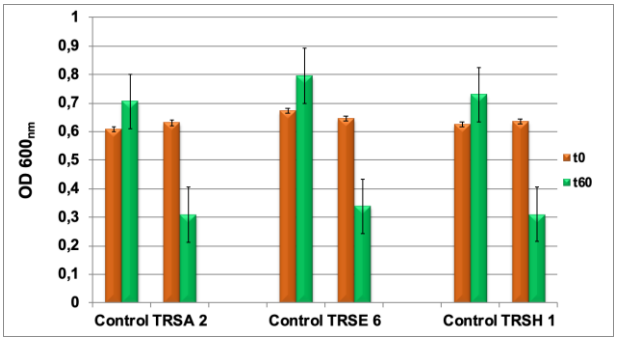

Figure 7. Demonstration of anti-staphylococcal activity of endolysins.

Anti-biofilm Activities of Endolysins

Crystal Violet Method: The *Staphyloccus* strains were grown in TSB medium (TSBG) containing 1% glucose overnight. The cultures were diluted 100 fold with TSBG medium and 200 µL was transferred to microplates with 96 wells, and a biofilm was formed for 24-48 hours as described before.[12] Following biofilm formation, the microwells were washed twice with PBS, followed by the addition of an equal amount of PBS containing varying concentrations of endolysin, and incubated at 37°C for 12 hours. Following the incubation, the wells were washed twice with PBS and stained with 200 µL of 0.1% crystal violet for 5-10 min at room temperature. The wells were washed twice with distilled water, biofilm was dissolved in 200 µL of 95% ethanol, and dissolved crystal violet was read in a microplate reader at OD_{595nm}. The amount of biofilm removed by endolysin was determined by comparison with wells treated with PBS. Experiments were repeated at least three times (Figure 8).

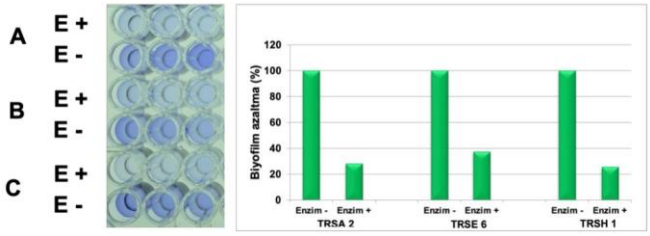

Figure 8. Removal of biofilms by endolysins. A; *S. aureus* TRSE 2, B; *S. epidermidis*, C; *S. haemolyticus*, E+; Endolysin added, E -; Endolysin not added.

Confocal Microscopy Method: Biofilms formed on the eight-well special glass coverslips were treated with endolysin for 12 hours. Then living and dead bacteria were stained with BacLight LIVE/DEAD and examined in three dimensions with Leica brand Confocal Laser Scanning Microscopy. Live bacteria were stained green with SYTO 9 dye and dead bacteria were stained red-orange with propidium iodide, and the biofilm removed by endolysin was evaluated qualitatively (Figure 9). The endolysin-treated biofilms from three different species of *staphylococci* were stained red indicating the death of the cells as compared to untreated biofilms. The rate of anti-biofilm activity was higher with the increase in endolysin concentration (Figure 9). Among all four strains, biofilms or biomass reduction of *S. aureus* TRSA 2, and *S. haemolyticus* TRSH 1 seemed to be higher by the lytic activity of the endolysins.

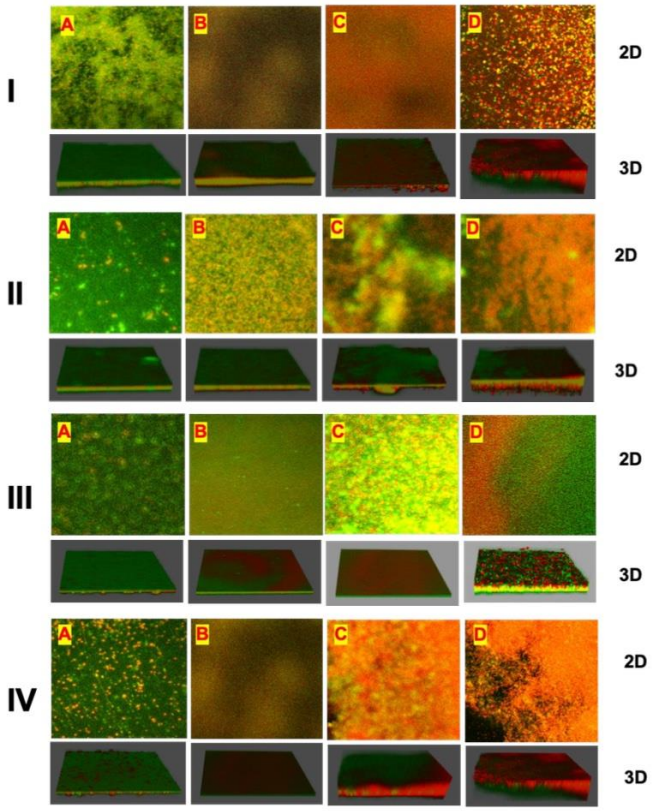

Figure 9. Demonstration of the anti-biofilm property of endolysin by confocal microscopy. Two (2D) and three-dimensional (3D) confocal microscopy (X630) of the concentration-dependent effects of recombinant endolysins on biofilms of *staphylococcal* strains. I; *S. aureus* TRSA 2, II; *S. aureus* TRSA 8, III; *S. epidermidis* TRSE 6, IV; *S. haemolyticus* TRSH 1, A; no endolysin, B; 1 µg/mL endolysin, C; 2.5 µg/mL endolysin, D; 5 µg/mL endolysin.

DISCUSSION

In this study, endolysin proteins of phages infecting *Staphylococcus aureus* strains isolated from various foods and personnel working with these foods were produced recombinantly in *E. coli*, and the potential anti-staphylococcal and anti-biofilm properties of these enzymes were observed in vitro. Here we show that *staphylococci* carry prophages widely and these prophages are suitable sources for endolysins and pET SUMO and pET-30b(+) vectors are suitable for cloning and recombinant production of endolysin genes. Although pET- series and several other vectors were used for the expression of phage endolysins, SUMO fusion was rarely used for this purpose. In this study, endolysin proteins of phages infecting *S. aureus* strains isolated from various foods and personnel working with these foods were produced recombinantly in *E. coli*, and the potential anti-staphylococcal and anti-biofilm properties of these enzymes were observed in vitro. Here we show that staphylococci carry prophages widely and these prophages are suitable sources for endolysins and pET SUMO and pET-30b(+) vectors are suitable for cloning and recombinant production of endolysin genes. A number of parameters have been used to increase the yield of recombinant endolysins. Neither the solubility tags nor co-expressed molecular chaperones were effective. Only significant yield improvement resulted from the induction temperature, and lysis buffer additives.[22] In this study, endolysins expressed in both pET SUMO and pET-30b(+) had indistinguishable SDS-PAGE band intensities and lytic activities, Figure 5 and Figure 6, respectively.

The growth of cultures in the presence of endolysin was found to be reduced by approximately 50% after 60 minutes compared to the control (Figure 7) as indicated by the turbidity reduction. Similarly, in the crystal violet method, biofilms formed with *S. aureus* TRSA 2, *S. epidermidis* TRSE 6, and *S. haemolyticus* TRSH 1 strains were reduced by at least 70% compared to the control biofilm without endolysins (Figure 8). The reducing activity of the endolysins, viable and dead cells was visible using confocal laser scanning microscopy, which is one of the valuable tools for biofilm studies.[23] The use of confocal microscopy not only provides the 3D structure of the biofilm but also provides valuable information about the penetration and action of the endolysin through the biofilm (Figure 9).

Although there are several limitations in this study, such as growing *E. coli* cultures in shake flasks in limited volumes instead of liters amount in more controlled fermentors, due to limited resources not being able to optimize the culture, induction, and cell lysing conditions, most of all not being able to obtain endolysins in high yield and purity.

The potential use of phage endolysins to prevent or remove both gram-positive and gram-negative bacterial biofilms has been reviewed in several recent studies. [24, 25, 26, 27, 28] Lysins act on the peptidoglycan layer of bacterial cell wall at the end of lytic cycles of the phages from within. Since the activity of lysin is compromized by the bacterial outer cell membrane of Gram-negative bacteria, membrane permeability to lysins are achieved by addition of membrane distabilyzing agents or fusion of lysin gene with a highly hydrophobic amino acid residue coding genes. In this study, lysin was expressed in its native form and was active only against members of staphylococci. There have been numerous recombinant phage lysins some of which been in clinical trials, for the treatment of bloodstream infections such as bacteremia and endocarditis. This is the first report on the phage endolysins from staphylococcal temperate phages from Türkiye.

In this work, endolysin encoded by a temperate bacteriophage in the chromosome of staphylococci showed hydrolytic activity against three species of staphylococci. A similar study was reported by phage DW2 from a bovine mastitis isolate of *S. aureus*.[29] These results indicate that functional endolysins could be cloned and recombinantly produced from both lytic and temparate phages, which increase the rate of finding ptentially broad host-range lysins.

It is noteworthy that endolysin genes originating from *S. aureus* strain and produced recombinantly in *E. coli* were able to lyse other species of staphylococci, most probably other relative gram-positive bacteria, which deserves further studies.

CONCLUSION

In this study, four phage endolysin genes belonging to the temperate phages integrated into the chromosomes of *S. aureus* strains, isolated from nose and hands, and food processing surfaces were amplified, cloned, and recombinantly expressed in *E. coli*. Their potential to be used as “enzybiotics” were demonstrated against different species of staphylococci by anti-bacterial, biofilm prevention, and biofilm removal experiments.

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Authorship contribution statement

Consept and desing: AOK.

Acquisition of data: SP, OBO, ID, UU.

Analysis and interpretation of data: AOK, SP and EK.

Drafting of the manuscript: AOK and SP.

Critical revision of the manuscript for important intellectual content: OBO and EK.

Statistical analysis: SP.

Declaration of competing interest

None of the authors have potential conflicts of interest to be disclosed.

Ethical approval

Ethics committee approval is not required.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

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