RESEARCH ARTICLE

Enhancement of Ciprofloxacin Activity against Uropathogenic Bacteria by Synergistic Effect of Purified Bacteriocin from *Staphylococcus epidermidis*

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ABSTRACT

Fourteen *Staphylococcus epidermidis* isolates were isolated from twenty-two skin samples collected from healthy persons. Some *S. epidermidis* isolates produced the bacteriocin by using *S. aureus* and *Pseudomonas aeruginosa* as indicator strains. A total of 30% acetone was applied to extract bacteriocin from *S. epidermidis.* Bacteriocin demonstrated strong bactericidal activity against all uropathogenic strains tested with values of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) between 8 and 128 µg/mL, while for ciprofloxacin, MIC and MBC levels ranged from 64 to 2056 µg/mL. The synergistic activity for bacteriocin with ciprofloxacin enhanced ciprofloxacin activity to high levels with *P. aeruginosa*, *Klebsiella pneumonia,* and *Escherichia coli*. These findings suggest that bacteriocin could effectively treat uropathogenic infections to help overcome the challenges of multi-antibiotic resistance.

Keywords: Bacteriocin*, Staphylococcus epidermidis,* Uropathogenic infections.

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INTRODUCTION

Staphylococcus epidermidis is a Gram-positive bacteria, one of twelve coagulase-negative *Staphylococcus* species found in the human integumentary system as commensals.[1] It is a part of the normal human flora, most commonly the skin and mucosal flora.[2] It's a facultatively anaerobic bacteria. *S. epidermidis* is usually not harmful, although people with compromised immune systems are at risk of developing the disease. Mostly acquired in hospitals.[3] *S. epidermidis* infections are common in catheters and other prothetic implants, as it is thought to produce biofilms that grow on these devices.[2,4]

S. epidermidis is a nonmotile, powerful microbe that forms grape-like cocci clusters. After 24 hours of incubation, it creates elevated, white, uniform colonies measuring 1–2 mm in diameter. On blood agar,[4] it is non-hemolytic, catalase-positive, and coagulase-negative.[5]

Lantibiotics are Staphylococcins generated by *S. epidermidis*. In general, *Staphylococci* bacteriocins have not been thoroughly studied. These bacteriocins have a broad and powerful antibacterial action against a variety of bacteria, including multidrug-resistant pathogens like Methicillin-resistant *Staphylococcus aureus* (MRSA).[6,7] The purpose of this study is to use a safe and bacteriocin-producing isolate of *S. epidermidis* as a probiotic, as well as to use bacteriocin to treat uropathogenic bacteria and to investigate its synergistic effect with antibiotics.

MATERIALS AND METHODS

Collection and Identification of *S. epidermidis* Isolates

BHI broth was used as an enrichment medium for this bacterium after skin samples (swaps) were taken from healthy people. After a 24 hours incubation period at 37°C, stepwise dilutions

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in normal saline were prepared, followed by a switch to the dilution 10^{-5} on mannitol salt agar. After a 24 hours incubation period at 37°C, the possible *S. epidermidis* candidates that met the morphological criteria were chosen and subcultured on mannitol salt agar to ensure purity. To determine the species, isolates were submitted to morphological testing and subsequently characterized by API Staph using a biochemical test and the VITEK 2 System.

Collection and Identification of Pathogenic Isolates

S. aureus and *P. aeruginosa* were among the pathogenic isolates employed as indicators, and they were isolated from infected wounds and burns. *S. aureus* was isolated and identified using a program identical to that used to identify *S. epidermidis*. However, *P. aeruginosa* was diagnosed using the VITEK system after being recognized principally by cultivating it on cetrimide agar and MacConkey agar.[8]

Screening of Isolates for Bacteriocin Production

All isolates that were identified as *S. epidermidis* were subjected to primary and secondary screening to determine which one produced the most bacteriocins, as described in the following sections:

Primary Screening

The following well diffusion assay was performed to assess bacteriocin production by *S. epidermidis* isolates: The bacteria were injected into BHI broth and incubated for 24 hours at 37°C. The culture broth was centrifuged at 10000 rpm for 15 minutes after incubation, and the cell-free supernatant (CFS) was collected and filtered under sterile conditions with 0.22 m Millipore filter paper.

The presence of bacteriocin in each isolate's CFS was determined using an agar well diffusion assay as follows: In a water bath, 100 µL of the indicator bacteria were introduced to 25 mL of sterilized MHA. The mixture was kept at the same temperature until it was transferred and solidified into sterile plastic Petri plates. Circular wells with a diameter of 5mm were drilled.

Secondary Screening

Bacteriocin activity was determined by testing a two-fold dilution series of *S. epidermidis* isolate cultures: The bacteriocin activity in each dilution was measured using an agar well diffusion test using *S. aureus* as the indicator isolate. The inhibition zone around each well was found after incubation. The bacteriocin activity was determined by the maximum dilution that produced an inhibitory zone. As a result, the bacteriocin activity was calculated as the reciprocal of the maximum dilution factor (DF), resulting in a detectable inhibitory zone. The following equation[9] was used to compute the bacteriocin activity, which is measured in arbitrary units (AU): AU/mL = 1/DF 1000/(volums spotted in µL).

Bacteriocin Partial Purification

Bacteriocin activity was determined after purification with organic solvent (acetone) precipitation in an ice bath with continuous stirring for 30 minutes to obtain saturation ratios of 20–70%, then left for 2 hours in the cooling conditions, centrifuged with cooling centrifugation for 30 minutes, and dissolved in phosphate buffer saline (PBS) buffer.

Reidentification of Urinary Tract Infection (UTI) Bacterial Isolates

Different genera of urinary tract infections causing were collected and diagnosed with VITEK 2 system.

Evaluation of Bacteriocin and Antibiotic Efficacy Against Uropathogenic Bacteria

Using a micro broth dilution test in 96-well flat-bottom sterile microplates, the effect of bacteriocin and ciprofloxacin on the causes of UTI infections was assessed by measuring the MIC and MBC for bacteriocin and ciprofloxacin.[10] The required microbial inoculum was created using an overnight growth culture that was standardized using the McFarland turbidity criterion of 0.5 McFarland. Each well was filled with 100 µL Mueller-Hinton broth, 20 l microbiological inoculum, and 80 l bacteriocin (1–2056 µg/mL) or ciprofloxacin (1–2056 µg/mL) at various doses. For observation, controls like sterility and growth were also taken into account. The microtiter plates were kept at 37°C for 24 hours. The MIC is a measure of how well a product performs in a given situation.

The Combination of Bacteriocin and Antibiotics has a Synergistic Effect Against Uropathogenic Bacteria

A micro broth dilution test was repeated to assess the MIC and MBC after the combination of pure bacteriocin and ciprofloxacin.

RESULTS AND DISCUSSION

Isolation and Identification of *Staphylococcus epidermidis*

Fourteen *S. epidermidis* isolates were isolated from twenty-two skin samples collected from healthy persons and treated with an isolation method. The majority of *S. epidermidis* isolates were obtained by examining the appearance of colonies on solid media and microscopic examination. On MSA agar, all isolate colonies were little pink or red, moist colonies with no color change to the medium and a spherical shape.

Screening of Bacteriocin Producers

A screening process was used on 14 isolates of *S. epidermidis* to select the isolate with the highest bacteriocin production that could be used in future experiments in this study. To test the antibacterial activity of *S. epidermidis* isolates, two isolates of *S. aureus* and *P. aeruginosa* were employed as indicator strains. These markers were chosen because they are all essential microorganisms that cause skin infections.

Primary Screening

The first step is determining whether or not a bacteria can produce bacteriocin. This was accomplished utilizing the good diffusion method, an antibacterial sensitivity test that used the cell-free supernatant of 14 *S. epidermidis* isolates to look for antimicrobial compounds. The main idea behind this procedure is to create an inhibitory zone around the well where no development may occur. Six isolates were able to

manufacture bacteriocin, even though some isolates displayed antibacterial activity against *S. aureus, P. aeruginosa*, or both, as shown in Table 1, while others did not. Antimicrobial activity against *S. aureus* is also higher than that against *P. aeruginosa.*

The use *S. epidermidis*, which produces bacteriocin as important cells to cure a variety of illnesses, including acne.[11] These bacteriocins can behave as colonizing peptides, allowing a producer to enter and/or dominate an already crowded niche.[12]

Secondary Screening

Four isolates were chosen and put through a further screening process. Compared to the other isolates, these isolates had stronger antibiotic activity against *S. aureus*, which was utilized as an indicator in the initial screening. The bacteriocin activity (AU/mL) was determined and found to be between 28 and 46 AU/mL, as shown in Figure 1.

Bacteriocin Partial Purification

The acetone was applied to extract bacteriocin from *S. epidermidis nine* isolate and the results appeared that 30% acetone led to precipitate of this protein at higher levels with activity 53 AU/mL (Figure 2).

Evaluation of the Efficiency of Bacteriocin and Antibiotic Uropathogenic Bacteria

The MIC and MBC of bacteriocin and ciprofloxacin against the bacteria isolated from UTI infections were shown in Figure 3. Bacteriocin demonstrated bactericidal solid activity against all pathogenic strains tested. In all experiments, the MIC and MBC of bacteriocin were between 8 and 128 µg/mL. Ciprofloxacin MIC and MBC levels ranged from 64 to 2056 µg/mL. As a result, bacteriocin alone had a substantially lower MBC than ciprofloxacin when compared to the tested antibiotic, because *P. aeruginosa* and *K. pneumonia,* as well as other bacteria, tested, exhibited high levels of antibiotic resistance.

Bacteriocins with significant antibacterial activity can be used as promising natural antimicrobials in a variety of industrial and food-related applications.[13] Bacteriocins offer a lot of potential in human health applications when compared to current antibiotics. Bacteriocins have several advantages, including minimal toxicity, a high target specific action mechanism, the presence of several forms in nature, and potency at nanomolar doses. Antibiotics are used to treat high blood pressure, inflammation, and allergies, as well as skin infections, herpes, and tooth infections.[14]

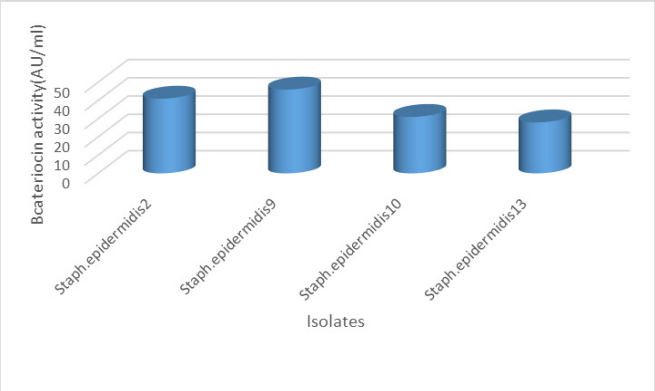

Figure 1: Secondary screening for bacteriocin production by selected *S. epidermidis* isolates

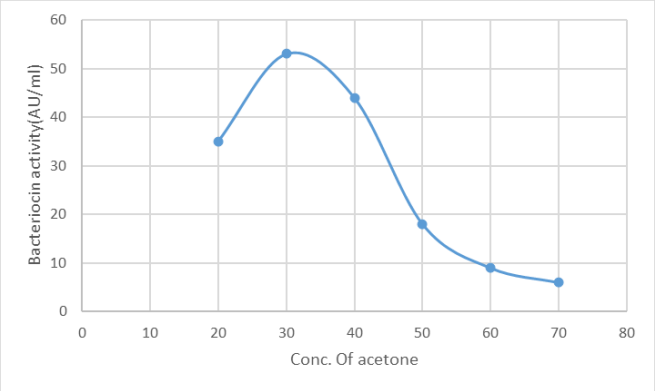

Figure 2: Purification of bacteriocin by different concentrations of acetone

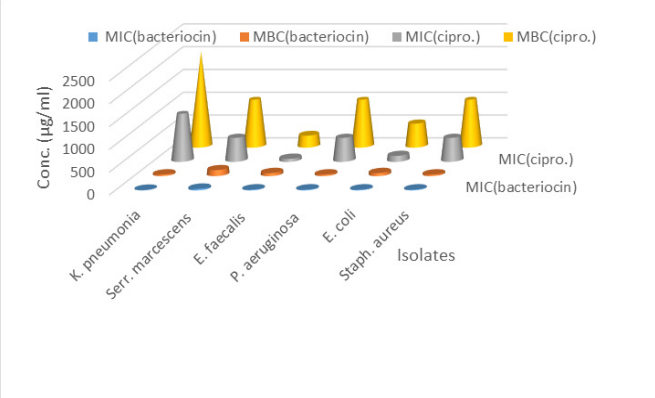

Figure 3: Detection of MIC and MBC for bacteriocin and ciprofloxacin against uropathogenic bacteria

Table 1: Primary screening for bacteriocin production by *S. epidermidis* isolates

Isolate	*S. aureus*	*P. aeruginosa*	*Isolate*	*S. aureus*	*P. aeruginosa*
S. epidermidis1	-	-	*S. epidermidis8*	-	18
S. epidermidis2	24	-	*S. epidermidis9*	26	22
S. epidermidis3	-	-	*S. epidermidis10*	20	17
S. epidermidis4	-	-	*S. epidermidis11*	-	-
S. epidermidis5	-	-	*S. epidermidis12*	-	21
S. epidermidis6	-	-	*S. epidermidis13*	20	15
S. epidermidis7	-	-	*S. epidermidis14*	-	-

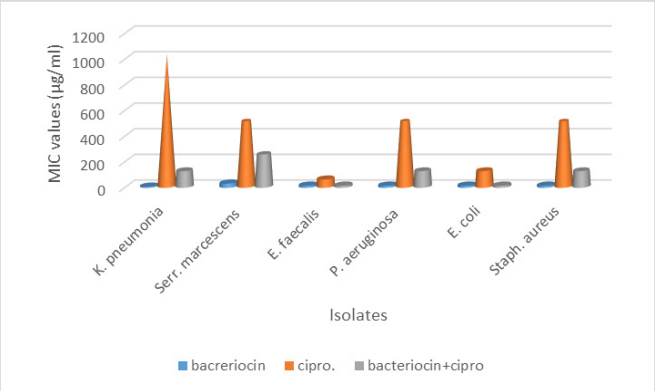

Figure 4: Synergistic effect of the combination of bacteriocin and ciprofloxacin

Synergistic Effect of a Combination of Bacteriocin and Antibiotic Against Uropathogenic Bacteria

When bacteriocin, ciprofloxacin, and ciprofloxacin were mixed, the bacteriocin enhanced the activity of ciprofloxacin to high levels with *P. aeruginosa*, *K. pneumonia,* and *E. coli*. In contrast, lower levels from enhancement in the activity were with *Serratia marcescens* as in Figure 4.

Bacteriocins from gram-positive bacteria (including staphylococcus) are often active against other gram-positive bacteria that are closely related, while some, like nisin, have shown activity against gram-negative bacteria.[15] Bacteriocins have been proven to be membrane-active peptides that cause membrane pores to develop, destroying the integrity of the cytoplasmic membrane.[16]

Gram-positive bacteria contain a high concentration of anionic lipids in their membranes, as is widely known. The bacteriocin's mode of action is derived from its initial association with the target's bacterial membrane. Electrostatic forces between negatively charged bacterial membrane lipids and positively charged bacteriocins guide the mechanism, which is restricted in the C- or N-terminal area. This is due to bacteriocin penetration into the lipid bilayer of the bacterial membrane and pore formation through the permeabilized bacterial membrane. The cell spills critical ions and metabolites that are required for survival, resulting in bacterial disease.[17]

CONCLUSION

These findings suggest that bacteriocin could effectively treat uropathogenic infections to help overcome the challenges of multi-antibiotic resistance.

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