Original Article

Isolation, Purification, and Identification of Novel Bacteriocin Produced by *Staphylococcus haemolyticus* Isolated from Semen Fluid

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Abstract

Background: Bacteriocins, which are ribosomally synthesized peptides, were initially classified as proteinaceous substances that affected the growth and/or viability of closely related organisms. **Objectives:** The study aimed to obtain an alternative antimicrobial compound, such as bacteriocins isolated from *Staphylococcus haemolyticus* (*S. haemolyticus*), effective against multidrug-resistant bacteria. **Materials and Methods:** *S. haemolyticus* isolates were recovered from 75 semen fluid samples of males aged 18–45 years and identified using macroscopic characteristics. Bacterial identification was confirmed using the Vitek 2 system. The optimization for bacteriocin production was carried out in a modified media with 1% glucose at 37°C for 72 h, with an inoculum size of 1.2×10^9 CFU/mL and pH 7. For screening the antibacterial activity of bacteriocin produced by *S. haemolyticus*, the filter paper disk method was used. **Results:** Bacteriocin was best produced by *S. haemolyticus*, and the bacterial activity of the bacteriocin was 70 and 40 AU/mL. After partial purification of bacteriocin by the ammonium sulfate method at saturation, the 70% with Mwt reached 29 kDa. **Conclusion:** The results of the production and purification of bacteriocin under optimal conditions showed, for the first time, an active local isolate of *S. haemolyticus* from semen fluid in Iraq.

Keywords: Bacteriocins, semen fluid, *Staphylococcus haemolyticus*

Introduction

Staphylococcus haemolyticus (*S. haemolyticus*) *is* a coagulase-negative and methicillin-resistant species that colonizes neonates admitted to intensive care units.[1] *S. haemolyticus* is a leading cause of late-onset sepsis and plays an important role in hospital-acquired infections.[2] *S. haemolyticus* is acquiring antibiotic resistance (MDR), which contributes to the emergence of virulent clones.[3] It has among newborn infants emerged as a frequently encountered late-onset sepsis pathogen.[4]

The most important virulent *S. haemolyticus* is the formation of biofilms, which are crucial for the development of infections.[5] It produces several types of toxins and enzymes that help in bacterial changing immunity.[5]

The causing hospital infections are characterized by the genome of *S. haemolyticus* insertion sequences.[6]

The hospital infections are characterized by the presence of *S. haemolyticus* insertion sequences.[7] Bacteriocin is a synthesized protein with antibacterial activity against other bacteria.[8]

Bacteriocin genes can be encoded or chromosomal genes that encode an active protein and genes encoding resistance to the protein, genes responsible for the export of the bacteriocin from the cell.[9]

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Submission: 19-Jan-2023 **Accepted:** 08-Feb-2023 **Published:** 30-Sep-2025

How to cite this article: Razaq RA, Ahmed ME, Shareef HK. Isolation, purification, and identification of novel bacteriocin produced by *Staphylococcus haemolyticus* isolated from semen fluid. Med J Babylon 2025;22:638-42.

Access this article online

Quick Response Code:

Website: https://journals.lww.com/mjby

DOI: 10.4103/MJBL.MJBL_60_23

Materials and Methods

Collection of samples

Seminal fluid samples were collected from 75 males suffering from UTI (aged 18–45 years) at a Laboratory Hospital, Baghdad. The clinical samples were cultured on different culture media (mannitol salt agar, blood agar, and skim milk agar) and incubated under aerobic conditions for 24 h at 37°C.[10] The bacterial identification was carried out using standard microbiological methods[11] and finally confirmed using Vitek 2 system.[12]

Detection of bacteriocin production

Assay method

Bacteriocin production was screened using the filter paper disk (FPD) method.[13] Bacteriocin activity was expressed as following:

AU/mL AU was calculated as 1000/100 × D,
where 1000 is a constant, 100 is the volume of the supernatant in a well (µL), and D is the dilution factor.

Partial purification and dialysis

The bacterial isolates were inoculated in brain heart infusion (BHI broth), and the cells were harvested by centrifugation at 6000 rpm for 15 min. The supernatant was then mixed with ammonium sulfate at 70% saturation. The purification of bacteriocin was conducted using the Sephadex G-50 column.[14]

Optimization of bacteriocin

1 pH: Production of bacteriocin was measured at a range of pH as in the following 5, 6, 7, 8, and 9 effects and measured the protein assay.
2 Inoculum size: Using 1 (3 × 108), 2 (6 × 108), 3 (9 × 108), and 4 (1.5 × 109) (1 × 108) CFU/mL for 24 h at 37°C.
3 Incubation period: Time difference between one to three days at strain (1.5 × 109) CFU/mL.
4 Temperature: Incubation temperature at 25, 30, 37, 40, and 45°C.
5 Optimum production medium: Production culture media include nutrient broth (NB), BHI broth, modified BHIB with 1% glucose, and Muller–Hinton broth.
6 Effect of nitrogen source and carbon source (sugars): Included (meat extract, yeast extract, sucrose, glucose, albumin, gelatines, tryptophan, and esculin) with concentrations of 1% for each one added to the BHIB.

Determination of the molecular weight
Tricin SDS-PAGE was carried out to determine the molecular weight of bacteriocin according to the method of Schagger and von Jagow.[14]

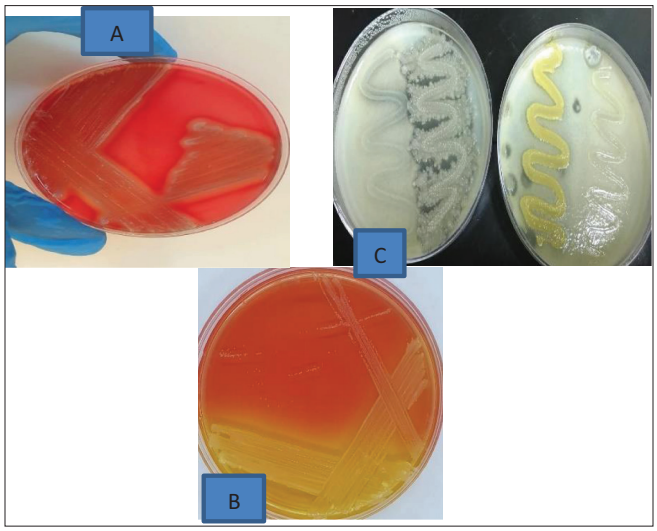

Figure 1: *S. haemolyticus on* (a) MSA (mannitol fermenters isolate), (b) blood agar, and (c) milk agar at 37°C for 24 h

Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients' verbal consent before the sample was taken. The study protocol and the subject information and the consent form were reviewed and approved by a local ethics committee according to document number 338 01/09/2023.

Results

A total of 100 *S. haemolyticus* strains were isolated on different culture media (mannitol salt agar, blood agar, and skim milk agar). Figure 1 shows the colony morphology on mannitol salt agar and yellow pigmentation on milk agar.

Production bacteriocin from *S. haemolyticus*

Figure 2 shows the results of the antibacterial activity of the bacteriocin produced by *S. haemolyticus* isolates against different bacterial species using the filter paper diffusion method.

Optimum conditions for bacteriocin

- The best pH 7 had the highest protein concentration production which reached to 3 µg/mL, while it reached to 2 µg/mL as the lowest pH 4 activity at the protein concentration [Figure 3].

Optimum inoculum

The results of optimal conditions for the extracellular bacteriocin production were at the bacterial inoculum of 1×10^8 CFU/mL.

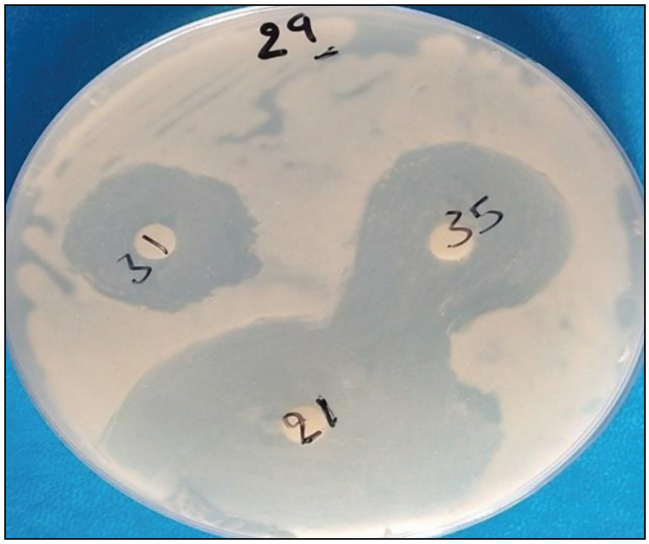

Figure 2: Screening of antibacterial activity of crude bacteriocins produced by *S. haemolyticus* on MHA at 37c for 48 h

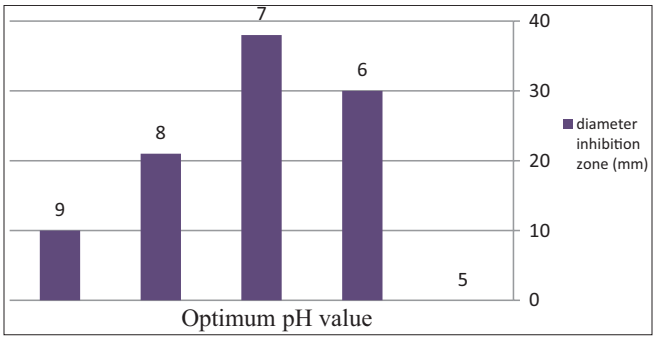

Figure 3: Effect of different pH on the production of bacteriocin

Incubation period

The results found that after 24 h, a high production of bacteriocin was obtained. After this range, the activity was decreased between 48 and 72 h. The bacteriocin production reached the maximum activity during the 24 h [Figure 4].

Optimum temperature

The optimum temperature for production was 37°C, while bacteriocin was reduced at the range between 25°C and 45°C with a maximum inhibition zone reaching 15 [Figure 5].

Optimum medium

The production of crude bacteriocins was tested on different culture media, including BHIB modified with 1% glucose. The lower inhibition zone reached 7 mm in the NB medium. This result may be due to the differences in the medium components of BHIB with a diameter of the inhibition zone reaching 15 mm [Figure 6].

Regarding the effect of different nitrogen and carbon sources on bacteriocin production, the results found that the optimum carbon and nitrogen sources yielded the greatest inhibition zones of 3.9 and 2.8 cm compared to other carbon sources and control (without any sources). Glucose was the best carbon source for the production of bacteriocin [Figure 7].

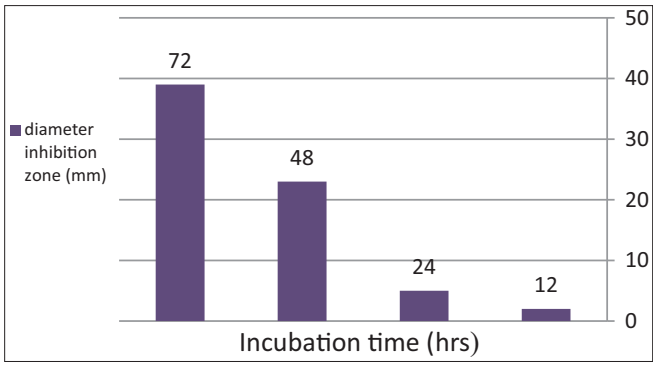

Figure 4: Effect of incubation time on bacteriocin production

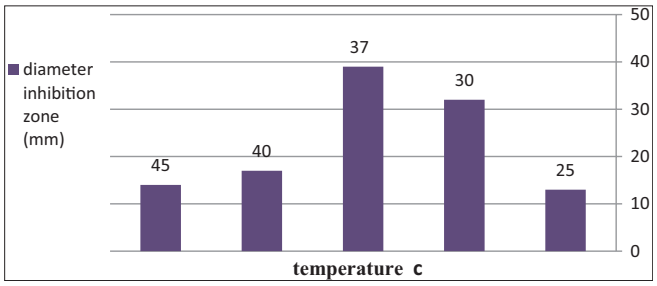

Figure 5: Effect of temperature on bacteriocin production

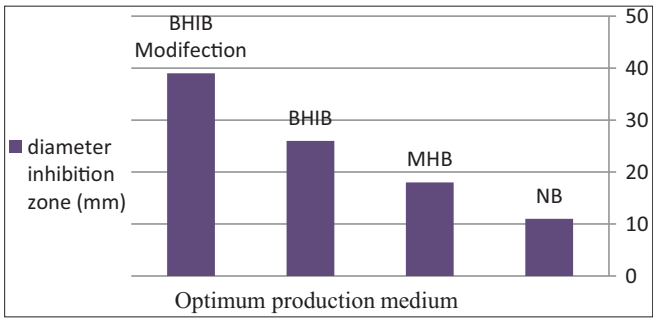

Figure 6: Bacteriocin production on different culture media

Ammonium sulfate precipitation and desalting by dialysis

The activity of the purified protein compared to the crude extract was observed by producing an inhibition zone of 37 mm. Purification was carried out by Sephadex G-50 column after several stages of precipitation and dialysis. Figure 8 shows the purification of bacteriocin by Sephadex G-50 column (red), and the absorbance fraction numbers yielded one protein peak at the fractions 20, 40, and 60 [Figure 8].

Molecular weight

Regarding the determination of the molecular weight of bacteriocin using SDS-PAGE, the results revealed that

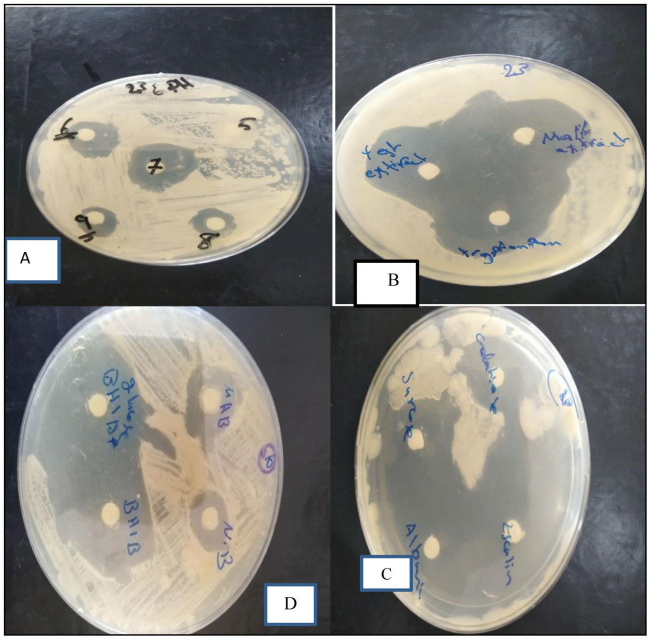

Figure 7: Antibacterial activity of Determination of the Optimal Conditions for Bacteriocin Production by *S. haemolyticus* at different A) pH, B) Nitrogen Source, C) Carbon (Sugars) Source and D) Medium on MHA, at 37°C for 72 h in pH = 7, using the FPD method

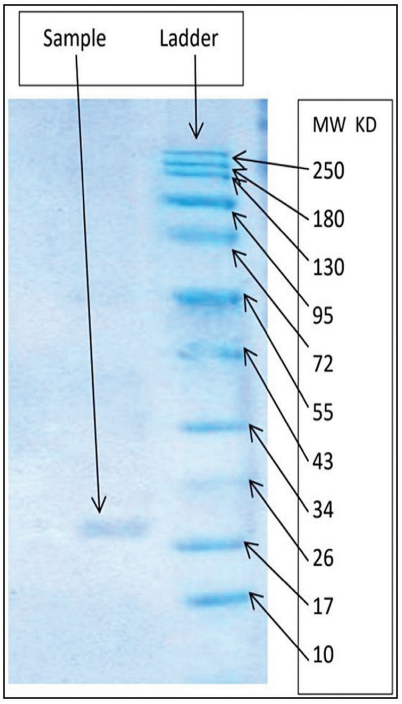

Figure 9: Molecular weight of purified bacteriocins produced from *S. haemolyticus*

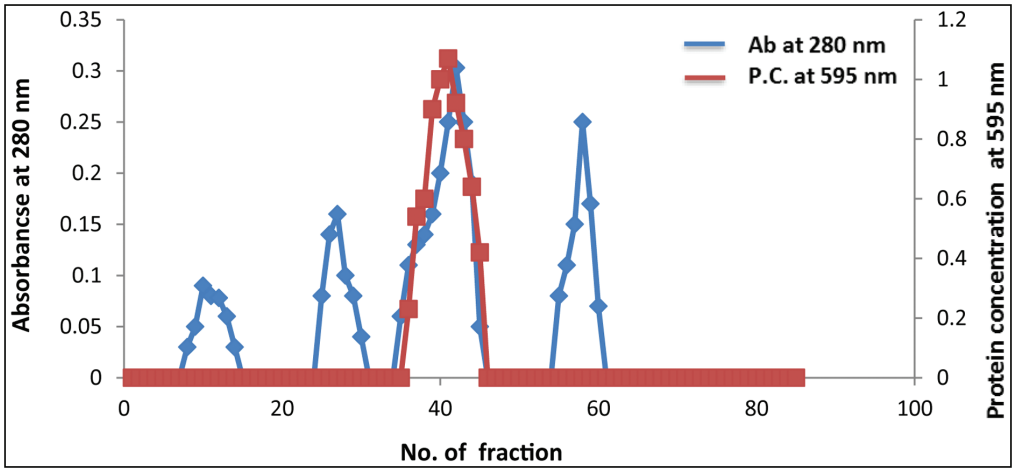

Figure 8: Purification of bacteriocin by Sephadex G-50 column (red)

there was a single target band at 29 kDa [Figure 9] of the bacteriocin. The calculation was done by estimating the length of the gel and the distance of migration compared with the standard protein of 10–250 kDa.

Discussion

S. haemolyticus was the most common among the other species isolated from urinary tract infections. This result was consistent with Obaid and Baiee.[15] The phenotypic results showed the fermentation of mannitol sugar to bacteria when cultured on a mannitol medium, and this was compatible with Kadhim *et al.*[16]

The results of the initial screening of *S. haemolyticus* isolates showed the highest diameter of inhibition, and this result coincided with the findings of Meade *et al.* who revealed obtaining a diameter of 10 mm.[17] The use of bacteriocins in the food industry has been proven effective and stable in different conditions.[18] The proteinase production in promising amounts along with its potential usage as a detergent makes it useful in industries, such as laundry. The best pH for the production of bacteriocin produced by Meade was neutral, and the result was compatible with Enterocin, which also exhibited high protein concentration at neutral pH.[19]

Regarding the best size of inoculum for the production of bacteriocin, the result of this study was consistent with Abbasiliasi *et al.*,[20] while the highest productivity was after three days of incubation period which was compatible with bacteriocins produced by lactic acid bacteria.[21] The activity of the bacteriocin at higher and lower temperatures was also compatible with Tulini *et al.*[22]

The variation in the temperature can affect bacteriocin production which led to the retardation of bacteriocin production. Raafat *et al.*[23] revealed a novel bacteriocin produced by *Enterococcus faecium* (*E. faecium*) at different temperatures compared to the control at 37°C.

The present result showed an increase in bacteriocin production when glucose was added to the culture medium. Lazreg *et al.* revealed that an increase in the biomass of *E. faecium* was observed when the concentration of glucose reached to 2%.[24] The practical purification of the bacteriocin in the present study was compatible with Oiao *et al.*[25] who used the same steps for the purification of a novel bacteriocin produced by E. faecium.

Conclusion

The results of the production and purification of bacteriocin under optimal conditions showed, for the first time, an active local isolate of *S. haemolyticus* from semen fluid in Iraq.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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