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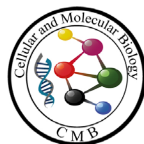

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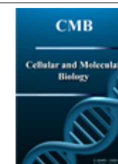

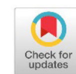

Original Article

Antimicrobial activity of *Enterococcus*-derived bacteriocins against multidrug-resistant *Pseudomonas aeruginosa*

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Abstract

Pseudomonas aeruginosa is a key concern in clinical settings due to its high level of resistance to antibiotics, making infections given rise to this bacterium very problematic to treat. The rise of multidrug-resistant bacteria poses a danger to treatments and stresses the necessity to find new antimicrobial drugs. In a neoteric study, *P. aeruginosa* was found in a suction machine tube, affirming the importance of identifying and managing potential sources of infection in healthcare facilities—many strains of *Enterococcus faecium* output bacteriocins, which are antimicrobial compounds. The study aimed to produce, isolate, purify, and characterize a new bacteriocin from *E. Faecium* found in stool samples and to investigate the effects of *E. Faecium* and its bacteriocin on multidrug-resistant *P. aeruginosa* in laboratory conditions. *E. faecium* is a kind of lactic acid bacteria (LAB) set up in the intestines of both humans and animals. In a study, stool specimens from 79 healthy individuals aged 5 to 35 were collected, yielding 70 isolates of *Enterococcus* spp. These bacteria exhibit growth in aerobic conditions and are identified through the API20 method. A crude preparation was made to extract the E. *faecium bacteriocin* by combining it with n-butanol in a 1:1 ml ratio in BHIB and then refining it using an ion exchange column. Through this purification process, the final specific activity of purified enterocin GH reached 38.19 U mg with a 4761.9 purification fold. The molecular weight of the *E. faecium* bacteriocin was determined using ion exchange chromatography. The study also examines how temperature and pH affect the activity of pure enterocin GH using *Staphylococcus aureus* and *P. aeruginosa* Type Culture Collection. Both crude and purified enterocin GH from *E. faecium* exhibited significant antibacterial activity against *P. aeruginosa* isolates when compared to the control ($p<0.05$). Additionally, the antibiofilm activity of enterocin GH was found to be more effective than penicillin ($p<0.05$) in preventing biofilm formation on the suction machine's tube.

Keywords: Enterococcus, Bacteriocins, *Pseudomonas aeruginosa*, Biofilm formation, Lactic acid bacteria (LAB)

1. Introduction

Fluid suction devices are vital in the variability of medical operations, mostly those covering surgery and wound care, as they aid in posit surplus fluids, blood, and other waste. But if not thoroughly emboldened and disinfected, these gadgets can turn to convert havens for bacteria. Patients may turn out contaminated and cultivate infections due to the warm, humid environment that fosters bacterial growth inside the suction tubes and canisters.

Typical devices that contain *Pseudomonas aeruginosa* [1] *Staphylococcus aureus* antibiotics [2] According to Kaper et al. [3], *Escherichia coli* can contaminate medical equipment in hospitals and *Klebsiella pneumoniae* [4].

Hospitals often have infections from fluid suction equipment and the tubing that supports them when performing procedures. These gadgets include organic matter and liquids that foster the growth of bacteria, such as *P. aeruginosa*, which can lead to serious infections, particularly in those with reduced immune systems [5].

Meanwhile, *P. aeruginosa* has a history of resistance to traditional antibiotics, and attention to the discovery of alternate therapy is increasing [6]. Microbes utilize various substrates as raw materials to produce a range of biological products, including enzymes, proteins, antioxidants, and pigments [7].

Enterococci are Gram-positive, facultatively anaerobic lactic acid bacteria located in the gastrointestinal tracts of humans and animals, as well as in dietary and environmental settings [8, 9]. Enterococci are a category of microorganisms that may be quite beneficial in applications of food. Through the processes of lipolysis, proteolysis, and citrate and pyruvate metabolism, enterococci enhance the flavor

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and texture of fermented dairy products. Moreover, they create bacteriocins, which help these products last longer on the shelf [10].

The term bacteriocin is also employed to encompass microbiome-derived antimicrobial molecules, such as non-ribosomally synthesized peptides [11]. Bacteriocins are classified into three categories: class I, comprising antibiotic families; class II, consisting of small non-modified peptides; and class III, which includes larger heat-labile proteins [12].

Bacteriocins can exhibit many mechanisms of action, such as inhibiting cell wall construction, exerting effects via DNase and RNase activities, and more commonly, creating pores in the target cell membrane [7, 13]. Enterococci synthesize bacteriocins, also known as enterocins. Enterocins are classified into four types: class I lantibiotics, class II non-lantibiotics, class III cyclic enterocins, and class IV high molecular weight proteins. Franz et al. [14] divide Class II into three subclasses: IIa is made up of pediocins, IIb doesn't have a leader peptide, and IIc is made up of other linear enterocins that aren't pediocin-like. The main *Enterococcus* spp. that output bacteriocins are *E. faecalis* and *E. faecium*. However, representatives of the *E. mundtii* species have also been discovered to produce bacteriocins.

Researching replacement antimicrobial treatments is vital for stopping infections linked to devices. Favorable strategies for tackling this trouble include bacteriophage virals that infect bacteria and bacteriocins antibacterial proteins made by bacteria such as *Enterococcus* [15].

Mostly, bacteriocins have demonstrated promise as vital protectors of the gastrointestinal system, presenting fresh avenues for successfully managing resistant bacteria in medical settings.

The study aims to purify enterocin from *Enterococcus faecium*, depict its physical and chemical characteristics, and test its antibacterial activity anti-*P. aeruginosa* strains extracted from surgical suction device tubes are the objectives of this study. To get better hygienic and safety procedures, this study looks into enterocin as a probable antibacterial worker to lessen bacterial contamination in surgical environments.

2. Methods and Materials

2.1. Isolation and identification of *P. aeruginosa*

Identifying clinical isolates of *P. aeruginosa* The Vitek2E compact system was used to identify sixteen *P. aeruginosa* isolates that were taken from a suction device used during surgeries at Al-Yarmouk Hospital.

2.2. Antibiotic susceptibility testing

An Atest for sensitivity of antibiotics was conducted to assess the susceptibility of *P. aeruginosa* isolates to various antibiotics, specifically CAZ, CL, TCC75/10, LEV, and PL. The disc diffusion method was employed to conduct an antibiotic sensitivity test. The assessment was conducted in accordance with the guidelines set forth by the Clinical Laboratory Standards Institute [16].

2.2.1. Bacterial isolation and identification of Enterococcus

E. faecium is isolated. Isolates of enterococci from healthy people's feces. The isolates were made by suspending one gram of excrement in two milliliters of 0.85% saline, spreading the mixture on Brain Heart Infusion agar, and then classifying the mixture according to its biochemical characteristics (API 20 Strep, bioMerieux France). They were incubated for 24 hours at 37°C after being cultured as usual in BHIB (Oxoid). The same medium with 20% (w/v) glycerol was used to keep all cultures alive at -80°C. Biochemical characteristics (API 20 Strep, bioMerieux France). Cultivation occurred in BHI broth (Oxoid) with incubation at 37°C for 24 hours. All strains were preserved in a medium containing 20% (w/v) glycerol at -80°C.

2.3. Detection of Enterococcus bacteriocin

We applied the agar well diffusion method to evaluate the creation of *E. faecium* bacteriocins [17].

2.4. Enterococcus bacteriocins activity assay

Using the good diffusion assay and Mahdi's [18] recommended technique, the antibacterial activity of enterocin LHMG was assessed.

2.5. Assessment of protein concentration

The protein concentration was measured using the Lowry et al. [19] method.

2.6. Synthesis of crude enterocin

E. faecium no. 6 was employed to produce crude enterocin. To purify against denaturant proteases and heat-labile proteins, crude enterocin extract (CEE) was heated at 80°C for 10 minutes, following the methodology established by Powell et al. [20].

2.7. Purification of Enterococcus bacteriocins

Purification of *Enterocin* from *E. faecium* Phase separation was achieved by thoroughly mixing enterocin, found in the supernatant fractions, with n-butanol at a 1:1 ratio, followed by purification using an ion exchange column (DEAE cellulose column) as outlined by Mahdi et al. [21]. The concentration of protein at each step was quantified using the Bradford method.

2.8. Description of produced bacteriocins

Description of the generated bacteriocins included determining the weight of molecular and investigating the effects of pH, temperature, and enzymes, on bacteriocins.

2.8.1. Bacteriocin molecular weight determination

The molecular weight of the bacteriocin was determined using an ion exchange column (DEAE cellulose column) based on the principles outlined by Whitaker and Bernhard [22]. The molecular weight of the generated bacteriocins was assessed using Tricine-SDS-Polyacrylamide gel electrophoresis.

2.8.2. pH Effectiveness on bacteriocins

The impact of pH on Enterococcus bacteriocins was assessed through the agar well diffusion method, utilizing *S. aureus* and *P. aeruginosa* as reference strains. The strains were sourced from the College of Sciences at Mustansiriyah University [23].

2.8.3. Temperature Effectiveness on bacteriocins

Temperature effectiveness on bacteriocins was evaluated by incubating them at different temperatures, as described by Mahdi et al. [23], and then assessing the residual

activity.

2.8.4. Effectiveness of enzymes on bacteriocin

Following the incubation of the bacteriocin with various enzymes (lipase, α-amylase, catalase, proteinase E, and Proteinase K) at 30°C for one hour, the enzymes were subsequently inactivated by heating at 100°C for three minutes. The residual enzyme activity was subsequently assessed [24].

2.9. Crude and purified bacteriocins antibacterial activity

Mahdi et al. [25] demonstrated the antibacterial efficacy of crude and purified bacteriocin against *P. aeruginosa* at a concentration of 32 µg/ml in Ref.

2.10. Antibacterial activity of Enterocin GH and Penicillin against *P. aeruginosa* in the tube of the suction machine

To cause biofilm formation on urinary catheters, a modified version of the Jones and Versalovic [26] approach was used. 1 cm long tube of suction machine segments were inserted into 10 milliliters of BHIB containing 1.5 \times 10^8 CFU/ml of *P. aeruginosa* strain no. 6. Over 24 hours, the setup was aerobically incubated at 37°C. Decantation was used to remove the media and planktonic cells, and distilled water (DW) was used twice to wash the tube fragments. A second 24-hour incubation was then conducted after adding 200 µl of BHIB mixed with Enterocin GH and Penicillin (32 µg). Tube of suction machine fragments were incubated for 30 minutes before being dried and twice cleaned with DW. The samples were then processed for inspection using atomic force and stained with gold. Sterile BHIB with a tube of suction machine pieces served as blank controls, and a bacterial culture free of bacteriocin was also prepared.

2.11. Analysis of statistical data

The simple one-way analysis of variance, or ANOVA, method was utilized to assess intergroup variation. The significance level was established at $P<0.05$. The statistical software Sigma State was utilized to do the one-way analysis of variance.

3. Results

3.1. Isolation of *P. aeruginosa*

Among the 70 samples analyzed from surgical suction device tubes, *P. aeruginosa* was the most predominant bacterium isolated, comprising 21.43% of the overall isolates. Following *P. aeruginosa,* other isolated bacteria involved *S. aureus* (7.14%), *Proteus mirabilis* (14.29%), *E. coli* (12.86%), and *K. pneumoniae* (15.71%). These findings underline the predominance of *P. aeruginosa* in the sampled surgical environments, emphasizing the critical need for infection control measures to mitigate bacterial contamination (Table 1).

3.2. Antibiotic susceptibility test for *P. aeruginosa* isolates

Strict protocols were followed to assess the antibiotic susceptibility of bacteria isolated from 15 samples. According to the data, bacteria in 60% of the samples (9 out of 15) showed resistance to antibiotic PEN, which was also the most resistant. On the other hand, 40% of the samples (6 out of 15) contained bacteria that were susceptible to antibiotic PEN, which showed the maximum sensitivity. As can be seen from (Fig. 1).

Other antibiotics (CL, CAZ, and TIT/CLA) showed total resistance in all isolates under examination. These results show how differently bacteria react to antibiotics and emphasize how important it is to test for bacterial susceptibility to guide effective treatment efforts. This is a crucial step in determining effective clinical therapy (Fig. 1).

3.3. Isolation and identification of *E. faecium*

The Vitek system, biochemical testing, and culture were used to examine 100 stool samples taken from healthy people. Fifty of the samples tested positive for Enterococcus species. Of these, 26 were well-known as *E. faecalis* and 24 as *E. faecium*. This study, which used thorough microbiological techniques for microbial identification, emphasizes the predominance of Enterococcus species in stool samples from healthy people (Table 2).

3.4. Detection of Enterocin GH production

All of the acquired *E. faecium* isolates could form enterocin, according to the results of the bacteriocin production screening. When enterocin was purified, *E. faecium* number 6 was used as the enterocin producer because it was the best isolate for producing enterocin. Enterocin is the term for the bacteriocin that is thus generated.

3.5. Purification of Enterocin GH

Bacteriocins were purified using ion exchange techniques. The overall yield and activity are summarized in

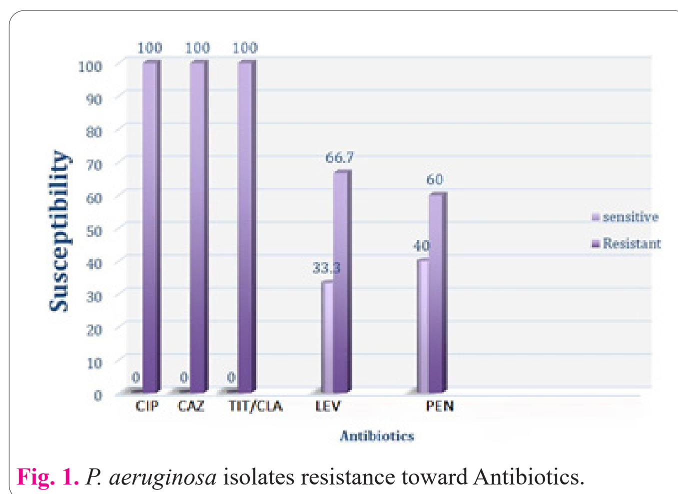

Fig. 1. *P. aeruginosa* isolates resistance toward Antibiotics.

Table 1. The most predominant bacterium isolated from surgical suction device tubes.

Bacterium	No.	%
Pseudomonas aeruginosa	15	21.43%
Staphylococcus aureus	5	7.14%
Proteus mirabilis	10	14.29%
Escherichia coli	9	12.86%
Klebsiella pneumoniae	11	15.71%

Table 2. The predominance of Enterococcus species in stool samples from healthy people.

Isolation from stool samples	No.	%
Enterococcus faecalis	26	52%
Enterococcus faecium	24	48%

Table 3 and Fig. 2.

3.6. Characterization of Enterocin GH

The characterization of Enterocin involves determining its molecular weight and examining the effects of pH, temperature, and five enzyme types on the activity of crude Enterocin.

3.6.1. pH stability for Enterocin GH

The activity of Enterocin GH remained stable at pH values 2 to 9. However, at pH 2, Enterocin GH lost 80% of its activity, and at pH 9, it lost 60% of its activity. This indicates that Enterocin GH is sensitive to alkaline conditions (Fig. 3).

3.6.2. Effect of temperature on Enterocin GH

E. faecium Enterocin GH was incubated for 15 minutes at 25, 30, 37, 40, 45, 50, 60, 70, 80, 90, 100, and 121°C to assess the influence of temperature. The indicator isolates were then used to measure any residual activity (Fig. 4).

3.6.3. Sensitivity Enterocin GH to some enzymes

Enterocin GH was inactivated upon exposure to proteolytic enzymes (proteinase K and proteinase E), although it remained active when subjected to α-amylase, catalase, and lipase (Fig. 5).

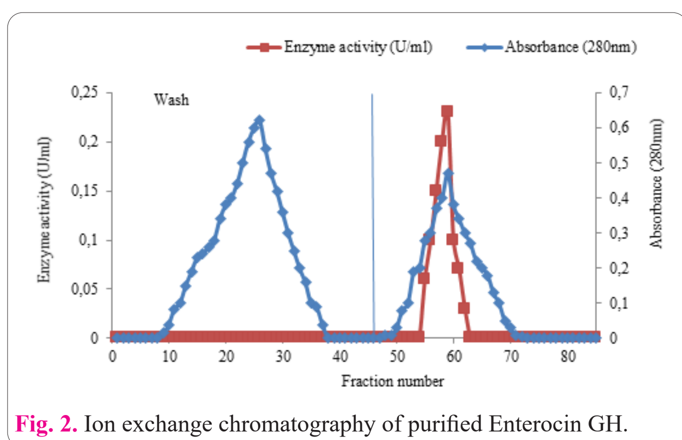

Fig. 2. Ion exchange chromatography of purified Enterocin GH.

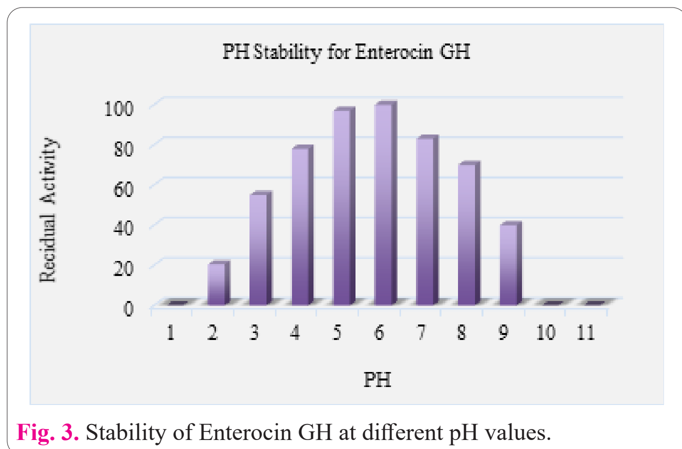

Fig. 3. Stability of Enterocin GH at different pH values.

3.7. Molecular weight of enterocin GH

The molecular weight of the enterocin GH protein was determined using SDS-PAGE gel electrophoresis (Fig. 6). Two protein bands were detected following Coomassie Blue staining.

3.8. Antibacterial activity of crude and purified Enterocin GH against *P. aeruginosa*

Table 4 illustrates the modification of the agar-well diffusion technique to assess the inhibitory effect of basic and cleansed Enterocin GH on *P. aeruginosa* isolates. At a dosage level of 32 mg/ml, the inhibiting region of crude ente-

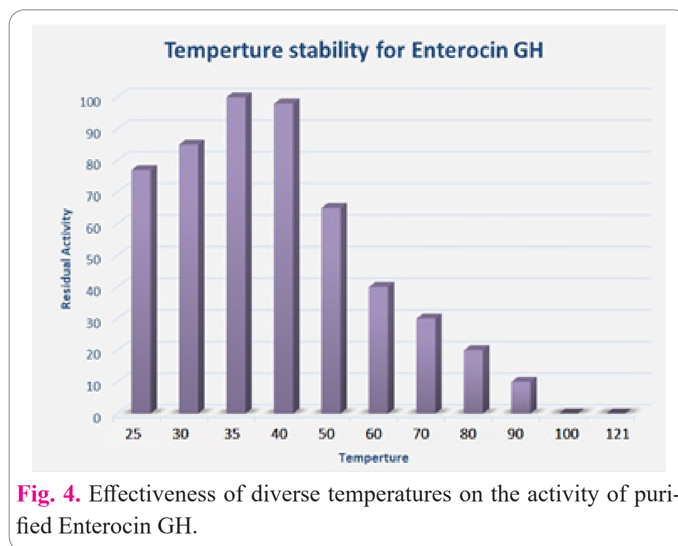

Fig. 4. Effectiveness of diverse temperatures on the activity of purified Enterocin GH.

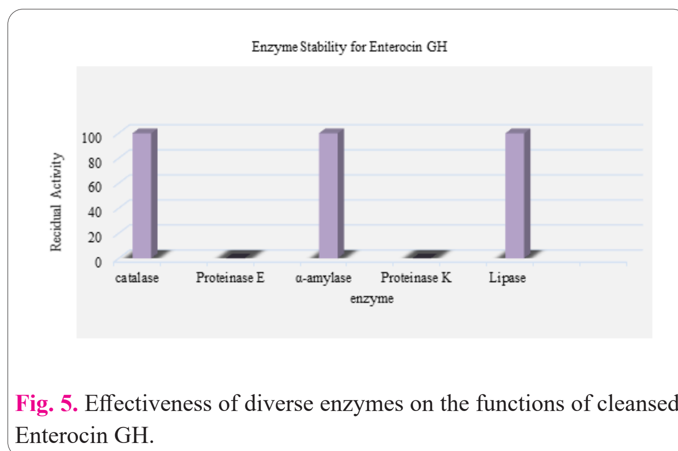

Fig. 5. Effectiveness of diverse enzymes on the functions of cleansed Enterocin GH.

Table 4. The modification of the agar-well diffusion technique to assess the inhibitory effect of basic and cleansed Enterocin GH on *P. aeruginosa* isolates.

Enterocin GH	Concentration of treatment (µg/ml)	Zone of inhibition (Mean ± SD)
Crude	32	34.67 ± 0.57
Purified	32	35.67 ± 0.57
Control (D.W.)	0	0±0
P-value	0.0019	

Table 3. Purification steps of bacteriocins produced by *Enterococcus faecium* isolate.

Step	V (ML)	Activity (AU/ML)	Protein concentration (mg/ml)	Specific activity (U/mg)	Total activity	Fold	Yield
Crude	250	125	0.32	124.68	31250	1	100
Butanol 1:1	35	450	0.16	449.84	15750	3.60	50.4
Ion exchange	30	400	0.084	4761.9	12000	38.19	38.4

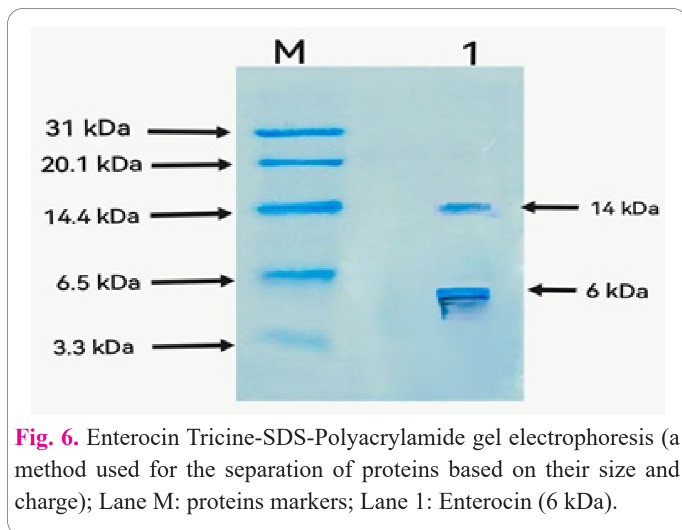

Fig. 6. Enterocin Tricine-SDS-Polyacrylamide gel electrophoresis (a method used for the separation of proteins based on their size and charge); Lane M: proteins markers; Lane 1: Enterocin (6 kDa).

rocin GH was measured at 34.67 ± 0.57, while the zone of the inhibition of purified enterocin GH was measured at 35.67 ± 0.57. Both crude and purified enterocin GH demonstrate antibacterial activity against *P. aeruginosa* isolates; however, purified enterocin GH shows significantly greater antibacterial activity compared to crude enterocin GH ($p<0.05$) (Fig. 7; Table 5).

3.9. Antibiofilm activity of Enterocin GH and Penicillin against *P. aeruginosa* in a tube of a suction machine

The findings from the atomic force microscope (AFM) analysis of biofilm growth on the surfaces of tube suction machines are summarized as follows:

Group A (No treatment or bacteria): The surface height of the tube measured 8.1 nm.

Group B (P. aeruginosa alone, untreated): The surface height of the tube increased to 36.8 nm.

Group C *(P. aeruginosa* treated with the antibiotic penicillin): The surface height was recorded at 17.5 nm.

Group D (*P. aeruginosa* treated with Enterocin GH): The surface height was further reduced to 12.0 nm.

These results indicate that Enterocin GH is a more effective antibiofilm agent compared to penicillin, as illustrated in Figures 8 and 9. This revision enhances readability and ensures that the information is presented in a clear, organized manner.

Table 5. Crude and purified bacteriocins' antibacterial activity anti-*Pseudomonas aeruginosa.*

Isolates	Crude	Purified
1	25	28
2	26	28
3	33	35
4	28	30
5	32	34
6	35	36
7	34	35
8	20	28
9	33	34
10	35	35
11	30	33
12	26	30
13	34	35
14	30	32
15	32	34

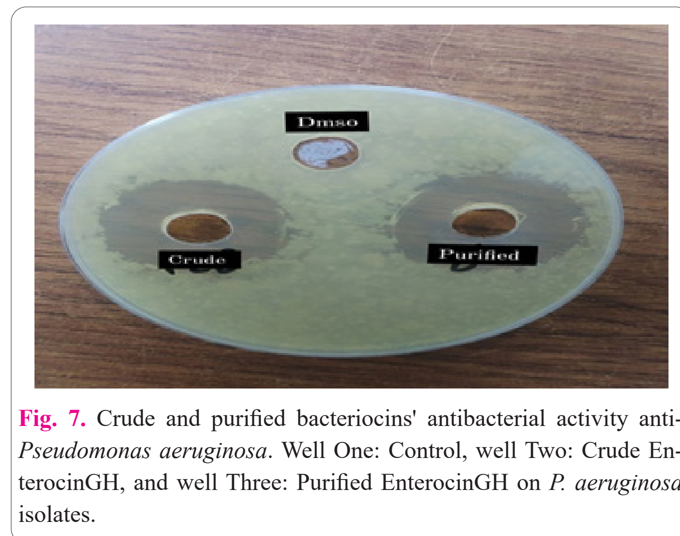

Fig. 7. Crude and purified bacteriocins' antibacterial activity anti-*Pseudomonas aeruginosa*. Well One: Control, well Two: Crude EnterocinGH, and well Three: Purified EnterocinGH on *P. aeruginosa* isolates.

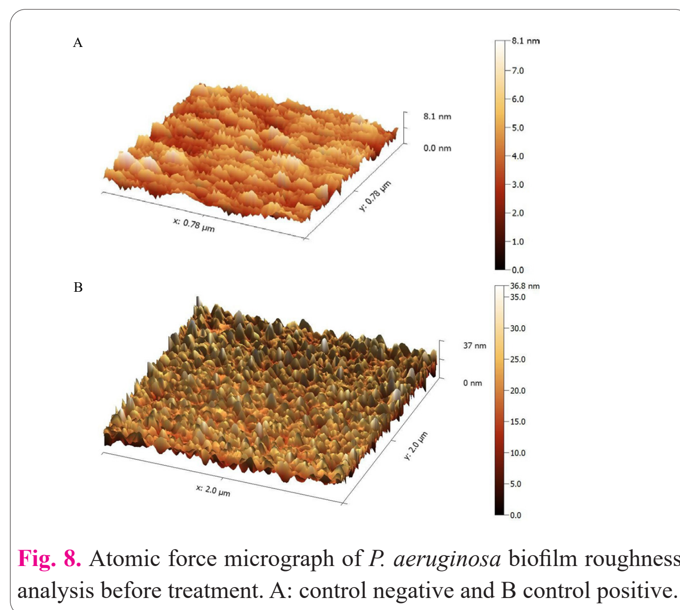

Fig. 8. Atomic force micrograph of *P. aeruginosa* biofilm roughness analysis before treatment. A: control negative and B control positive.

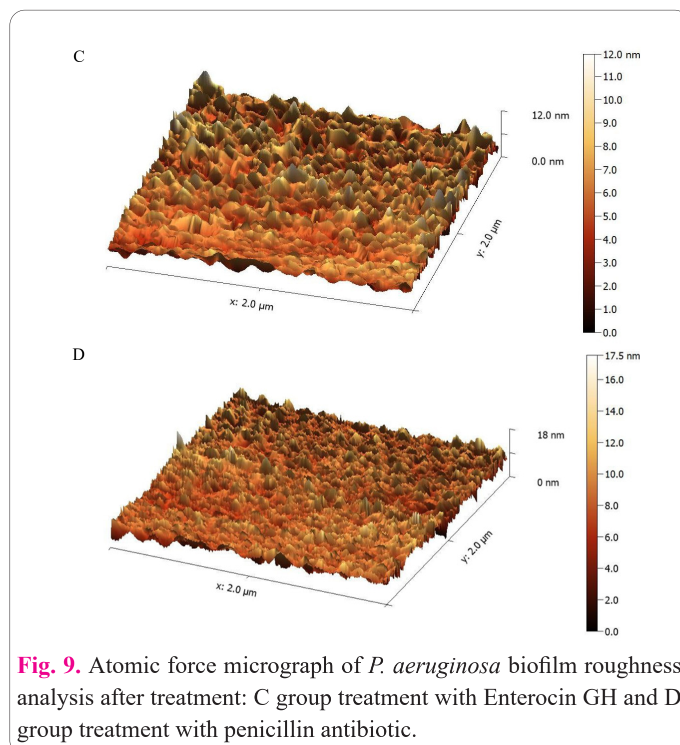

Fig. 9. Atomic force micrograph of *P. aeruginosa* biofilm roughness analysis after treatment: C group treatment with Enterocin GH and D group treatment with penicillin antibiotic.

4. Discussion

In a study of 70 surgical suction device tube samples, *P. aeruginosa* accounted for 21.43% of all isolates, making it the most common bacteria. It is common in healthcare environments and very resistant to antibiotics, especially carbapenems [27].

P. aeruginosa's capacity to form biofilms boosts its resistance to antibiotics and disinfectants, creating it a persistent threat in surgical settings. The biofilms shield the bacteria, increasing their survivability and making illnesses harder to treat. This bacterium is a significant cause of nosocomial infections, especially in intensive care units, and is frequently involved in hospital outbreaks. Strict infection control measures are crucial, according to recent studies [28, 29].

P. aeruginosa has a multidrug susceptibility phenotype, as exposed in a novel study by Khadhom [30] in Iraq, as well as in this work and another investigation by Hirsch and Tam [31] action it challenging to treat this bacterium in addition to its significance biofilm development. Prior research has indicated that *P. aeruginosa* isolates can develop biofilm on surfaces such as stainless steel and ceramic [32-34]. Consequently, researchers are trying to identify a substitute for common antibacterial drugs [35, 36]. Research conducted by Al-Samaree and Al-Khafaji [37] indicates that the resistance to amikacin among Iraqi isolates is relatively low, as demonstrated in this study.

LAB are Gram-positive, catalase-negative, non-spore-forming bacteria that exhibit high pH tolerance. Lactic acid is produced from glucose along with inhibitory substances akin to bacteriocins, hydrogen peroxide, and diacetyl, which serve to prevent food spoilage [38]. This experiment's results indicate the presence of bacteriocin-producing LAB in colostrum samples from sheep [39] and goats [40]. *E. faecium* and other gram-positive lactic acid bacteria exhibit optimal growth on MRS media, which is specifically formulated to enhance the proliferation of lactic acid bacteria at a pH of approximately 5.7. Common media utilized for the cultivation of lactic acid bacteria comprise glucose, yeast peptone (GYP), tryptone glucose yeast extract (TGE), and MRS medium [41].

In dairy products, and other ecological niches, enterococci are attending and are well-known to contribute to the organoleptic properties of the product [42]. Additionally, it is known that they generate one or more bacteriocins, which inhibit a variety of foodborne pathogens, such as *Listeria* species [43].

According to Corr et al. [44] and Ness et al. [43], this characteristic may affect niche competition and help regulate pathogen infections.

E. faecium sp. is the source of enterocins, which are potent reducing agents that stop the growth of *E. coli* and *Salmonella* strains [45].

E. faecium accounted for around half of the stool isolates in this investigation. All the isolates could produce enterocin GH, although the capacity was diversified. The early step in identifying bacteriocin-producing strains is to screen and select those that generate an inhibition zone against anti-indicator bacteria. To achieve this objective, direct or indirect methods may be employed to identify strains that produce bacteriocins. The determination of bacteriocin production involves the observation of inhibition zones formed by the strains against anti-indicator bacteria. The presence of a distinct and well-defined inhibition zone surrounding the colony or well suggests that the bacteria under examination are likely to produce bacteriocins [46].

Generality *E. faecium* strains produce bacteriocins that belong to subclasses IIa, IIb, and IId. Since most bacteriocins are thermally stable, heat treatments to food won't harm them. Certain devices can function at low pH and temperatures, making them potentially valuable in the processing of food and low-temperature applications [47].

These features were common to many of the enterocins that have previously been identified, including enterocin A [48], enterocin B [49], enterocin P [50], and enterocin 1071A and 1071B, generated by *E. faecalis* [51].

The stability of these enterocins is crucial because they are used for preserving fermented foods. Their antimicrobial activity decreases after treatment with proteolytic enzymes, indicating that they are protein-based. These enterocins are classified as class 2a and have a molecular weight under 10 kDa [52].

A lot of the gram-positive bacteria are susceptible to the broad inhibitory spectrum displayed by several Enterocin and other members of the pediocin-like family of bacteriocins [48-50]. Together with *S. paratyphi*, *L. monocytogenes* strains *L. plantarum*, *L. innocua*, *S. typhi*, and *E. faecalis*, our bacteriocin also significantly reduced these activities. Certain strains of Enterococcus bacteria were found to be susceptible to our bacteriocin, even though Gram-negative bacteria are typically thought to be resistant to numerous bacteriocins from these strains. Certain LAB bacteriocins, particularly the class 2 bacteriocin pediocin, have been found in some reports to support the inhibition of a limited number of Gram-negative bacteria, such as *Shigella* sp., *Salmonella* sp., *Pseudomonas*, and *Shigella flexneri* [53].

E. faecium strain screening the best crude and purified antimicrobial activities' spectrum as each of those had activity against multidrug-resistant *P. aeruginosa.*

Enterocin GH may act anti-bacteria by interfering with vital metabolic processes or by rive the integrity of cell membranes. The antibacterial activity of purified Enterocin GH is higher than that of crude formula. This results from the purification process's elimination of impurities and inactive ingredients, which concentrates the active bacteriocin molecules that reign antibacterial activity [54].

Antimicrobial activity appraisal is commonly done using the agar well diffusion method, which was utilized in this work. To measure the amount of bacterial inhibition, wells containing bacteriocin were placed on agar plates that were injected with bacteria. The diameter of the clear zone surrounding the well reflects this [55].

The results further corroborate the findings of Cintas et al. [50] and Lemos Miguel et al. [56] regarding the antimicrobial efficacy of enterocin *P against* these bacteria.

The inhibitory zone widths of crude and pure Enterocin GH differ significantly ($p<0.05$), emphasizing the practical implications for food safety and pharmaceutical applications. Because of their increased specific activity and stability, purified bacteriocins are more effective and dependable when used as natural preservatives or medicinal agents [57].

Research indicates that bacteriocins typically cause hole expansion and cell lysis by attaching to certain receptors on bacterial membranes [58].

The antibiofilm properties of Penicillin and Enterocin

GH anti-*P. aeruginosa* biofilms on suction machine tubes are assessed in this work. The habit of biofilm on these tube surfaces was investigated using atomic force microscopy (AFM). Enterocin GH may have a stronger potential to enter and breach biofilms, as seen by the larger drop in surface height visible in Group D rather than Group C. The ability of bacteriocins, such as Enterocin GH, to breach bacterial cell membranes more effectively than conventional antibiotics is consistent with Cotter et al. [58].

According to the research, enterocin GH may be a powerful treatment for medical device-related infections, which are over and over made worse by the expansion of biofilm. The capacity of Enterocin GH to efficiently decrease biofilm development on suction machine tubes underscores its potential as a useful therapeutic alternative since biofilm-associated infections are known to be challenging to treat [59].

Atomic Force Microscopy (AFM) on Foley catheters showed that *E. faecalis* output enterocin GLHM and demonstrated substantial anti-*K. pneumoniae* biofilm activity, according to Mahdi et al. [60].

The following heights were determined by measuring the catheter tubes' surfaces: Group A: 7.33 nm (control; neither treatment nor bacteria) Group B: 20.31 nm (exclusively *K. pneumoniae*, untreated) - *K. pneumoniae* treated with enterocin GLHM in Group C: 7.34

5. Conclusion

In conclusion, the study highlights the critical role of *P. aeruginosa* and *E. faecium* in healthcare-associated infections, emphasizing their antibiotic resistance and biofilm formation abilities. *P. aeruginosa*, the most prevalent bacteria isolated from surgical suction device tubes, demonstrates significant resistance to antibiotics and disinfectants, posing challenges in surgical and intensive care settings. Further research into bacteriocin production, purification, and application will be crucial for developing effective interventions against resistant bacterial pathogens.

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