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Biomedical Applications of Bacteriocin Purified from *Lacticaseibacillus paracasei* as Antimicrobial and Antibiofilm Compound against Pathogenic Bacteria and Yeast

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Abstract: Gastrointestinal systemic infections caused by bacteria and fungus as well recognized to be the main reason for causing acute gastroenteritis; Bacteriocins have a wide spectrum of antibacterial and antifungal efficiency. Hence the present experiment was designed to examine the antibacterial, antifungal and antibiofilm eligibility of Bacteriocin over locally isolated pathogens including *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. Bacteriocin was purified from local strain of *Lacticaseibacillus paracasei* that identified by the *16S rRNA* gene, the identification of *Candida albicans* isolate was confirmed by *ITS* gene. The antimicrobial activity was examined by well diffusion assay and MIC method by the concentration 0.54 mg/ml for Bacteriocin. Meanwhile, antibiofilm dispersal consideration have been completed by Microtiter plate method. The outcomes revealed to highest inhibition observed over *S. aureus* isolates ranged from 23.5 mm to 39 mm, the minimum inhibitory concentrations were 0.05 mg/ml for all pathogenic indicators, while biofilm suppression show high reduction in biofilm against *C. albicans* with OD (0.13). In conclusion, Bacteriocin has obvious antibacterial, antifungal effect and biofilm dispersal ability and could be used towards Gram- positive, Gram- negative and yeast.

Keywords: *ITS* gene, *16S rRNA*, Bacteriocin, antimicrobial, antibiofilm.

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Introduction

Gastrointestinal infections (GIs), mostly obvious as clinical syndromes, involving enteric fever, acute vomiting, and diarrhea, induced by the digestion of pathogens as well as the deactivation of normal microbiota, GIs are caused by bacteria, viruses, fungi, protozoa all together with parasites (1). Enteropathogenic microbes including *E. coli* recorded an essential global health burden especially in developing nations and a major cause for children mortality and morbidity (2). The probiotic *Lacticaseibacillus paracasei*, as the previous name is *Lactobacillus paracasei* is a Gram-positive and facultative heterofermentative lactobacilli, mainly from dairy products, this bacterium is able to survive under acidic conditions and shows bile salts resistance as well (3). Bacteriocins are multi efficacious, ribosomally made, proteinaceous components with prominent antimicrobial efficiency when used with specified concentrations (4). Bacteriocins antimicrobial peptides (5), have various

action modes including the formation of pores, the Inhibition of peptidoglycan mechanism in addition to the interaction with Lipid II as a biosynthesis mediated activity, moreover bacteriocins able to bind to teichoic and lipoteichoic acids and displace enzymes were involved in cellular lysis(6). Bacteriocin purified by the beneficial *Lacticaseibacillus paracasei* isolated from milk yoghurts considered to be safe and boost tendency in phagocytic activity (7).

Materials and methods

Collection and isolation of pathogenic samples

Exactly two hundred and three clinical samples (*Staphylococcus aureus, Escherichia coli* and *Candida albicans*) were obtained from children's patients for both genders aged between two- ten years old suffering from diarrhea and gastrointestinal infections who settled in Al-Yarmouk Teaching Hospital, Baghdad, Iraq. All collected clinical specimens that represent three pathogenic indicators were grown, individually, each on its selective medium and then incubated under each required condition, *S. aureus* has been grown on Mannitol salt agar at 37°C for 24hrs (8), *E. coli* specimen was incubated aerobically at 37°C for 24hrs on MacConkey agar and *C. albicans* cultured on Sabouraud dextrose agar (SDA) at 37°C for 24hrs. Furthermore, after incubation and depending on colonies morphology (shape, size, color and texture), isolates suspected to belonging to *S. aureus, E. coli as* well as *C. albicans* were taken for primary identification (9). Pathogenic samples also subjected for macroscopic, microscopic and biochemical, also isolates were subjected for VITEK 2 system identification (10).

Identification of *Candida albicans* by *ITS* gene

Candida albicans isolates were subjected for an advance confirmative molecular identification by *ITS* gene (11), fungal DNA extracted, quantified then the electrophoresis has been done to determine DNA pieces after the process of extraction to detect the result of the interaction of PCR during the presence of the standard DNA to distinguish the bundle size of the outcome of the interaction of PCR on the Agarose gel. The primers have been used according to (12) used included within (Table 1).

Table (1): The specific primer of gene *ITS* gene for *Candida albicans.*

Primers	Sequence	Tm (°C)	GC (%)	Product size	Ref.
Forward	5′- TCCGTAGGTGAACCTGCGG -3′	60.3	50	500-650 base pair	(Zarrin *et al*., 2016).
Reverse	5′ TCCTCCGCTTATTGATATGC-3′	57.8	41		

Collection and isolation of *Lacticaseibacillus* spp.

A total of thirty-six samples were gathered for obtaining *Lacticaseibacillus* species from different non-clinical sources (homemade yoghurt and cheese), cultivated in sterile tubes containing de Man, Rogosa and Sharpe (MRS) broth and then incubated at 37ºC for 24hours in microaerophilic conditions in a candle jar then re-cultured as single colony and spread in the selective MRS agar medium plate (pH 6.5) at 37ºC for 24hours in same manner with (13).

Identification of *Lacticaseibacillus* spp. by *16S rRNA* gene

Lacticaseibacillus isolate that are able to produce bacteriocin subjected for an advance confirmative

molecular identification by *16 S rRNA* (14), bacterial DNA extracted, quantified then the electrophoresis has been done to determine DNA pieces after the process of extraction to detect the result of the interaction of PCR during the presence of the standard DNA to distinguish the bundle size of the outcome of the interaction of PCR on the Agarose gel. The primers have been used according to (15) used included within (Table 2).

Table (2): The sequences of primers of *16S rRNA* gene for *Lacticaseibacillus* isolate.

Primers	Sequence	Tm (°C)	GC (%)	Product size	Ref. (12)
Forward	5'- AGAGTTTGATCCTGGCTCAG- 3'	54.3	50.0	1250 base pair	(Srinivasan *et al.*, 2015
Reverse	5'- GGTTACCTTGTTACGACTT- 3'	49.4	42.1		

Bacteriocin partial purification

The Precipitation of bacteriocin peptide was carried out via ammonium sulphate (16), the cell free supernatant (CFS) of *lacticaseibacillus paracasei* culture was prepared from 400 ml of 24hrs grown culture. Afterward, the CFS was transferred into a beaker placed in an ice bath on a magnetic stirrer. Additionally, an exact amount (3.3, 11.3, 18.05, 21.8. and 25.8 grams) of ammonium sulphate $(NH4)_2$ SO_4 according to the specific addition standard related to the experiment was added gradually to the CFS in order to achieve 30, 40, 50, 60, 70, and 80 % of saturations, respectively, through slow and constant stirring at 4ºC. Notably, the stirring process was continued for an additional 30min. Furthermore, the precipitate formed in every saturation level was subjected for centrifugation for 30min at 10000 rpm. Afterward, supernatant was decanted and the precipitates were redissolved in buffer of phosphate (pH 7.2, 0.1M) in an appropriate volume. Further purification was carried out by gel filtration chromatography using Sephadex G-150 gel. The gel was loaded carefully in the column to obtain 2.6 × 90 cm (diameter: height). The column was equilibrated with 0.1M phosphate buffer with 7.2 pH. The dialyzed protein sample was then applied to the column and proteins were eluted using 0.1M phosphate buffer pH 7.2 within room conditions. Forty fractions were obtained and the absorption of these fractions was tested via 280 nm by UV-spectrophotometer. Moreover, each fraction was examined for antibacterial and antifungal impact against the indicator isolates by the well diffusion method. The fractions that showed antimicrobial activity were gathered in one tube, and the protein concentration and bacteriocin activity has been determined (17).

Antimicrobial assays

The agar well diffusion method was applied to screen the antimicrobial activity of bacteriocin with concentration 0.54 mg/ml (obtained from Table 3) against three pathogenic microbes (*S. aureus*, *E. coli* and *C. albicans*). Following (18), the inhibition zones measurements were measured in millimeter (mm) as: (Inhibition zone (mm)= Diameter of growth inhibited zone- Diameter of the well). Additionally, The Minimum inhibitory concentration (MIC) has been completed following (19) steps.

Formation of pathogenic microorganisms' biofilm

The qualitative method, Congo red agar assay (CRA) used for the detection of biofilm producing

pathogens which depends on the change in colonies color grown on CRA medium (20). Moreover, the quantitative assay, Microtiter plate method is a used to determine biofilm using the microplate reader, data were calculated exactly as (20).

Antibiofilm assay

The dispersal activity of Bacteriocin against the formation of biofilm by *S. aureus*, *E. coli* and *C. albicans* isolates was determined by using the wells polystyrene plate method (20).

Detection of microbial viability after dispersal of biofilm

According to the culture-based detection method, the three pathogenic indicators *S. aureus*, *E. coli as* well as *C. albicans* were tested for their viability after the treatment with Bacteriocin to confirm the restrict activity of Bacteriocin for the removal of biofilm only. Moreover, the three pathogenic isolates were grown, separately, each on its selective medium and then incubated under each required condition. Furthermore, after incubation and depending on colonies morphology (shape, size, color and texture), isolates were identified by their growth (21).

Results and discussion

Identification of pathogenic microbes

According to macroscopic, microscopic and biochemical tests (catalase, oxidase and VITIK 2 system) isolates of pathogenic microorganisms proved to be *S. aureus*, *E. coli* and *C. albicans* (22,23).

Molecular identification of *Candida albicans* by the detection of *ITS* gene

Depending on the *ITS* gene - based molecular identification of clinical isolates and to confirm the identification of *C. albicans*, *ITS* gene amplification was performed using PCR technique to detect the positive result as imaged in (Figure 1) and compatible 100% with reference strain ID: OK030637.1 in Gen Bank this assured the correct diagnosis (24).

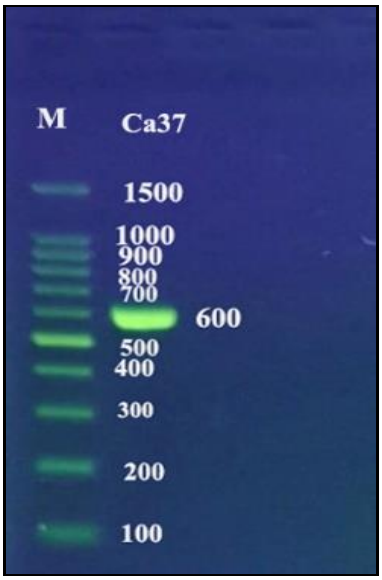

Figure (1): The PCR product of *ITS* gene using *Candida albicans* primers (Ca 37), the band size 600 bp. The product was electrophoresis on 1.5 % agarose gel at 75 volt/cm2. 1x TBE buffer for 40 min. M: ladder.

Molecular identification of *Lacticaseibacillus* spp. by *16S rRNA* gene

All colonies of isolation MRS agar were white, rounded in shape and range in consistency from creamy white in color to glossy white and moist-mucoid colony appearance on the surface (25). The results of microscopic examination showed that the isolates were Gram positive and were negative to catalase and oxidase biochemical tests. According to the *16S rRNA*-based molecular recognition technique of clinical isolates and to confirm the identity of *Lacticaseibacillus paracasei*, *16S rRNA* gene amplification was performed via the Polymerase Chain Reaction technique to detect the positive result as imaged in (Figure 2), a new strain of *L. paracasei* namely (MOIQ35) with identity percentage 99% were obtained from local homemade yoghurt sample have been registered in The National Center for Biotechnology Information (NCBI)(26).

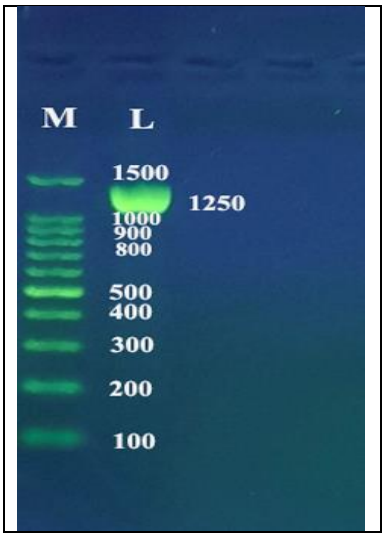

Figure (2): The PCR product of *16S rRNA* gene using *Lacticaseibacillus paracasei* (L) primers, the band size 1250 bp. The product was electrophoresis on 1.5 % agarose gel at 75 volt/cm2. 1x TBE buffer for 40 min. M: ladder.

Bacteriocin partial purification

The outcomes illustrated to maximum bacteriocin precipitation was obtained at 80% saturation level as indicator strains, *S. aureus, E. coli and C. albicans* were used to confirm the effectiveness of the formula confirmed by (Figure 3), the obtained concentration of purified Bacteriocin was 0.54 mg/ ml illustrated within (Table3) (27).

Table (3): The purification steps of Bacteriocin produced from *Lacticaseibacillus paracasei*.

Purification stage	Volume by (ml)	Bacteriocin activity (AU/ml)	Total activity (AU)	Protein Conc. (mg/ml)	Specific activity (AU/mg)	Purification (folds)	Yield concentration%
The crude Extract	40	40	1600	0.23	173.9	1	100
Ammonium sulfate precipitation 80%	12	40	480	0.25	160	0.92	30
Gel- filtration Sephadex G-150	15	40	600	**0.54**	74.07	0.425	37.5

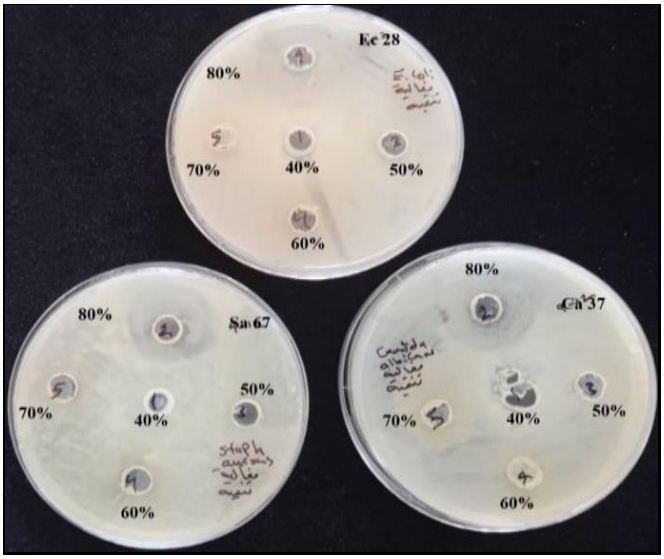

Figure (3): Bacteriocin activity precipitated with different concentrations of ammonium sulfate over three indicators (*Staphylococcus aureus, Escherichia coli and Candida albicans*) by well diffusion assay at 37ºC for 24hrs.

Antimicrobial effect of bacteriocin

The inhibitory effect of Bacteriocin over the three *S. aureus* isolates explicated that that all isolates were affected as shown there in (Table 4) and (Figure 4), the highest inhibition area (39 mm) in average dimeter was registered, while the lowest inhibition circle diameter was (23.5 mm). Moreover, the inhibitory effect towards *E. coli* isolates depending on the well diffusion method illustrated that all isolates were affected based on (Table 5) and (Figure 5), the highest inhibition zone (21.5 mm) in dimeter was recorded and much close diameter recorded (18 mm). The inhibitory impact of Bacteriocin over *C. albicans* isolate leaded to inhibition circle measured exactly (34 mm) diameter (28) as Shown by (Figure 6).

Table (4): inhibition zone data for *S. aureus* isolates after treatment with Bacteriocin by well diffusion process.

S. aureus Isolates	Inhibition zone (mm) by Bacteriocin	Results
St 2	34.5	+++
St 17	23.5	+++
St 44	39 (average)	+++

+++= Strong inhibition

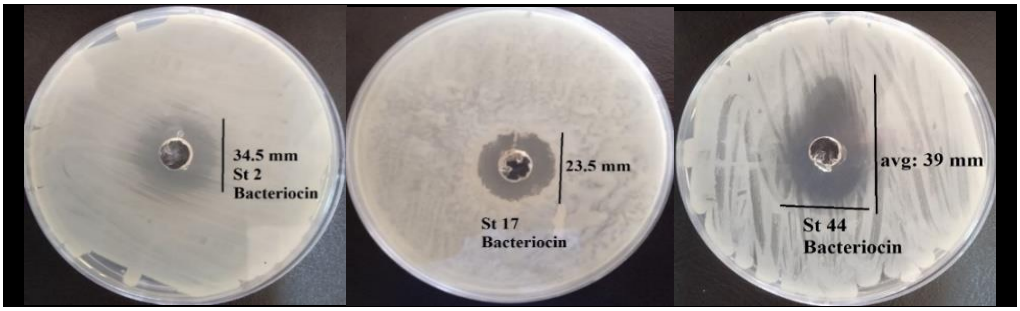

Figure (4): Antibacterial activity of Bacteriocin against *S. aureus* isolates symbolled (St 2, St 17 and St 44) by well diffusion assay at 37ºC for 24hrs.

Table (5): Inhibition zone data for *E. coli* isolates after treatment with Bacteriocin by well diffusion process.

E. coli Isolates	Inhibition zone (mm)	Results
Ec 28	18	+++
Ec 51	21.5	+++
Ec 67	12.5	+++

+++= Strong inhibition.

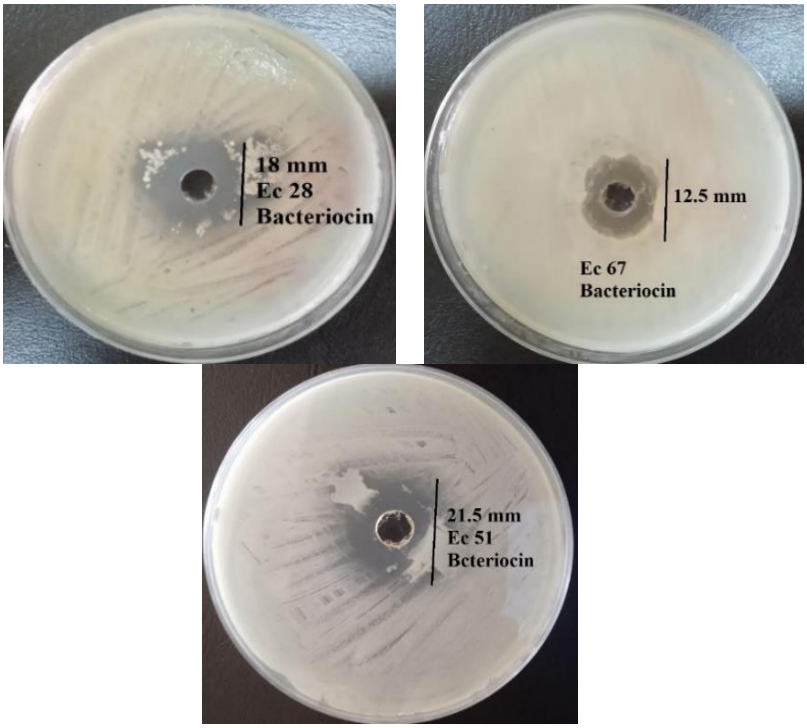

Figure (5): Antibacterial activity of Bacteriocin against *E. coli* isolates symbolled (Ec 28, Ec 51 and Ec 67) by well diffusion technique at 37ºC for 24hrs.

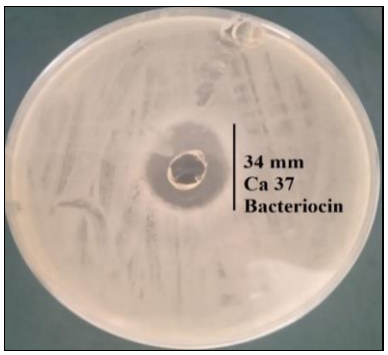

Figure (6): Antibacterial activity of Bacteriocin against *C. albicans* strain symbolled (Ca 37) by well diffusion process at 37ºC for 24hrs

The minimum inhibitory concentration (MICs)

By using microtiter plate method, Bacteriocin had minimum inhibitory concentration 0.5 mg/ml for all tested isolates for the three pathogenic indicators *S. aureus, E. coli and C. albicans*. Conformationally, the colors had transformed from blue to pink or pale pink (29).

Biofilm formation

After incubation of three *S. aureus* isolates, three *E. coli* isolates, and one isolate of *C. albicans* were found have the capability to form biofilm when black colonies were observed for the biofilm production via Congo red agar (30). Additionally, the outcomes of the Microtiter plate (MtP) method for biofilm formation by *S. aureus*, *E. coli* and *C. albicans* are shown within (Tables 6,7 and 8) illustrated the strong formation of chosen isolated (31).

Table (6): Detection of biofilm formation by *S. aureus* isolates depending on microtiter plate method

Isolate NO	Isolate OD	Results
St 2	0.58	+++
St 17	0.33	++
St 44	0.15	+
NC	0.05	0

0= No biofilm, + = Weak biofilm, ++= Moderate biofilm, +++= Strong biofilm; NC= Negative control; SD= 0.001.

Table (7): Detection of biofilm formation by *E. coli* isolates depending on microtiter plate method

Isolate No.	Isolate OD	Results
Ec 28	0.33	++
Ec 51	0.32	++
Ec 67	0.58	+++
NC	0.05	0

0= No biofilm, + = Weak biofilm, ++= Moderate biofilm, +++= Strong biofilm; NC= Negative control; SD= 0.001.

Table (8): Detection of biofilm formation by *C. albicans* strain depending on microtiter plate method

Strain No.	Strain OD	Results
Ca 37	0.60	+++
NC	0.05	0

0= No biofilm, + = Weak biofilm, ++= Moderate biofilm, +++= Strong biofilm; NC= Negative control; SD= 0.001.

Antibiofilm efficiency of bacteriocin

Biofilm produced by *S. aureus* isolates has been eradicated by Bacteriocin when the documented averages of biofilm OD values were minimized from averages of each isolate control mentioned via (Figure 7). However, highest dispersal in biofilm OD (0.13). Moreover, highest reduction in biofilm of *E. coli* OD (0.15) was registered, drawn within (Figure 8). Also, Biofilm produced by *C. albicans* strain has been eradicated in same manner when recorded an average of biofilm OD value (0.12) was reduced from the average of the isolate control (0.49) according to (Figure 9). In same manner, (32) proved by their study the ability of bacteriocin to eradicate biofilm formed by *S. aureus*. Moreover, (33) mentioned antibiofilm efficiency of bacteriocin over *E. coli* isolates.

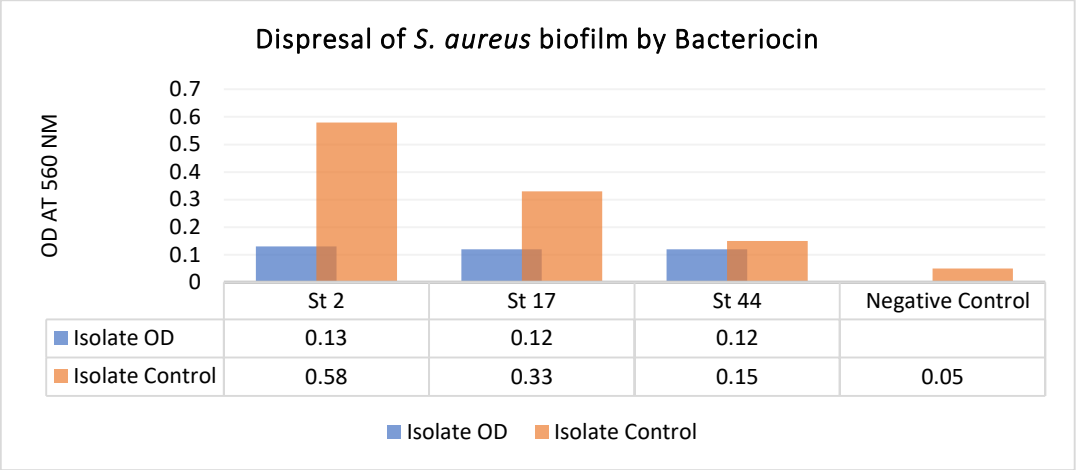

Figure (7): The antibiofilm effect of *Lacticaseibacillus paracasei* Bacteriocin on *Staphylococcus aureus* isolates biofilm using microtiter plate assay.

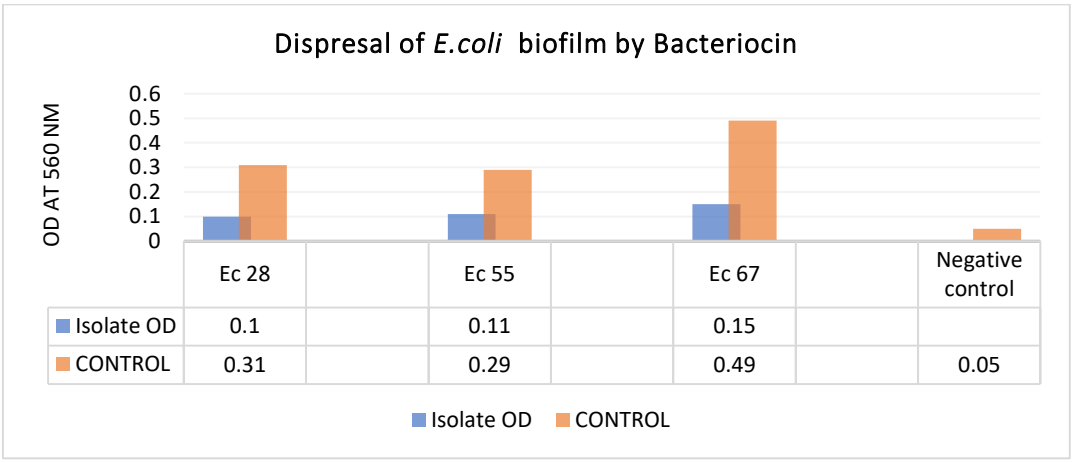

Figure (8): The antibiofilm effect of *Lacticaseibacillus paracasei* Bacteriocin on *Escherichia coli* isolates biofilm using microtiter plate assay.

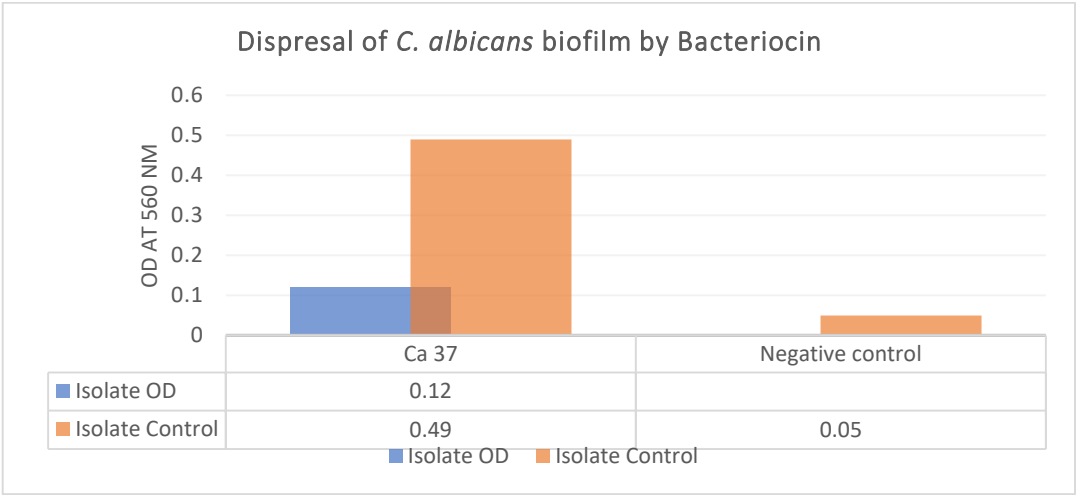

Figure (9): The antibiofilm effect of *Lacticaseibacillus paracasei* Bacteriocin on *Candida albicans* biofilm using microtiter plate assay

Microbial viability after biofilms dispersal

The results for the viability of the three pathogenic indicators *S. aureus*, *E. coli as* well as *C. albicans* after the treatment with antibiofilm compound (Bacteriocin) showed that all microorganisms isolates were viable depending on colonies morphology (shape, size, color and texture) when grown separately, each on its selective medium as pointed within (Figure 10). In same pattern, (34) obtained viable cells of *Pseudomonas aeruginosa* after antibiofilm application of curcumin.

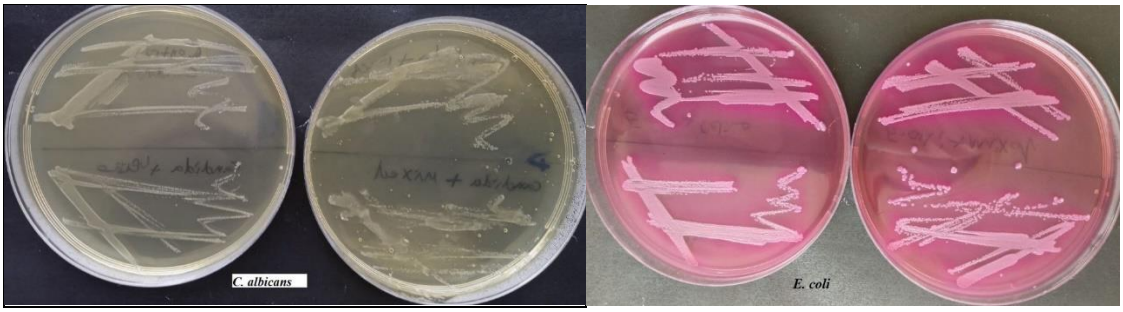

Figure (10): Pathogenic isolates of *C. albicans* and *E. coli* shows viability after treatment with Bacteriocin

Conclusion

Considering the results of the current work, the outcomes were showed that a significant efficiency of Bacteriocin as antimicrobial and antibiofilm metabolites against the Gram negative, Gram positive and yeast microorganisms as well, it might consider a promising new aera that could be used within pharmaceutical purposes in instead of common more resisted drugs.

References

1. Ariyoshi, T.; Hagihara, M.; Takahashi, M. and Mikamo, H. (2022). Effect of *Clostridium butyricum* on gastrointestinal infections. Biomedicines, 10(2): 483.
2. Ali, F. (2018). Distribution of CTX-M gene among *Escherichia coli* strains isolated from different clinical samples in Erbil City. Iraqi Journal of Biotechnology, 17(1): 78-90.
3. Cui, Y.; and Qu, X. (2021). Genetic mechanisms of prebiotic carbohydrate metabolism in lactic acid bacteria: Emphasis on *Lacticaseibacillus casei* and *Lacticaseibacillus paracasei* as flexible, diverse and outstanding prebiotic carbohydrate starters. Trends in Food Science and Technology, 115 (1): 486-499.
4. M'hamed, A.; Ncib, K.; Merghni, A.; Migaou, M.; Lazreg, H.; Snoussi, M., *et al*. (2023). Characterization of probiotic properties of *Lacticaseibacillus paracasei* L2 isolated from a traditional fermented food "Lben". Life, 13(1): 21.-38.
5. Abd, F. and Luti, K. (2016). Improvement of Bacteriocin Production by *Bacillus subtilis* NK16via Elicitation with Prokaryotic and Eukaryotic Microbial Cells. Iraqi journal of biotechnology, 15(2): 59-73.
6. Pérez-Ramos, A.; Madi-Moussa, D.; Coucheney, F. and Drider, D. (2021). Current knowledge of the mode of action and immunity mechanisms of LAB-bacteriocins. Microorganisms, 9(10): 2107.
7. Pérez, V.; da Costa, G.; Guimarães, A.; Heinemann, M.; Lage, A. and Dorneles, E. (2020). Relationship between virulence factors and antimicrobial resistance in *Staphylococcus aureus* from bovine mastitis. Journal of global antimicrobial resistance, 22 (1): 792-802.
8. Ahmed, Z. and Al-Daraghi, W. (2022). Molecular Detection of medA Virulence Gene in *Staphylococcus aureus* Isolated from Iraqi Patients. Iraqi Journal of Biotechnology, 21(1): 8-18.

9. Abbas, A., and Hamim, S. (2019). Serological and Molecular detection of *Staphylococcus aureus* isolated from UTI patients. University of Thi-Qar Journal, 14(3): 64-79.
10. AL-Jubouri, S. and Shami, A. (2022). Molecular Detection of Cephalosporin Resistance Genes in *Escherichia coli* Isolated from Urinary Tract Infections in Baghdad Hospitals. Iraqi Journal of Biotechnology, 21(2):145-152.
11. Zarrin, M., Ganj, F. and Faramarzi, S. (2016). Analysis of the rDNA internal transcribed spacer region of the *Fusarium* species by polymerase chain reaction-restriction fragment length polymorphism. Biomedical Reports, 4(4): 471-474.
12. Angaali, N.; Vemu, L.; Padmasri, C.; Mamidi N. and Teja, V. (2018). Direct identification and susceptibility testing of Gram-negative bacilli from turbid urine samples using VITEK2. Journal of laboratory physicians, 10(03): 299-303.
13. AL-Kadmy, M.; Musafer, K. and Sajid, M. (2015). Detection of 16S ribosomal RNA methylation in extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* clinical isolates from Baghdad hospitals. Iraqi Journal of Biotechnology, 14(1): 53- 62.
14. Srinivasan, R.; Karaoz, U.; Volegova, M.; MacKichan, J.; Kato-Maeda, M.; Miller, S., *et al*. (2015). Use of 16S rRNA gene for identification of a broad range of clinically relevant bacterial pathogens. PloS one, 10(2):117.
15. Tareq, I. and Luti, K. (2022). An application of Bacteriocin-Producing Vaginal *Lactobacillus Crispatus* IS30 in A Gel Formula Against Some Vaginal Pathogens. Iraqi Journal of Science, 63 (2): 491-507.
16. Ahmed, M.; Mousa, I.; Al-Halbosiy, M. and Jabar, E. (2018). The anti-Leishmaniasis activity of Purified Bacteriocin Staphylococcin and Pyocin Isolated from *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Iraqi Journal of Science, 28: 645-653.
17. Majda, B.; Barchan, A.; Aarab, A.; Bakkali, M.; Arakrak, A. and Laglaoui, A. (2022). Evaluation of the antibacterial activity of essential oils against *E. coli* isolated from rabbits. Iraqi Journal of Agricultural Sciences, 53(4): 802-809.
18. Pan, X.; Chen, F.; Wu, T.; Tang, H. and Zhao, Z. (2009). The acid, bile tolerance and antimicrobial property of *Lactobacillus acidophilus* NIT. Food Control, 20(6): 598-602.
19. Teh, C.; Nazni, W.; Nurulhusna, A.; Norazah, A. and Lee, H. (2017). Determination of antibacterial activity and minimum inhibitory concentration of larval extract of fly via resazurin-based turbidometric assay. BMC Microbiology, 17(1): 1-8.
20. Kırmusaoğlu, S. (2019). The methods for detection of biofilm and screening antibiofilm activity of agents. Antimicrobials, antibiotic resistance, antibiofilm strategies and activity methods. Intechopen, 7: 99-113.
21. Kumar, S. and Ghosh, A. (2019). Assessment of bacterial viability: a comprehensive review on recent advances and challenges. Microbiology, 165(6): 593-610.
22. Kranjec, C.; Ovchinnikov, K.; Grønseth, T.; Ebineshan, K.; Srikantam, A. and Diep, D. (2020). A bacteriocin-based antimicrobial formulation to effectively disrupt the cell viability of methicillin-resistant *Staphylococcus aureus* (MRSA) biofilms. npj Biofilms and Microbiomes, 6(1): 1-13.
23. Rodenes, A.; Casinos, B.; Alvarez, B.; Martinez-Blanch, J.; Vijayakumar, V.; Superti, G., *et al*. (2022). Reclassification of probiotic *Lactobacillus acidophilus* NCIMB 30184 as *Lactobacillus helveticus* and *Lactobacillus casei* NCIMB 30185 as *Lacticaseibacillus paracasei*. *bioRxiv*, 2022-10.
24. Al-Asadi, H. and Luti, K. (2023). Antibacterial activity of *Lactobacillus plantarum* bacteriocin as a dermal probiotic against *Pseudomonas aeruginosa* isolated from diabetic foot ulcer. Journal of Madenat Alelem University College, 15(1): 38-46.
25. Yaaqoob L. (2022). Effectivity of Iron Oxide Nanoparticles Synthesis by Intracellular *Lactobacillus* as Antibacterial Agent against Pseudomonas aeruginosa. Iraqi Journal of Biotechnology, 21(2); 428-438.
26. Malkoc, A. and Wong, D. (2021). Methicillin-Resistant *Staphylococcus aureus* -Associated Diarrhea in a Critically Ill Burn Patient. Journal of Medical Cases, 12(7): 257.

27. Hassan, H. and Hanna, S. (2019). Extended-spectrum beta-lactamases (ESBLs) detection in some uropathogenic bacteria and their correlation with biofilm formation. Zanco J. Med. Sci., 23(3): 375-382.
28. Călinoiu, L. and Vodnar, D. (2019). Thermal processing for the release of phenolic compounds from wheat and oat bran. Biomolecules, 10(1): 21.
29. Naji, S. and Awadh, H. (2022). A Study of Urinary Tract Infections Prevalence, Antibiotics Resistance, and Biofilm Formation Capability of the Bacterial Causal Agents. Tikrit Journal of Pure Science, 27(6): 11-17.
30. Hassan, H. and Hanna, S. (2019). Extended-spectrum beta-lactamases (ESBLs) detection in some uropathogenic bacteria and their correlation with biofilm formation. Zanco J. Med. Sci., 23)3), 375-382.
31. Kranjec, C.; Ovchinnikov, K.; Grønseth, T.; Ebineshan, K.; Srikantam, A. and Diep, D. (2020). A bacteriocin-based antimicrobial formulation to effectively disrupt the cell viability of methicillin-resistant *Staphylococcus aureus* (MRSA) biofilms. npj Biofilms and Microbiomes, 6(1): 58.
32. Sharma, G.; Dang, S.; Kalia, M. and Gabrani, R. (2020). Synergistic antibacterial and anti-biofilm activity of nisin like bacteriocin with curcumin and cinnamaldehyde against ESBL and MBL producing clinical strains. Biofouling, 36(6): 710-724.
33. Salman, A. and Khadam, Z. (2022). The Influence of Bacteriocin on The Soft Liner Tensile Strength And Its Inhibitory Effect On *Candida Albicans*. Mustansiria Dental Journal,18(1):30- 40.
34. Abdulrahman, H.; Misba, L.; Ahmad, S.; and Khan, A. (2020). Curcumin induced photodynamic therapy mediated suppression of quorum sensing pathway of *Pseudomonas aeruginosa:* An approach to inhibit biofilm in vitro. Photodiagnosis and Photodynamic Therapy, 30: 101645.