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The antibiofilm activity of bacteriocin produced by *Proteus mirabilis* against some bacterial species

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ABSTRACT

Bacteriocins are antibacterial proteins produced by bacteria and it's active against other strains bacteria which are closely related to the producing strains and some species from the same family. The aim of this research was to investigate the antibacterial activity of bacteriocin from clinical *Proteus mirabilis* isolates from Baghdad /Iraq. The antibacterial activity of bacteriocins from *P. mirabilis* isolates against different pathogenic species of Gram-negative bacteria (by well assay method) was investigated, however antibiofilm activity determined also by tissue culture plate method against pathogenic species before and after biofilm formation. The results showed that bacteriocin was effective against pathogenic species in different concentrations by producing different inhibition zones. Results also revealed that bacteriocin had a broad antibiofilm activity on many pathogenic species of Gram-negative bacteria. In addition to these results it was observed that the effect of bacteriocin against preformed biofilm of *Klebsiella* was higher than the effect of it against other bacterial species while it was affected on mature biofilm of other bacterial species in addition to *Klebsiella*.

Keywords: Bacteriocins, antibiofilm activity, *Proteus mirabilis, Escherichia coli, Klebsiella pneumoniae*.

1. INTRODUCTION

Proteus mirabilis is more commonly associated with urinary tract infections (UTIs). It is the third most common cause after *Escherichia coli* and *Klebsiella pneumoniae* of complicated UTI and the second most common cause after *Providencia stuartii* of catheter-associated bacteria in the group of long-term catheterized patients [1, 2]. Besides UTI, *P. mirabilis* have been described as opportunistic etiological agents in infections of the respiratory tract, wounds, burns, skin, eyes, ears, nose, throat, and gastroenteritis resulting from the consumption of contaminated meat or other food [3]. *P. mirabilis*, once attached to urinary tract it infects the kidney more commonly than *E. coli* and urinary tract infections. It is best known for its ability to form stones in the bladder and kidney, as well as its ability to form crystalline biofilms on the outer surface and in the lumen of indwelling urinary catheters [4].

Biofilm is a microbial derived sessile community characterized by cells that are attached to an abiotic or living surface and embedded in a matrix of extracellular polymeric substances that they have produced. This polymicrobic community has an altered phenotype and it is physiologically different from planktonic microorganisms [5]. It has been observed that the resistance of biofilms to antibiotics is increased compared with what is normally seen with planktonic cells. In fact, when cells exist in a biofilm, they can become 10–1000 times more resistant to the effects of antimicrobial agents [6].

Bacteriocins are a kind of ribosomal synthesized antimicrobial peptides produced by bacteria, which can kill or inhibit bacterial strains closely-related or non-related to produced bacteria, but will not harm the bacteria themselves by specific immunity proteins [7]. Bacteriocins differ from traditional antibiotics in one critical way: They have a relatively narrow killing

spectrum and are only toxic to bacteria closely related to the producing strain. These toxins have been found in all major lineages of Bacteria and, more recently, have been described as universally produced by some members of the Archaea [8]. Accordingly to Klaenhammer [9], nearly 99% of all existing bacteria at least make one bacteriocin and, indeed, a plethora of new bacteriocin classes and families has been identified by genome sequencing that led to an enormous progress in the field of ribosomal synthesized and post-translationally modified compounds. Interestingly, these novel bacteriocins are often widely distributed over all bacterial lineages [10]. The objective of the present study was to investigate the potential of *Proteus mirabilis* to produce bacteriocin, bacteriocin extraction in crude form and study the effect of bacteriocin crude extract against pre-formed and mature biofilm of *Proteus* and some pathogenic species.

2. MATERIALS AND METHODS

2.1 Isolation and identification

All samples were streaked on Blood agar, MacConkey agar and Xylose lysine deoxychoclate agar (XLD agar) plates, which is a selective and differentiation medium used with the other mentioned media to approach the identification of *Enterobacteriaceae*. The plates were incubated aerobically at 37° C for 24 hours.

The isolates were identified depending on the microscopical feature by using Gram stain to detect their response to stain, shape and arrangement [11]. In addition, the morphological features on culture media such as Swarming on blood agar, Non lactose fermented growth on MacConkey agar and colorless growths on (XLD) agar were examined [12].

2.2 Detection Bacteriocin producing isolates

The *Proteus mirabilis* indicator isolates used in this study was taken from different urine samples and each one of these isolates were tested against each other. Well diffusion method was used to investigate the ability of these bacteria for bacteriocin production, overnight cultures of tested bacteria were added in wells made on Muller hinton agar that cultured previously with indicator bacteria so the results appeared as clear zones after 24 hrs from incubation.

2.3 Extraction of Bacteriocin from producing isolates

Bacteriocin crude extract was achieved according to [13].

The overnight culture of bacterial isolates in volume 2.5 ml of LB broth was used to inoculate100 ml of sterile LB broth supplemented with 5 % glycerol and in shaker incubater.At cell density of about $3x10^8$ (14hr incubation of late log phase), Mitomycin C was added at concentration of 2 µg / ml,incubation continued with shaking for another 3 hr.The culture was centrifuged at 5000Xg for 30 min in cooling centrifuge.The supernatant was taken for assay of bacteriocin activity and protein determination. For estimation of protein concentration in colicin crude extract,the same steps of Bradford method [14] were followed and the protein concentration was calculated depending on BSA standard curve.

2.4 Detection the activity of klebocin crude extract against different isolates

Different dilutions were prepared from bacteriocin crude extract and tested against *P.mirabilis, K.pneumoniae and E.coli,* well diffusion method was used to detect the activity of these extracts.

2.5 Biofilm assay

Method described by [15] was followed to achieve biofilm formation:

To study the ability of adherence, *K.pneumoniae, P.mirabilis* and *E.coli* isolates, were grown in Tripticase soya broth containing 1% glucose in 96-well polystyrene tissue culture plates and incubated at 37◦C for 24 h under aerobic conditions. After incubation, the planktonic cells were washed three times with deionized water, and the adhering bacterial cells in each well were fixed with 200 µl of absolute methanol for 20 min. The plates were emptied and left to dry overnight. The adhering cells were stained with 200 µl of 0.1% crystal violet for 15min, and excess stain was rinsed off. The plates were washed with distilled water and air-dried overnight. The crystal violate dye bound to the adherent cells was dissolved with 200 µl of 96% ethanol per well, and the plates were read at 490 nm using a spectrophotometer. The experiment was performed in triplicates, and the absorbance of wells containing sterile TSB was used as the negative control the result calculated as in table below.

Table 1: Classification of bacterial adherence by tissue culture plate method (18)

OD values	Adherence	Biofilm formation
< OD c	Non	Non
OD < OD ≤ 2*ODc	Weakly	Weak
2*ODc < ODt≤ 2*ODc	Moderately	Moderate
4ODc<OD t	Strong	High

2.6 Detection antibiofilm activity of bacteriocin crude extracts on pre-formed biofilm

Method followed according to [16] for the inhibition of biofilm, the isolates of *K. pneumoniae, P.mirabilis* and *E.coli,* were selected to be assayed according to inhibition activity of bacteriocin against planktonic cells of it on plate agar. Same protocol described earlier was followed to produce a biofilm but 100 µl of bacteriocin extract was added to each well. The plate was incubated for 24hr at 37 ºC. After incubation

period all wells were washed and stained, then absorbance was determined at 490 nm in an ELISA reader. Controls were performed with crystal violet binding to the wells exposed only to the culture medium with bacteria.

2.7 Detection antibiofilm activityof bacteriocin crude extract on mature biofilm

Methods mentioned earlier was also followed, then befor the staining step,the crude extract of bacteriocin was added with volume 200 µl in each well,incubated for another 24 hr at 37 ºC, after that all wells were washed, stained,and read O.D at 490 nm (17).

2.8 Statistical Analysis

The Statistical Analysis System- SAS [18] program was used to effect of difference factors in study parameters. Least significant difference –LSD test was used to significant compare between means in this study

3. RESULTS AND DISCUSSION

3.1 Isolation and identification

Proteus species, (Gram negative) are well known for causing urinary tract and nosocomial infections. According to Betty *et al*. [12], three differential media were used to isolate and identify *Proteus spp.* from other species of *Enterobacteriaceae* as a primary identification based on the most common characters, which are:

- Swarming phenomenon with no hemolytic activity on blood agar media.
- Non- lactose fermenter isolates that appeared as pale, convex, circular and smooth colonies, with special fish-like odour on MacConkey agar, Colourless colonies of isolates on XLD agar media.

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3.2 Detection Bacteriocin producing isolates

The bacterial isolates were screened for their bacteriocin mediated bioactivity by well diffusion method. The results showed that highly producing isolates were (P1 and P8), so these isolates were chosen for bacteriocin extraction and testing against different pathogenic isolates in both planktonic and biofilm states (Table 2). Interestingly, a strong bioactivity was manifested against gram negative bacteria including *K.pneumoniae* and *P.mirabilis* by *proteus* bacteriocins. These findings are well supported with the findings of Kusek & Herman [19], who demonstrated significant bacteriocin production by clinical *Proteus* isolates.

Table 2: The diameters of inhibition zones of bacteriocin produced by *P. mirabilis*

	Producing isolates							
Sensitive isolates	**P1**	**P8**	**P9**	**P15**	**P16**	**P17**	**P18**	**P20**
P1	–	–	–	–	–	–	–	–
P8	–	–	–	–	–	–	–	–
P9	–	30mm	–	–	–	–	–	–
P15	–	–	–	–	–	–	–	–
P16	–	–	–	–	–	–	–	–
P17	–	–	–	–	–	–	–	–
P18	–	–	–	–	–	–	–	–
P20	40mm	15mm	–	–	–	–	–	–

3.3 Extraction of bacteriocin from producing isolates

The bacteriocin was extracted from 2 bacterial isolates (P1 and P8) by using inductive material called Mitomicin C, Numerous studies carried out for induction of different bacteriocins , have demonstrated that increased synthesis of bacteriocin occurs in response to a wide range of DNA damaging physical agents such as ultraviolet (UV) radiation [20]; antibacterial agents, such as Mitomycin C (MMC) [13]; The phenotypic assay for mitomycin C-inducible bacteriocin production detects virtually all strains bearing a colicin gene [21,22]. In aprevious studies by cavard [23], and Debroy [24], addition of 300 ng/ml of mitomycin found to increase colicin production. Glycerol was used as an enhancing agent where Asenio, [25] and Pugsely, [26] observed that the production of these antagonists was best by using minimal glycerol media.

The results of bacteriocin concentrations were detected by Bradford assay. The concentration of bacteriocin crude extract was 59.925 and 90.796 µg/ml (Table 3). The crude extract of bacteriocin isolated from producer local isolate showed awide activity spectrum on other gram negative bacteria such as *E.coli* and *P.mirabilis* in different concentrations. These results were in agreement with previous results of Khalaf and Flayyih, [27] who reported that bacteriocin extracted from uropathogenic *E.coli* was effective against other bacterial species such as *Klebsiella, Proteus* and *Salmonella*.

Table 3: The concentration of bacteriocin crude extracts that estimated by Bradford method

Bacteriocinogenic isolates	Concentration µg/ml
P1	59.925
8 **P**	90.796

Table 4: Effect of bacteriocin crude extract against different isolates

	Indicator isolates				
Producing isolates	***Proteus 9***	***Proteus 20***	***E.coli 20***	***Klebsiella 10***	***Klebsiella 14***
Proteus 1	no	40mm	no	20mm	10mm
Proteus 8	30mm	15mm	no	15mm	20mm

3.4 Detection of the bacteriocin activity against other pathogenic species

The isolated organisms were screened for their bioactivity against other bacteria. The results revealed that bacteriocin crude extract was effective against different bacterial isolates by producing clear zones around indicator isolates (figure 1). The isolates that gave largely inhibition zones was choosed for using its extract against biofilm of *Proteus* and other bacterial species (Table 4).

From the table above it can noticed that bacteriocin crude extract of *Proteus* was affected against *Proteus* and *Klebsiella* but there was no effect against *E.coli.* This finding is supported by previous studies. There were little studies about bacteriocin of *Proteus* and effect of it against different bacterial species. Naz and Rasool [28], in your research reported that *Lactobacilli, Pseudomonas, Proteus* and *Staphylococci* that isolated from different indigenous ecological nichs displayed good potential of bacteriocin production and this finding was inagreement with present study.

Figure 1: The antibacterial effect of bacteriocin crude extract against *P.mirabilis*

3.5 Detection antibiofilm activity of bacteriocin crude extracts on pre-formed biofilm

Bacteriocin of *Proteus* has asignificant effect (P<0.05) against Klebsiella 10 biofilm in all its concentrations but against *Klebsiella* (14 in all concentrations except 1/2 that gave no significant effect (P>0.05). The results showed that bacteriocin has no effect against *Proteus* (9) and *Proteus* (20) in all its concentrations and no significant effect (P>0.05) against *E.coli* biofilm (Table 5 and Figure 2). From all of these results it can concluded that bacteriocin of *Proteus* gave significant effect against *Klebsiella* biofilm but there was no significant effect against *Proteus* and *E.coli* biofilm.

Table 5. Effect of bacteriocin extract from *P. mirabilis* against bacterial biofilm in first stage of formation

	Treatments/ Concentration µg/ml					LSD value
Bacterial spp.	**Control**	**45.39**	**22.69**	**11.34**	**5.67**	
***Klebsiella* 10**	0.165 ±0.008	0.084 ±0.003	0.076 ±0.002	0.069 ±0.002	0.074 ±0.002	0.071 *
Klebsiella 14	0.259 ±0.013	0.297 ±0.008	0.197 ±0.004	0.153 ±0.003	0.148 ±0.004	0.096 *
***Proteus* 9**	0.158 ±0.008	0.094 ±0.003	0.134 ±0.004	0.126 ±0.003	0.152 ±0.003	0.078 NS
Proteus 20	0.148 ±0.006	0.098 ±0.003	0.092 ±0.002	0.092 ±0.002	0.103 ±0.005	0.068 NS
E. coli 20	0.113 ±0.004	0.085 ±0.003	0.091 ±0.003	0.072 ±0.004	0.076 ±0.003	0.070 NS
LSD value	0.062 *	0.074 *	0.088 *	0.069 *	0.062 *	---

* (P<0.05), NS: Non-significant.

In other experiment, the effect of bacteriocin against mature biofilm was studied . The results showed that bacteriocin gave significant effect (P<0.05) against *Klebsiella* 14 biofilm in concentration (**90.796** and**5.67**) **µg/ml**, but other concentrations gave no significant effect (P>0.05), in the other hand the mature biofilm of *Proteus* (9) was inhibited by bacteriocin extract in the following concentrations (**90.796**, **45.39** and **5.67**) **µg/ml**.

The effect of bacteriocin against *Proteus* (20) was also observed in all its concentrations but the best effect by concentration (**90.796** and **45.39**) **µg/ml**. The highly effect was observed against *E.coli* mature biofilm in all bacteriocin concentration but the best effect in (**90.796** and **45.39**) **µg/ml**, so the results of current study showed the ability of bacteriocin produced by *P. mirabilis* for biofilm inhibition but the best effect achieved against mature biofilm in compare with biofilm in first stages of formation (Table 6 and Figure 3).

Table 6: Effect of bacteriocin extract from *P. mirabilis* against bacterial mature biofilm

Bacterial spp.	Treatments/ Concentration µg/ml						LSD value
	Control	90.796	45.39	22.69	11.43	5.67	
***Klebsiella* 10**	0.165 ±0.008	0.136 ±0.006	0.102 ±0.002	0.074 ±0.001	0.096 ±0.003	0.106 ±0.002	0.061 *
Klebsiella 14	0.387 ±0.017	0.186 ±0.004	0.362 ±0.007	0.568 ±0.015	0.314 ±0.005	0.147 ±0.005	0.113 *
***Proteus* 9**	0.311 ±0.009	0.107 ±0.004	0.123 ±0.003	0.316 ±0.008	0.223 ±0.005	0.177 ±0.003	0.095 *
Proteus 20	0.227 ±0.008	0.096 ±0.003	0.094 ±0.003	0.123 ±0.004	0.128 ±0.003	0.143 ±0.002	0.091 *
E. coli 20	0.672 ±0.037	0.097 ±0.003	0.096 ±0.002	0.307 ±0.009	0.158 ±0.005	0.185 ±0.004	0.106 *
LSD value	0.209 *	0.076 *	0.106 *	0.117 *	0.109 *	0.092 NS	---

* (P<0.05), NS: Non-significant.

The results of the present study showed the ability of bacteriocin produced by *P.mirabilis* for biofilm inhibition for different bacterial isolates.

There are little studies about the effect of bacteriocin of *Proteus* on biofilm of different bacterial species, but many studies reported the effect of colicin (bacteriocin of *E.coli*) against biofilm. Khalaf and Flayyih [27] reported in their study that crude colicin extract from 6 isolates was affected against all isolates biofilm as treatment, these results showed that colicin (bacteriocin of *E.coli*), was active against mature biofilm for different pathogenic isolates.Furthermore, Lyon *et al.,* [29] reported in their patent that the colicin (bacteriocin of *E.coli*) was had the ability of inhibiting the growth of one or more species and/or strains of pathogenic Enterobacteriaceae, as for example, *Shigella* spp. such as *Shigella dysenteriae, Shigella flexneri, Shigella boydii, Shigella sonnei*, and the like; *Salmonella* spp. such as *S. typhi, S. typhimurium, S.paratyphi* A, *S. choleraesuis*, and the like; *Escherichia* spp. such as *E.coli* strain 0157:H7, *E. afreundii*, and the like; and *Enterococcus* spp.this report was in an agreement with present study. colicins H and G were shown to exhibit inhibitory activity against *Salmonella* strains isolated from clinical cases [30]. Other studied showed the ability of different species of *Lactobacillus* for biofilm inhibition, one of these studies reported by Buommino *et al.* [31], suggest the ability of the molecules released by *L. plantarum* to act both on preformed biofilm of *P.mirabilis* and *Candida albicans.*

A small number of studies suggest that colicinlike bacteriocins may be useful in the treatment and/or prevention of biofilm-mediated bacterial infections. For example, Trautner *et al.* [32] have shown that pre-growth of colicin-producing *E. coli* K-12 on catheters is able to prevent colonization by a colicin-susceptible *E. coli* clinical isolate, indicating that colicin production may act as a potent inhibitor of *E. coli* biofilm formation. Furthermore, colicinproducing *E. coli* strains have been shown to persist for longer periods in the murine gastrointestinal tract which is likely to involve inhibition of other enteric bacterial biofilms [33].

In addition, Saeidi *et al.* [34] have also suggested that pyocins could be utilized as anti-biofilm therapies. So using klebocin extract as effective antibacterial agent gave a candidate for using it as treatment for infections that caused by this pathogen and other multi-resistance organisms.

Although antibiotic therapy generally reverses the symptoms caused by planktonic cells, microrganisms within a biofilm can display up to 1000-fold increased resistance to antibiotic or biocide treatment [7]. For this reason biofilm infections typically show recurring symptoms, even after cycles of antibiotic therapy. In this context, identification of natural compounds which can limit formation of bacterial biofilms represent an important task for the researchers working in this field.

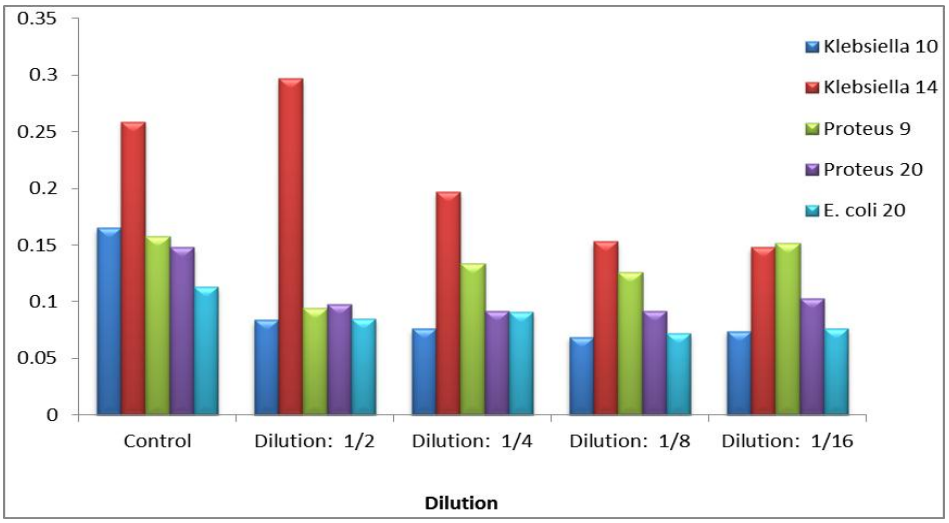

Figure 2: Effect of Bacteriocin extract from *P.mirabilis* against bacterial biofilm in first stage of formation.

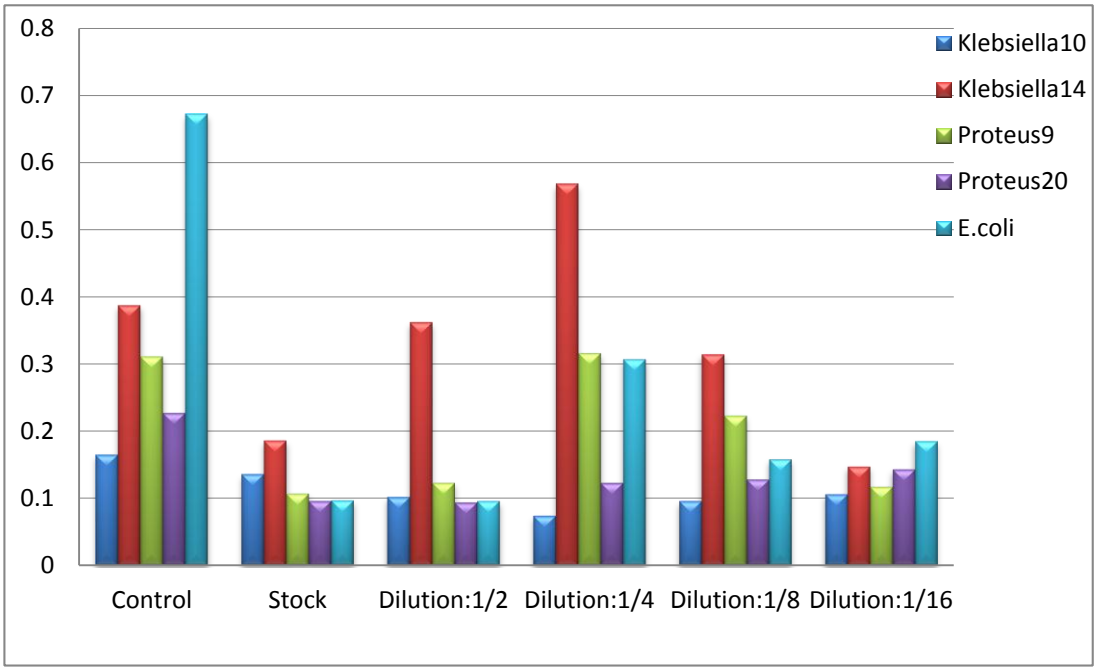

Figure 3: Effect of Bacteriocin extract from *P.mirabilis* against bacterial biofilm mature biofilm.

4. CONCLUSION

The results of current study reported that bacteriocin crude extract of *Proteus mirabilis* had antibiofilm effects against different bacterial species, furthermore the bacteriocin was highly effective against mature biofilm incampare with pre-mature biofilm. The finding of this paper showed that the effects of bacteriocin against *Klebsiella* higher than the effects of it against other bacterial species in premature and mature biofilm.

5. REFERENCES

1. Wassif C, Cheek D, and Belas. A. (1995). Molecular Analysis of a Metalloprotease from *Proteus mirabilis*. *J. of Bacteriology*, 177(20): 5790–5798.
2. Bradford, P. A. (2001). Extended spectrum β-lactamases in the 21st century: characterization, epidemiology, and detection of this important threat. Clinical microbiology reviews. American society for microbiology. Oct. P: 933-951. 3-Jacobsen S.M. and M. E. Shirtliff. (2011). *Proteus mirabilis* biofilms and catheter-associated urinary tract infections. Landes Bioscience, 2(5):1-6.
3. Jacobsen S.M. and M. E. Shirtliff. (2011). Proteus mirabilis biofilms and catheter-associated urinary tract infections. Landes Bioscience, 2(5):1-6
4. Pearson, M. M.; M. Sebaihia; C. Churcher; M. A. Quail; A. S. Seshasayee; N. M. Luscombe; Z. Abdellah; C. Arrosmith; B. Atkin;T. Chillingworth; H. Hauser; K. Jagels; S. Moule; K. Mungall, H. Norbertczak; E. Rabbinowitsch; D. Walker; S. Whithea; N. R. Thomson; P. N. Rather; J. Parkhill and H. L. T. Mobley. (2008). Complete Genome Sequence of Uropathogenic *Proteus mirabilis*, a Master of both Adherence and Motility. Journal of bacteriology, (190)11:4027–4037.
5. Vranes, J. and Maric, S. (2007). Characteristics and significance of microbial biofilm formation. Period. Biol. 109:2.
6. Mah, T. C. and O Tool, G. A. (2001).Mechanisms of biofilm resistance to antimicrobial agents.Trends. Microbiol. 9(1): 34-9.
7. Yang,Shih-chun;Lin,Chin-hung;Sung, C.T. and Fang, Jia-you.(2014) Antibacterial activities of bacteriocins:application in foods and pharmaceuticals.Front Microbiol. 5:241.
8. Riley, M.A. and Wertz, J.E. (2002). BACTERIOCINS: Evolution, Ecology, and Application (2002). Annual Reviews in Microbiology. 56:117-137.
9. Klaenhammer, T. R. (1988): Bacteriocins of lactic acid bacteria. In: Biochimie 70 (3), pages 337–349
10. Schmidt, E. W. (2010): The hidden diversity of ribosomal peptide natural products. In: BMC Biol 8, 83.
11. Cappuccino JG, and Sherman N. (2008). Microbiology a laboratory manual, 8th ed. Pearson education, Inc. London.
12. Betty, A. F; Sahm DF, and Weissfeld A S. (2007). Bailey and Scott`s Diagnostic microbiology. 12th ed. Mosby, Inc., an affilinate of Elsevier Inc.
13. Herschman,H.R. and Helinski, D.R.(1967).Purification and characterization of colicin E2 and colicin E3. The J. biological Chemistry. 212(22):5360-5368.
14. Bradford,M.M.(1976).Adye binding assay for protein .Anal.Biochem.72:248_254.
15. Atshan, S.S.; Shamsudin, M.N.; Sekawi,Z.; Lung, L.T.T.; Hamat, R.A.; Karunanidhi, A.; Mateg, Ali, A.; Ghaznavi-Rad, E.;Ghasemzadeh-Moghaddam, H.; Chong Seng, J.S.;Nathan, J.J.Pei,C. (2012). Prevalence of Adhesion and Regulation of Biofilm-Related Genes in Different Clones of *Staphylococcus aureus*. *J. Biomed. Biotech*. 3: 10-17.
16. Harry,C.O. and Walker,D.(2013). Cytotoxic activity of colicin E1, E3 and E9 against *E.coli* BW25113 in the planktonic and biofilm states.Int.J.Curr.Res.Aca.Rev.1 (2):55-71.
17. Merritt, J.H.; Kadouri, D.E. and O'Toole, G.A. (2005). Growing and analyzing static biofilms. Curr. Protocols Microbiol. 1B.1.1–1B.1.17.
18. SAS. 2012. Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.
19. Kusek, J.W. and L.G. Herman. 1980. Typing of Proteus mirabilis by bacteriocin production and sensitivity as a possible epidemiological marker. J. Clin. Microbiol., 12: 112-120.
20. Ozeki, H.; Stocker, B.A.; De Margerie, H.(1959). Production of colicine by single bacteria. Nature.184:337–9.Mol.Microbiol. 30: 295–304.
21. Gordon, D. M.; Riley, M. A. and Pinou, T. (1998). Temporal changes in the frequency of colicinogeny in *E. coli* from house mice. Microbiology. 144: 2233–2240.
22. Gordon, D.M. and O'Brien, C.L. (2006). Bacteriocin diversity and the frequency of multiple bacteriocin production in

Escherichia coli. Microbiology (Reading, Engl). 152(11):3239–3244.
23. Cavard, D. (2002). Assembly of colicin A in the outer membrane of producing *Escherichia coli* cells requires both phospholipase A and one porin, but phospholipase A is sufficient for secretion. J.Bacteriol. 184(13):3723-33.
24. DebRoy, C. and Jayarao, B.(2003). Competitive Exclusion of *E. coli* O157:H7 using non-pathogenic bacteriocins producing *Escherichia coli* strains. The Pennsylvania State University, University Park, PA 16802
25. Asensio, C. and Perez-Diaz. (1976).Anew family of low molecular weight antibiotics from enterobacteriaceae. Biochem.Biophs.Research. communication. 69 (1):7-14.
26. Pugsely,A.P.(1985).*Escherichia coli* K12 strains for use in the identification and characterization of colicins .J.Gen.Microbiol. 131:369-376.
27. Khalaf, Z.Z. and Flayyih, M. T. (2015).Inhibitory activity of colicin crude extract against different isolates of enterobacteriaceae in planktonic and biofilm state. Iraqi, J. Biotechnol.14(2): 414- 428.
28. Naz, S. A. and Rasool, S. A.(2013). Isolation, production and characterization of bacteriocins produced by strains from indigenous environments. Pak. J. Bot., 45(1): 261-267.
29. Lyon, W. L. and Murano, E. A. (1996). Method and colicin composition for inhibiting *Escherichia coli* 0157:H7 in food products.USA.Pat.US5559895 A.Aug-27.
30. AL- Charrakh, A.H.; Yousif, S.Y. and AL-Janabi, H. S. (2011).Antimicrobial spectrum of the action of bacteriocins from Klebsiella isolated from Hilla- Iraq. Iran.J.Microbiol..2(5):1-11.
31. Buommino, E.; Fusco, A.; Rizzo,A.; Alfano, A.; Schiraldi,C and Donnarumma,G.(2015). *L. Plantarum* Supernatant Affects *P. Mirabilis* and *C. Albicans* Biofilm Formation.Intern.J.Med.Science.Clinic.Invent.2(11):1436-1442.
32. Trautner, B.W.; Hull, R.A. and Darouiche, R.O. (2005). Colicins prevent colonization of urinary catheters. J. Antimicrob. Chemother. 56:413–415.
33. Lagos, R.; Villanueva, J. and Monasterio, O. (1999). Identification and properties of the genes encoding microcin E492 and its immunity protein. *J. Bacteriol*., 181(1):212-217.
34. Saeidi, N.; Wong, C.K.; Lo, T.-M.; Nguyen, H.X.; Ling, H.; Leong, S.S.J.; Poh, C.L. and Chang, M.W. (2011). Engineering microbes to sense and eradicate *Pseudomonas aeruginosa*, a human pathogen. Mol. Syst. Biol. 7:521.

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