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SYNERGISTIC EFFECT OF PARTIALLY PURIFIED BACTERIOCIN FROM CRONOBACTER SAKAZAKII AND ANTIBIOTICS AGAINST STAPHYLOCOCCUS AUREUS

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ABSTRACT

The antibacterial activity of partially purified bacteriocin produced by *Cronobacter sakazakii* was studied by well diffusion method. Results showed that bacteriocin produced by *C. sakazakii* have the antibacterial activity according to the inhibition zones against *E. coli, S. aureus, K. penumoniae, S.dysenteriae, P. vulgaris* and *S. marcescens*. Antibiotic susceptibility of *S. aureus* was examined against different antibiotics. The minimum inhibitory concentration of bacteriocin, amikacin and tetracycline against *S.aureus* was determined, then synergistic effect of bacteriocin and antibiotics against *S.aureus* was analyzed. Result showed that the synergistic effect of bacteriocin with both amikacin and tetracycline in different ratios. The highest synergistic effect was obtained when bacteriocin was mixed with both amikacin and tetracycline in a ratio of 3:1, where the inhibition zones were increased to 29.4 mm and 27.3 mm respectively.

KEYWORDS: Cronobacter sakazakii, bacteriocin, synergistic effect.

INTRODUCTION

The microorganisms resistant antibiotic strains, such as *Staphylococcus aureus* (MRSA) and others bacterium are the public health problem worldwide, and this has stimulated researchers to looking for a new conventional drugs at new pharmaceuticals. One strategy is the possibility of combination of conventional drugs with natural antimicrobials, that can serve as alternatives to treatment. Antimicrobial peptides are produced by bacteria and called bacteriocins, they are defined as a heterogeneous group with bactericidal action on various types of bacteria. Combination between natural antimicrobials and antibiotic support to

potentially eliminate resistant strains, delay the evolution of drug resistance, reduce the dosage of individual drugs, and diminish side effect.[1,2] Bacteriocin are antimicrobial peptides with different sizes, microbial target and mechanisms of action produced by large variety of bacteria. They are characterized by a bactericidal or bacteriostatic activity against strains of the same species or closely related species and differ from most therapeutic antibiotics due to their narrow activity spectrum and their proteinaceous nature.[3] *Cronobacter sakazakii* is an emerging opportunistic human pathogen associated with bacterial infections in infants. The microorganism belongs to the family *Enterobacteriaceae*, and is a Gram-negative, motile, facultatively anaerobic, non-sporeforming organism.[4] The ability of bacteria to production bacteriocin so many important in designer pharmaceutical, genetic engineering, agricultural, biological control, and medicine according to these findings this study was aimed to determine the synergetic effect of partially purified bacteriocin with antibiotics.

MATERIALS AND METHODS

Test microorganisms

Pathogenic bacteria (*Escherichia* coli, *Staphylococcus aureus, Klebsiella penumoniae,* supplied from Al-Zafaraniyah General Hospital.

Antibacterial activity of partial purified bacteriocin on pathogenic bacteria

Partial purification of bacteriocin from *Cronobacter sakazakii* was performed by ion exchange chromatography, then antibacterial activity of bacteriocin produced by *C. sakazakii* was studied by detection the inhibitory effect of bacteriocin against *E. coli, S. aureus, K. penumoniae, S. dysenteriae, P. vulgaris* and *S. marcescens*, grown on BHI agar plates. The antagonistic effect against the test microorganism was done according to the well diffusion assay method described by[5] as follows: Brain heart infusion broth was inoculated with fresh culture of test microorganism (*E. coli, S. aureus, K. penumoniae, S. dysenteriae, P. vulgaris* and *S. marcescens*) and incubated at 37 °C for 24 hrs. Then 100 µL of overnight culture of test microorganisms was taken and spread on BHI agar plates by sterile cotton swabs. Immediately wells were made in the medium using sterile cork borer and filled with 100 µl of crude filtrate of the *C. sakazakii*. Plates were incubated at 37 °C for 24 hrs. The inhibitory effect of bacteriocin measured using the diameter of inhibition zones around bacteriocin extract.

Antibiotic susceptibility test

Antibiotic susceptibility of *S. aureus* was done according to[6] by an antibiotic disk (amikacin, tetracycline, vancomycin, chloramphinicol, penicillin, and methicillin) was fixed in the middle of Muller-Hinton agar plate that already was streaked with *S.aureus*. Each plate was incubated for overnight at 37˚C, the diameter of inhibition zone was measured and recorded.

Minimum Inhibitory Concentration Determination

Minimum inhibitory concentration (MIC) for bacteriocin, tetracycline and amikacin was determined by tube-dilution method[7], twofold dilutions were prepared (4, 8, 12, 16, 20, 24, 28 and 32) for bacteriocin, tetracycline and amikacin as illustrated in table (2-5) using distal water for dilution and distributed in test tube. *S.aureus* was propagated in BHI broth medium and incubated at 37 ˚C for 3-5hrs until the optical density was 0.5 (each tube contains approximately 5 x 10^5 CFU/ml), then 2 ml of *S.aureus* suspension was added to each dilution of antibacterial substances tube and incubated at 37 ˚C for 24hrs, then tubes were examined for visible bacterial growth as evidenced by turbidity. The lowest concentration of antibacterial substances that prevented growth represented the minimal inhibitory concentration (MIC). Antibacterial activity of bacteriocin, tetracycline and amikacin (24 µg/ml) was studied by well diffusion method (). *S.aureus* was propagated in BHI broth medium and incubated at 37 ˚C for 3-5hrs until the optical density was 0.5, then *S.aureus* was spread on Muellar Hinton agar plates containing wells were made in the medium, then wells were filled with 100 µl of each concentration of antibacterial compound, and incubated at 37 ˚C for 24hrs. Result was recorded after 24 hrs by measuring the diameter of the inhibition zone.

Synergistic effect of bacteriocin and antibiotics against *S.aureus*

In order to detect the synergetic effect of partially purified bacteriocin with antibiotics (tetracycline, amikcin). Partially purified bacteriocin was mixed with tetracycline and amikacin separately in one, two and three folds respectively as indicated in table (1), and synergistic effect was determined according to the well diffusion method as in the above paragraph.

RESULTS AND DISCUSSION

Antibacterial activity of partial purified bacteriocin on pathogenic bacteria

Antibacterial activity of partially purified bacteriocin against some pathogenic bacteria was assayed depended on diameter of inhibition zone. Result indicated in figure (1) showed that

the activity of bacteriocin increasing when partially purified, The maximum inhibition zone was observed in *S.aureus* diameter of inhibition zones was reached to 41mm.

Antibiotic susceptibility test

Antibiotic susceptibility pattern of *S.aureus* against different antibiotics was studied. Results indicated in table (1) showed that *S. aureus* was resistant to two antibiotics (methicillin and penicillin), while it was sensitive to the other four antibiotics (vancomycin, chloramphenicol, amikacin and tetracycline). Methicillin and Penicillin are inactivated by β-lactamase, a serine protease that hydrolyzes the β-lactam ring. Production of β-lactamase is widespread in *S aureus* and may be conferred by the blaZ gene.[8] Chopra and Roberts,[9] referred that *S.aureus* was sensitive to tetracycline because tetracycline inhibit bacterial protein synthesis. They have also been shown to cause damage to the bacterial cytoplasmic membrane.[10] Another study showed that vancomycin acts by interfering with the synthesis of the cell wall in gram-positive bacteria such as *S.aureus*.[11] Chloramphenicol and amikacin inhibiting protein synthesis by irreversibly binds to small ribosomal subunit.[12,13]

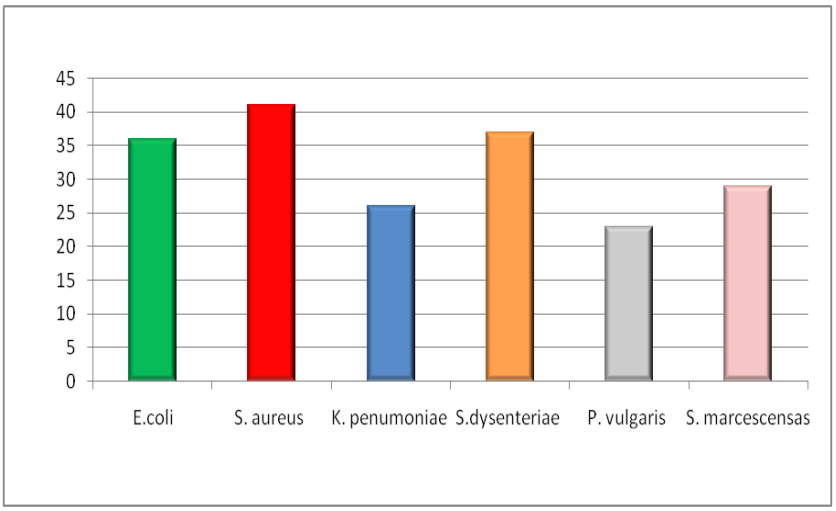

Figure 1: Inhibitory effect of partially purified bacteriocin produced by locally isolated *C.sakazakii* against pathogenic bacteria.

Minimum Inhibitory Concentration Determination

Minimum inhibitory concentration of bacteriocin, tetracycline and amikacin against *S.aureus* was studied. Results indicated in table (2) showed that MIC of bacteriocin, tetracycline and amikacin was 20 **µ**g/ml, 20 **µ**g/ml and 16 **µ**g/ml respectively. Rubin *et al*.[14] found minimal inhibitory concentration range of tetracycline against *S.aureus* 2-32**µ**g/ml, while results dose not accordance with what it says Trzcinski *et al*.[15] that the minimal inhibitory concentration

of tetracycline against *S.aureus* 32-128 **µ**g/ml. On the other hands result mentioned by Caron *et al.*[16] showed that minimal inhibitory concentration of amikacin 16µg/ml. Antibacterial activity of bacteriocin, tetracycline and amikacin (24 µg/ml) was studied. Results indicated in table (3) showed that the inhibitory effect of bacteriocin, tetracycline and amikacin (24µg/ml) was 26.3, 20 and 26 respectively.

Table 1: Antibiotic susceptibility of *S.aureus* .

Antibiotic	Susceptibility
Vancomycin	**S**
Chloramphenicol	**S**
Methicillin	**R**
Tetracycline	**S**
Penicillin	**R**
Amikacin	**S**

R: resist S: sensitive

Figure 2: Minimal inhibitory concentration of bacteriocin, tetracycline and amikacin against *S.aureus.*

Antibacterial substances	Concentration (µg/ml)	Growth	Antibacterial substances	Concentration (µg/ml)	Growth
Bacteriocin	4	+++	Tetracycline	20	-
Bacteriocin	8	+++	Tetracycline	24	-
Bacteriocin	12	++	Tetracycline	28	-
Bacteriocin	16	+	Tetracycline	32	-
Bacteriocin	20	-	Amikacin	4	+++
Bacteriocin	24	-	Amikacin	8	++
Bacteriocin	28	-	Amikacin	12	+
Bacteriocin	32	-	Amikacin	16	-
Tetracycline	4	+++	Amikacin	20	-
Tetracycline	8	+++	Amikacin	24	-
Tetracycline	12	++	Amikacin	28	-
Tetracycline	16	+	Amikacin	32	-

(+++): heavy growth, (++): moderate growth, (+): low growth, (-): no growth

Table 3: Diameter of inhibition zones of *S.aureus* under the effect bacteriocin, tetracycline and AMIKACIN.

Antibacterial substances	Concentration (µg/ml)	Inhibition zone diameter(mm)
Bacteriocin	24	26.3
Tetracycline	24	20
Amikacin	24	26

Synergestic effect of bacteriocin and antibiotics against *S.aureus*

The bacteriocin and two antibiotics in our experiment (Tetracycline, Amikicin) were analyzed for their potential synergy against *Staphylococcus aureus* by used different concentration of bacteriocin and antibiotics. Results indicated in table (4) showed the synergistic effect of bacteriocin with both amikacin and tetracycline in different ratios. The highest synergistic effect was obtained when bacteriocin was mixed with both amikacin and tetracycline in a ratio of 3:1, where the inhibition zones were increased to 29.4 mm and 27.3 mm respectively because of the higher concentration of bacteriocins act as membrane lysis that lead to enhance the actions of antibiotic. Van den *et al*.[17] found the lethal action of cloacin DF13 is the result of several activities residing within this protein: (a) interaction of cloacin DF13 with sensitive cells results in potassium leakage across the cytoplasmic membrane. and (b) binding of cloacin DF13 molecules to a specific outer membrane receptor protein. In addition, inactivation of ribosomes by cleavage of 16S rRNA near its 3'-end. Another study showed that cloacin DFI3 containing rRNase to non specific digest RNA of bacteria.[18] The bacteriocin in higher concentration can potentiate the actions of antibiotic and reduce side effect of antibiotic and diminish resistant antibiotic strains. Bacteriocins were reported to boost the activities of antibiotics with a great synergistic effect by modes of action which can be categorized into two types: membrane lysis and no membrane lysis (intracellular targets). Usually, dual mechanisms are reported for several AMPs and are concentration dependent. AMPs cause membrane lysis at high concentration and no-membrane lysis at low concentration.[19] Many researches reported that use synergistic action between antibiotic and bacteriocin gain highly activity against resistant strains such synergistic effect on *P. fluorescens* was apparent when nisin Z or pediocin PA-1/AcH was used in combination with antibiotics. Combinations of antimicrobial peptides with penicillin G, streptomycin; lincomycin appeared to be highly synergistic against esistant variants of *P. fluorescens*[20], another study showed that a combination of haloduracin which isolated from *Bacillus halodurans* and chloramphenicol have synergistic activates against different clinically important microorganisms such *S. aureus* (CCUG 2354), *S. aureus* (CCUG 15915), *E. faecium* (CCUG 36804), *E. faecalis* (CCUG 9997), *Streptococcus* group G (ATCC 12394), *Streptococcus* group C (ATCC 12388), Group A *Streptococcus* Type12 (CCUG 4207) and Group B *Streptococcus* (CCUG 4208).[2] Another study reported that Synergy was seen between polymyxin B and garvicin KS produce from *L. garvieae* against *Acinetobacter* strains.[21]

Table 4: diameter of inhibition zones of *S.aureus* under the synergistic effect of synergistic bacteriocin and antibiotics.

Antibacterial substances	Concentration (µg/ml)	Ratio	Inhibition zone diameter(mm)
Bact + Tet	24	3:1	27.3
Bact + Tet	24	2:1	26.8
Bact + Tet	24	1:1	25.2
Bact + Amik	24	3:1	29.4
Bact + Amik	24	2:1	29
Bact + Amik	24	1:1	28.8

(Bact): Bacteriocin (Tet): Tetracycline (Amik): Amikacin

REFRENCES

1. Hassan, M., Kjos, M., Nes, I. F., Diep, D. B., and Lotfipour, F. Natural antimicrobial peptides from bacteria: characteristics and potential applications to fight against antibiotic resistance. *Journal of applied microbiology*, 2012; *113*(4): 723-736.
2. Danesh, A., Ljungh, Å., Mattiasson, B., and Mamo, G. Synergistic effect of haloduracin and chloramphenicol against clinically important Gram-positive bacteria. *Biotechnology Reports*, 2017; *13*: 37-41.
3. Riley, M. A., and Chavan, M. A. *Bacteriocins*. Springer-Verlag Berlin Heidelberg. 2007
4. Drudy, D., Mullane, N. R., Quinn, T., Wall, P. G., and Fanning, S. Enterobacter sakazakii: an emerging pathogen in powdered infant formula. *Clinical Infectious Diseases*, 2006; *42*(7): 996-1002.
5. Todorov, S. D., and Dicks, L. M. Bacteriocin production by Pediococcus pentosaceus isolated from marula (Scerocarya birrea). *International Journal of Food Microbiology*, 2009; *132*(2): 117-126.
6. Bauer, A. W., Kirby, W. M., Sherris, J. C., and Turck, M. Antibiotic susceptibility testing by a standardized single disk method. *American journal of clinical pathology*, 1966; *45*(4): 493.
7. Patel, R. M. The guiding principles on antimicrobial susceptibility testing. *Bulletin of Pharmaceutical Research*, 2012; *2*(3): 146-53.
8. Akanbi, O. E., Njom, H. A., Fri, J., Otigbu, A. C., and Clarke, A. M. Antimicrobial Susceptibility of Staphylococcus aureus Isolated from Recreational Waters and Beach Sand in Eastern Cape Province of South Africa. *International journal of environmental research and public health*, 2017; *14*(9): 1001.

9. Chopra, I., and Roberts, M. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiology and molecular biology reviews*, 2001; *65*(2): 232-260.
10. Kaddora, I. (2010). Antibiotic sensitivity patterns of hospital-acquired and community-acquired methicillin resistant Staphylococcus aureus.
11. Kang, H. K., and Park, Y. Glycopeptide antibiotics: Structure and mechanisms of action. *Journal of Bacteriology and Virology*, 2015; *45*(2): 67-78.
12. Jensen, S. O., and Lyon, B. R. Genetics of antimicrobial resistance in Staphylococcus aureus. *Future microbiology*, 2009; *4*(5): 565-582.
13. Magalhães, M. L., and Blanchard, J. S. Aminoglycosides: Mechanisms of action and resistance. In *Antimicrobial Drug Resistance* Humana Press, 2009; 171-181
14. Rubin, J. E., Ball, K. R., and Chirino-Trejo, M. Antimicrobial susceptibility of Staphylococcus aureus and Staphylococcus pseudintermedius isolated from various animals. *The Canadian Veterinary Journal*, 2011; *52*(2): 153.
15. Trzcinski, K., Cooper, B. S., Hryniewicz, W., and Dowson, C. G. Expression of resistance to tetracyclines in strains of methicillin-resistant Staphylococcus aureus. *Journal of Antimicrobial Chemotherapy*, 2000; *45*(6): 763-770.
16. Caron, J. P., Bolin, C. A., Hauptman, J. G., and Johnston, K. A. Minimum Inhibitory Concentration and Postantibiotic Effect of Amikacin for Equine Isolates of Methicillin-Resistant Staphylococcus aureus In Vitro. *Veterinary surgery*, 2009; *38*(5): 664-669.
17. van den Elzen, P. J., Walters, H. H., Veltkamp, E., and Nijkamp, H. J. J. Molecular structure and function of the bacteriocin gene and bacteriocin protein of plasmid Clo DF13. *Nucleic acids research*, 1983; *11*(8): 2465-2477.
18. Yang, S. C., Lin, C. H., Sung, C. T., and Fang, J. Y. Antibacterial activities of bacteriocins: application in foods and pharmaceuticals. *Frontiers in microbiology*, 2014; *5:* 241.
19. Shah, P., and Chen, C. S. Combinational Synergy of Antibiotics and Antimicrobial Peptides. *Journal of Translational Proteomics Research*, 2017; *3:* 6-11.
20. Naghmouchi, K., Le Lay, C., Baah, J., and Drider, D. Antibiotic and antimicrobial peptide combinations: synergistic inhibition of Pseudomonas fluorescens and antibiotic-resistant variants. *Research in microbiology*, 2012; *163*(2): 101-108.

21. Chi, H., and Holo, H. Synergistic Antimicrobial Activity Between the Broad Spectrum Bacteriocin Garvicin KS and Nisin, Farnesol and Polymyxin B Against Gram-Positive and Gram-Negative Bacteria. *Current microbiology*, 2017; 1-6.