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Original Research Article

The inhibitory activity of Bacteriocin produced from *Lactobacillus casei* against *Staphylococcus aureus* and *Klebsiella pneumoniae*

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Abstract

This study was conducted in the laboratories of the Department of Biological Science University of Samarra/College of Education for the period from 1/11/2020 to 10/4/2021 AD. Samples of *Lactobacillus casei* were collected from milk and various food sources, and they were grown on a selective nutrient medium; De Man Rogosa Sharpe (MRS) was then isolated and diagnosed in the Microbiology laboratory, and two types of pathogenic bacteria were used: *Staphylococcus aureus* and *Klebsiella pneumoniae* to determine the effect of bacteriocin on it. The study also included the quantitative detection of the protein of bacterial filtrate produced from *Lactobacillus casei*, as well as the study of the effect of Bacteriocin on the pathogenic bacterial species under study in three concentrations (1.64, 3.28, 6.56 mg/ml). The bacterial filtrate showed inhibitory activity against the bacterial species under study, which showed sensitivity to bacteriocin, and the inhibitory activity of the bacterial filtrate increased with increasing its concentration, as it gave the concentration (6.56) mg /ml. The highest inhibitory activity against the bacterial species under study was with an inhibitory diameter of (15.1 mm) for *S. aureus* and an inhibitory diameter of (14.2 mm) for *K. pneumoniae*. The Minimum Inhibitory Concentration (MIC) of bacteriocin was determined in this study at concentrations (0.21, 0.41, 0.82, 1.64, 3.28, 6.56 mg/ml). The MIC value varied according to the type, source, and concentration of the bacterial filtrate, as well as the type of bacteria. The (MIC) value of *S. aureus* was (1.64 mg /ml), while the (MIC) value of *K. pneumoniae* was (3.28 mg/ml).

Keywords *Staphylococcus aureus* and *Klebsiella pneumoniae* , Bacteriocin , *Lactobacillus casei*

Introduction

The human body contains a huge number of microorganisms, including types of bacteria, fungi, and viruses, and they form the Microbiome, which lives in several places in the body and grows on the surface of the skin or inside the body such as the mouth, nose, genitals, urinary system, and intestines (Rudolf et al., 2006). Bacteria are the largest groups within the intestine and have an important role in the pathogenicity. *S. aureus* bacteria cause acute Tonsillitis and the infection progresses, leading to complications and pain that extends to the ear, causing Otitis media, Bronchitis, Meningitis, Arthritis, and. Glomerulonephritis, Rheumatic Heart Diseases, Scarlet Fever, Toxic Shock Syndrome (Rudolf et al., 2006; Jawetz et al., 1991; AL-Samarraie et al., 2020). *Klebsiella pneumoniae* is one of the most famous and most dangerous opportunistic bacterial pathogens and is fatal to human health because it has resistance mechanisms and virulence factors that make it secrete more virulent and severe toxins, including Epidemic

diarrhea. (Rashid and Ebringe, 2007; Majeed et al., 2020), and many hospital-acquired infections caused Nosocomial infection, such as pneumonia, urinary tract infection, septicemia, and inflammation of wounds and burns (Rassol et al., 2003; Mustafa et al., 2020; Podschun and Ullmann 1998).

The studies have proven that bacteria can acquire resistance to antibiotics after several generations, as they transfer resistance genes among themselves, and it is difficult to treat them. Scientists have concluded that excessive use of such drugs is the chief aim for the appearance of drug-resistant strains (Dimopoulous and Falagas, 2007; Chatterjee et al., 2021 Dimri et al., 2018). The spread of antibiotic resistance between Gram-positive and negative bacteria has prompted researchers interested in the field of treatment to discover therapeutic alternatives. Among these alternatives are inhibitory proteins secreted by some types of *Lactobacillus* bacteria known as Bacteriocin.

The current study aimed to know the inhibitory effect of bacteriocin against *Staphylococcus aureus* and *Klebsiella pneumoniae*.

Materials and Methods

Preparation of bacterial isolate for *L. casei*

The bacterial isolate of *Lactobacillus casei* was obtained from the different food sources which included raw cow's milk, pickles, and local soft cheeses, and they were brought to the laboratory using sterile and airtight plastic tubes. The isolate was grown on MRS medium. Then it was purified several times until a pure colony was obtained (Al- Khafaji, 1990).

Preparation of bacterial filtrate from *L. casei*

The method (Hammadi, 2017) was adopted, where the isolated and identified *Lactobacillus casei* bacteria used in this study was recultivated by transferring a full loop from the isolate grown on solid MRS agar medium to MRS broth liquid medium, incubated in the incubator at 37°C for a period of (24-48) hours and under anaerobic conditions, then the samples were centrifuged to obtain a bacteria-free filtrate (Extract cell-free). The pH was adjusted to (pH=7) (utilizing sterile NaOH and at a concentration of 1 standard), the bacterial filtrate was filtered through Millipore filter paper (0.22) µm utilizing a sterile syringe for sterilization, and a phosphate buffer was added to the bacterial filtrate to overcome the inhibition of amino acids, and then kept in the refrigerator at 4° C until use.

Experiment the inhibitory effect for bacteriocin against *S. aureus* and *K. pneumoniae*

The agar well diffusion method was used for the sensitivity of bacteria under study (*Staphylococcus aureus* and *Klebsiella pneumoniae*) at concentrations (1.64, 3.28, 6.56) mg/ml, in this study the method of diffusion by digging in bacterial culture was used according to what was indicated by (Ogunbanwo et al., 2003; Kaushik and Chauhan, 2008; Chauhan et al., 2010), and the bacterial species were activated on Nutrient broth medium for one hour and incubated at 37°C, after 100 µL is taken and spread on Muller-Hinton agar and incubated at 37°C for 30 minutes until spreading and drying. Three holes are made with a diameter of 6 mm with a sterile cork perforator of equal dimensions in the culture medium inoculated with bacteria. The holes are filled with the bacterial filtrate of 100 µL, and then the culture dishes are left for two hours until spreading and drying occurs, and then incubated at a temperature of 37°C for 24 h. then observation are read and recorded by measurement the diameter of the inhibition is

measured through a clipper (Chauhan et al., 2010; Goyal et al., 2010; Kaushik et al., 2008)

Results and discussion

The results shown in Table 1 indicate that The effect of bacteriocin had a significant effect on the inhibition of the bacterial species under study, as the inhibitory diameter increased with the increase in the concentration of the bacterial filtrate, and the effect of bacteriocin differed with the different concentrations used and the type of bacteria, as it was directly proportional to the increase in concentrations, and with significant differences between the rates of the diameters of inhibition and the type of bacteria at the level of probability (0.05). The results showed that the rates of inhibition diameters ranged between (15.1-14.2 mm) at a concentration of (6.56 mg/ml), and the highest inhibitory diameter of the bacterial filtrate was (15.1mm) against the growth of *S. aureus* bacteria, followed by *K. pneumoniae* with a diameter of (14.2 mm), and is evidence of the ability of bacteriocin to affect the pathogenic bacterial species through the work of pores holes in the cytoplasmic membrane, causing its destruction and the exit of the contents of the cell and thus its death (Wiedemann et al., 2004; Ray and Daeschel, 1992). It also affects the biosynthesis of nucleic acids, type DNA, RNA (Zacharof and Lovitt, 2012). As for the concentration (3.28 mg/ml), the inhibitory diameters ranged from (13.2 - 9 mm), where *S. aureus* bacteria showed an inhibitory effect with the inhibitory diameter of (13.2 mm), followed by *K. pneumoniae* bacteria with an average inhibitory diameter of (9 mm), as for the concentration of bacterial filtrate at the concentration (1.64 mg/ml), the rates of inhibitory diameters ranged from (10- 6.3 mm), where *S. aureus* showed an inhibitory effect with an average inhibitory diameter of (10 mm), while the lowest rate of inhibition diameter was shown by *K. pneumoniae* bacteria (6.3 mm). The low concentration of the bacterial filtrate was this led to a weak inhibitory effect of bacteriocin on the pathogenic bacteria under study.

Table 1. Shows the inhibitory activity of Bacteriocin against *S. aureus* and *K. pneumoniae* in mm/mg

Average bacterial inhibition	Concentration of Bacteriocin mg/ml			Concentration Bacterial species
	6.56	3.28	1.64	
± 12.82.58 B	15.1 ab	13.2 bc	10.0 d	<i>S.aureus</i>
9.8 ± 4.02 C	14.2 b	9.0 de	6.3 e	<i>K.pneumoniae</i>

*The values shown in the table represent Mean (± SE).

*Similar letters mean that there are no significant differences between them, with a probability level of P < 0.05

In addition, *K. pneumoniae* showed a high resistance to bacteriocin. The reason for this was it possesses a capsule consisting of a thick layer of viscous mucous Lipopolysaccharides that blocks or prevents the penetration of the antibiotic or bacterial filtrate into the bacterial cell (Tenover, 2006; Jasim et al., 2020; Nayyef, et al., 2020).

Table 2. Shows the inhibitory activity of Minimum Inhibitory Concentration for Bacteriocin (MIC) against *S. aureus* and *K. pneumoniae*. There is no inhibitory effect (-), There is inhibitory effect (+)

Concentration of Bacteriocin mg/ml						Type of bacteria	N
6.56	3.28	1.64	0.82	0.41	0.21		
+	+	+	-	-	-	<i>S. aureus</i>	1
+	+	-	-	-	-	<i>K. pneumoniae</i>	2

Minimum inhibitory concentration for bacteriocin

The results showed the anti-bacterial activity of the filtrate, which increases by increasing its concentration in the medium (Table 2). The results showed that the value of the minimum inhibitory concentration (MIC) varied depending on the type of bacteria as well as on the incubation period. The (MIC) value for bacteriocin for *S. aureus* was (1.64 mg/ml), while the (MIC) value for *K. pneumoniae* was (3.28 mg/ml).

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