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Research Article

Antibacterial activity of Lactic acid bacteria producing Bacteriocins

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Abstract

The aim of this study was to evaluate how effective bacteriocins, which are produced by lactic acid bacteria, are against *Staphylococcus aureus*, *Eneterococcus fecalis*, and *Escherichia coli*. *Lactobacillus fermentum, Lactobacillus acidophilus,* and *Lactobacillus rhamnosus* were isolated from milk, dairy animal milk products, and the intestinal contents of healthy broiler chickens. Conventional methods were used to identify these isolates morphologically and biochemically. By using the precipitation process, bacteriocins were extracted from these bacteria. The disc diffusion method was used to test the antibacterial activity of bacteriocins. Isolated bacteriocin had inhibitory effect against Staphylococcus *aureus* and *Enterococcus fecalis*, but not against Eschrechia coli (2mm). The findings of this study revealed that bacteriocins are effective against common food pathogens and can thus be utilized to preserve food and food items.

Keywords

Food pathogens, Lactic acid bacteria, Bacteriocins, Antibacterial activity

Declaration of Conflicting Interest

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Introduction

Fermentation is the oldest known food preservation technique, having been used to preserve milk, meat, and vegetables for thousands of years. The antimicrobial action of their antibacterial goods enables man to extend the shelf life of many foods and food products by using lactic acid bacteria as a natural or controlled micro flora (Fox, 1993; Stiles, 1996). Lactic acid bacteria have been demonstrated to exhibit strong antagonistic activities against a variety of pathogenic and food-spoiled microorganisms. Because of their ability to create bacteriocins, several strains have been employed for food preservation (Brink et al., 1998). Lactic acid bacteria have the ability to create several lactose compounds such as organic acids, diacetyl, hydrogen peroxide, and proteins during lactic acid fermentation (Brink et al., 1998; Ouwehand, 1998; Zhennai, 2000; Oyetayo et al., 2003). Gram positive and Gram negative bacteria both produce bacteriocins. The bacterial cell ribosome releases these outside the cell in the form of low molecular weight peptides or proteins that have bacteriostatic or bactericidal effects on other closely related species (Tagg et al., 1976; Clevelan et al., 2001; Chen and Hoover 2003; Cotter et al., 2005). Bacteriocins have a lot of attention as food bio-preservatives because of their sustained antibacterial action at high temperatures and diverse pH levels. Nisin and Pediocin PA-1 are two well-known bacteriocins generated by lactic acid bacteria that can be used as food preservatives (Montville and Chen, et al., 1998; Galvez et al., 2007). Many commensal and lactic acid bacteria are thought to carry antibiotic resistance genes that are comparable to those found in human pathogenic and food-spoiled bacteria (Ammor et al., 2007). The increased demand for less processed, safe, and chemically additive-free foods, as well as the resistance of many bacteria to antibiotics, has sparked interest in using naturally safe products such as bacteriocins to replace these goods (Parada et al., 1980; Chopra et al., 1998; Rao, 1998). As a result, the purpose of this study is to determine the antibacterial activity of bacteriocins against widely encountered food pathogens, as well as to see if using bacteriocins for food preservation is advantageous.

Materials And Methods

Lactic Acid Bacteria Isolation and Identification Lactic acid bacteria were recovered from 15 distinct samples of raw milk from a home cow, yoghurt from various commercial locations, and the intestinal contents of broiler chicken from five different farms in Lahore, Pakistan (n = 5 each). For subsequent processing, all samples were kept at +4°C. Separately, phosphate buffer saline was used to dilute yoghurt, milk, and the supernatant of intestinal contents (1:10). (PBS). DeMan's Regosa and Sharpe (MRS) agar plates were used to isolate lactic acid bacteria, as previously described (De Man Rogosa et al., 1960; Tufail, et al., 2011). Lactobacilli species were identified using standard methods based on morphological and biochemical properties after cultures were purified using the streak plate method (Kalalou et al., 2004; Adesokan et al., 2008). Lactobacillus fermentum, Lactobacillus rhmanosis, and Lactobacillus acidophilus were isolated and identified, then kept at -80 °C in MRS broth medium with 25ml glycerol/L. (De Man Rogosa et al., 1960). Bacteriocin Isolation Using a Cell-Free Culture the isolated lactic acid bacteria were grown in 500 mL MRS broth at pH 7.0 for 48 hours at 37°C. By centrifuging (10,000 rpm) bacteriocin for 30 minutes at 4 °C, a cell-free solution was obtained. The isolated bacteriocin solution was precipitated with ammonium sulphate, and the impact of organic acids was inhibited with phosphate buffer saline (PBS). In 50 mL of 0.1 M potassium phosphate buffer, these precipitates were suspended (pH 7.0). As previously disclosed, precipitates were collected and employed in a disc diffusion experiment (Savadogo et al., 2004). Bacteriocin Antibacterial Activity Determination Bacteriocins were tested for antibacterial action against microorganisms that cause food spoilage, including E. coli, Enterococcus faecalis, and Staphylococcus aureus. The disc diffusion experiment (Savadogo et al., 2004; Tagg and McGiven, 1971) was used, with 50 g of each extract deposited on each disc. Muller-Hinton agar was used for all of the assays. Pathogenic bacteria (5107 CFU/ml) were injected onto Muller Hinton plates, which were then left undisturbed for 2 hours. The inoculation plates were then incubated for 24 hours at 37°C. The zones of inhibition were measured according to the instructions (Tagg and McGiven, 1971).

Discussion and Results

All of the isolated bacterium's morphological properties were determined, and they all showed that they are common features for lactic acid bacteria (Galvez et al., 2007; Krieg, 1984). (Outlined in Table 1).

Parameter	Isolate A	Isolate B	Isolate C
Colony	[a] Circular, Irregular, whitish white, 1-3 mm diameter	[b] Small, entire margins 1-3 mm diameter	[c] Small, Sharpe edges 2-5 mm in diameter
Gram's reaction	[a] Positive	[a] Positive	[a] Positive
Gas from glucose	[a] Positive	[b] Negative	[b] Negative
Catalase	[a] Negative	[a] Negative	[a] Negative
Cell Morphology	[a] Rod shape non spore forming single cell	[b] Rods, varying length, in pairs or chains Non spore forming	[c] Short rods in chain Non spore forming

[a, b, c], Denotes significant difference in rows ($P<0.05$).

Table 1: Morphological and Biochemical Characteristics of Isolated LAB

On the basis of morphological and biochemical features, isolates A and B were identified as *Lactobacillus fermentum, Lactobacillus acidophilus*, and *Lactobacillus rhmanosus*, respectively. A little amount of bacteriocins was separated due to adsorption on the cell surface in a study by Yang et al. As a result, a better approach for protein precipitation was developed using ammonium sulphate; nonetheless, this method has numerous drawbacks. The findings revealed that the lactic acid bacteria in both the fermented product and the gut were diverse. The disc diffusion assay revealed that bacteriocin's inhibitory spectrum was mediated against Gram negative and Gram positive harmful bacteria. The extracts revealed zones of inhibition against a variety of recognized organisms, including harmful bacteria strains *(Enterococcus faecalis, Escherichia coli and Staphylococcus aureus)*. Against indicator strains, a wide range of inhibition zones were detected, which were quantified in millimeters (inhibition diameter, mm) (Table 2).

Lactic acid bacteria	Indicator strain	Zone of Inhibition (mm)
Isolate A	Enterococcus faecalis, Staphylococcus aureus, Escherichia coli	[a] 11, 9, 5
Isolate B	Enterococcus faecalis, Staphylococcus aureus, Escherichia coli	[b] 9, 7, 3
Isolate C	Enterococcus faecalis, Staphylococcus aureus, Escherichia coli	[c] 4, 3, 2

[a, b, c,] Denotes significant difference in rows ($P<0.05$).

Table 2: Antibacterial activity of isolated bacteriocins (mm)

This inhibition suggests that they have the potential to improve the sanitary quality of foods and food products. Disk diffusion was used to verify inhibitory activity, and Vernier's calliper was used to measure inhibition zones. The diameters of inhibition ranged from 2 to 11 mm, with *Lactobacillus fermentum* showing the highest diameter of 11 mm on the indicator strain Enterococcus faecalis, indicating the strain's diversity and sensitivity to isolated bacteriocins. *Lactobacillus rhamnosus bacteriocins* inhibited the indicator strain of E. coli in the narrowest zone of inhibition. Staphylococcus aureus and Enterococcus faecalis were the strains that were most inhibited, while only one strain of E. coli was inhibited by the bacteriocin, and it was at a very low level. It was discovered that Gram positive bacteria were far more sensitive to our lactic acid bacteria's bacteriocin than Gram negative bacteria. As demonstrated in the picture, isolate A (11 mm) had the highest inhibition while isolate C (2 mm) had the lowest. These findings are similar to those of (Aslamet.al. 2011), who extracted bacteriocin from *Streptococcus thermophilus* and tested it against Gram positive bacteria such as *Staphylococcus aureus* and *Bacillus subtilis*, as well as Gram negative bacteria such as Escherichia coli and *Pasteurella multocida*. In comparison to the data given in this investigation, their results indicated low efficacy against Gram negative bacteria. Our findings are also consistent with prior research by (Savadagoet al 2004). They have, on the other hand, discovered eight strains of lactic acid bacteria that make bacteriocins from fermented milk. *Enterococcus faecalis*, *Bacillus cereus, Staphylococcus aureus*, and *Escherichia coli* were all inhibited by the isolated bacteriocins (Fig. 1). Gram-positive indicator bacteria were the most susceptible to inhibition. These findings suggest that isolated lactic acid bacteria strains can produce inhibitory compounds against pathogenic bacteria. On harmful reference indicator bacteria, these inhibitory compounds have varied effects. By examining the effect of bacteriocin on *Staphylococcus aureus*, *Pseudomonas aeroginosa, and Escherichia coli*, (Adesokan et al. 2008) discovered less inhibitory activity against Gram negative bacteria than Gram positive bacteria. Lactic acid bacteria create a variety of inhibitory compounds, the majority of which are proteins (Klaenhammer, 1993; Vandenberg, 1993). The inhibitory spectrum, on the other hand, differs from one strain to the next, depending on the strains employed. Gram positive pathogenic bacteria are more susceptible to bacteriocin generated by lactic acid bacteria than Gram negative bacteria, which have no growth inhibitory effect. Gram negative bacteria's resilience was owing to the precise quality of their cellular envelope, which bacteriocins affect through adsorption phenomena.

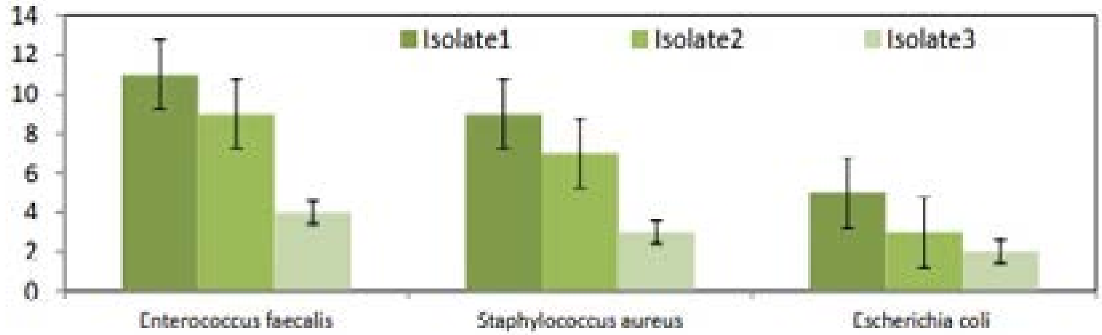

Figure 1: Effect of bacteriocin on the growth of three different pathogenic bacteria.

This could be owing to the bacterial cell wall's resistance to this bacteriocin (the presence or lack of peptidoglycan). Lactic acid bacteria are typically found in milk and vegetable fermented products. LAB is found naturally in a variety of raw materials used in food production, including milk, meat, and flour (Garrity, 1984; Rodriguez et al., 2000). Lactic acid bacteria create bacteriocins, which are chemical substances. The pervasive nature of LAB may be advantageous for natural food and food product preservation.

The ribosomal produced protein bacteriocin is a physiologically active complex protein with antibacterial activity against other bacteria, primarily closely related bacterial species. To minimize misunderstanding and worry with therapeutic antibiotics, which can cause allergic reactions in humans and other animals, they are not labelled as antibiotics. Bacteriocin, on the other hand, has no documented negative effects. Because most LAB have a safe status (GRAS) and are known as probiotics, they are easily digested in the digestive tract by enzymatic action. Except for *Escherichia coli*, which demonstrated resistance to bacteriocins, varying diameters of zone of inhibition revealed that pathogenic bacteria had different ranges of susceptibility to bacteriocins, but the exact cause is unknown. Several studies have looked at the level of inhibition in various bacteria with varying pathogenicity; the findings are consistent with earlier research (Kalalou et al., 2004; Schillinger and Lucke, 1989).

Conclusions

The Bacteriocin of LAB has the potential to be employed as an antibacterial and bio-preservative in foods and food items with no toxicity or negative effects for consumers. Lactic acid bacteria including *Lactobacillus fermentum, Lactobacillus acidophilus*, and *Lactobacillus rhamnosus* can create bacteriocin under anaerobic circumstances. Bacteriocins, which are antibacterial chemicals produced by LAB, have conferred a competitive advantage over other microbiota. The genetic modification and characterisation of amino acids and nucleotide sequences of these antibacterial substances should be the focus of future research. These items must also be tested for compatibility with other food preservatives or additives used to improve the quality and texture of the meal.

Conflict Of Interests

The authors declare that they have no conflict of interest with respect to the research, authorship, and/or publications of this article.

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