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Antimicrobial and therapeutic properties of bacteriocins from *Lactobacillus casei* isolated from goat milk

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Abstract

Lactic acid bacteria (LAB) bacteriocins are renowned for their broad spectrum of antimicrobial activity. These organisms are generally recognized as safe and are predominantly utilized in food preservation, effectively suppressing harmful bacteria. The present study aims to isolate LAB from goat milk, purify bacteriocins and analyze its therapeutic applications. Of the 26 isolates, isolate GO3 showing Enhanced antimicrobial activity against food-borne pathogens was identified using 16s rRNA sequencing. The organism was identified as *Lactobacillus casei* GO3 with 100% similar to *Lactobacillus casei* strain NR115322.1. Cystathionine gamma-synthase gene (*MetB*) with high homology to *Lacticaseibacillus casei* strain *MetB* gene was detected in the isolate GO3. The partially purified bacteriocin from *Lactobacillus casei* GO3 demonstrated a broad spectrum of antibacterial activity, achieving 76.4% inhibition against gram-positive *B. subtilis* and 46.2% against Gram-negative *Salmonella typhi* and antifungal activity, with maximum against *Phytophthora infestans* (47.7%) and a minimum against *Fusarium oxysporum* (42.2%). In addition to its antimicrobial activities, the bacteriocin demonstrated significant anti-inflammatory, α-amylase inhibition, antioxidant and anticancer activity. Further studies are required to analyze its mechanism of action and potential therapeutic applications in real-world scenarios.

Keywords: Bacteriocins, Lactic acid bacteria, Antibacterial, Antifungal, Anti-inflammatory, Antidiabetic, Anticancer, Antioxidative.

1. Introduction

The global rise in antibiotic resistance is progressing at a concerning rate, while the identification of new antibiotics is concurrently facing a notable decline. The rise of infections caused by gram-negative bacteria, particularly ESKAPE pathogens, is concerning due to their impermeable outer membrane and resistance mechanisms that hinder the entry of many antibiotics, resulting in limited treatment alternatives [1].

Bacteriocins present a promising alternative therapeutic approach for addressing multidrug-resistant and chronic bacterial infections. Bacteriocins are ribosomally synthesized peptides of low molecular weight, typically >10 kDa, and consist of 30 to 60 amino acids in length. Their nature is cationic, yet they also exhibit amphipathic characteristics due to the higher presence of lysine and arginine amino acid residues. These are linear polypeptides featuring secondary and tertiary structures; however, when dissolved in organic solvents such as trifluoroethanol or in phospholipid membranes, they adopt secondary helical structures[2,3]. Both Gram-positive and Gram-negative bacteria have a vast variety of these antimicrobial peptides. The bactericidal mechanism of bacteriocins primarily relies on their capacity to bind to receptors located on the bacteria cell surface, leading to cytotoxic effects on the bacterial cells[4] It is noteworthy that bacterial cells that produce bacteriocins exhibit resistance to their antimicrobial peptides, a phenomenon facilitated by specific immunity proteins generated by the host cells [2] The genes responsible for bacteriocin production and immunity are typically arranged in operon clusters and can be found on mobilizable elements, such as chromosomes in association with transposons or on plasmids [5].

The first bacteriocin was reported in 1925 and was produced by *Escherichia coli* and named "colicins" to indicate its microbial origin (6). In subsequent years, the bacteriocins produced by lactic acid bacteria have garnered considerable importance, particularly as the American Food and Drug Administration has conferred GRAS designation upon these microorganisms. Therefore, the first bacteriocin named Nisin produced from *Lactococcus lactis* has been widely used in commercial applications as an antimicrobial agent, anticancer agent and food preservative [7-9].

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Lactic acid bacteria (LAB) are a class of fastidious, catalase-negative, gram-positive, non-spore-forming, cocci or rods that have a high threshold for low pH. The LAB group includes more than 60 taxa, such as *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Lactococcus*, *Enterococcus*, *Weisella*, *Streptococcus* and others (10). LAB bacteriocins are well-known for their action throughout a broad pH range and for their intrinsic tolerance to high heat stress (11). Many authors reported that LAB isolates from various sources have antimicrobial properties in cell-free extracts (12). Concerns about antibiotic resistance, a need for natural food preservatives, and the possibility of using these microbes in sectors such as cosmetics, pharmaceuticals, and food all point to the necessity for novel lactic acid bacteria and bacteriocins with versatile uses. These microbes would provide a safer alternative to chemical additives while still being effective against a wider range of pathogens and spoilage organisms. Hence the present study, a novel bacteriocin-producing *Lactobacillus casei* isolated from goat milk was characterized and its antibacterial, antifungal, anti-inflammatory and anticancer activity was analyzed.

2. Material and methods

2.1. Screening and isolation of novel lactic acid bacteria

Thirty-five different dairy and non-dairy samples were collected from Ernakulam District in Kerala and screened for Lactobacillus species using MRS medium (HiMedia, India). The obtained lactic acid bacteria (LAB) isolates were categorized based on their sources of origin. The antimicrobial activity of the isolates was evaluated using agar well diffusion method against foodborne pathogens *Bacillus subtilis, Escherichia coli, Klebsiella pneumonia, Salmonella typhi, Staphylococcus aureus* and *Pseudomonas aeruginosa* along with Tetracycline as a standard.

2.2. Morphological and molecular identification of isolated bacteria using 16srRNA sequencing

Morphological identification of the organism isolated from goat milk showing enhanced antimicrobial activity was done using microscopic and biochemical analysis. The organism's genomic DNA was extracted using the QIAGEN genomic kit. 16S rDNA was amplified with 27F and 1492R primers. Amplified amplicon (1500 bp) was purified using a gel elute cleanup kit, Sigma and sequenced on a Sanger sequencing-based genetic analyzer. BLAST studies against the NCBI Genbank database using Aligner Geneious software yielded the consensus 16S rDNA gene sequence from forward and reverse sequencing. The top 10 sequences were aligned using Clustal W based on the greatest identity score. The Maximum Likelihood method and Tamura-Nei model were employed to infer the evolutionary history. The tree with the highest log-likelihood (-2090.79) is displayed, indicating the percentage of trees supporting taxa clustering. Initial trees were generated using Neighbor-Join and BioNJ algorithms, selecting the topology with superior log likelihood. Site proportions with unambiguous bases in each clade were shown. Analyses were performed in MEGA X.

2.3. Molecular characterization of bacteriocin gene-Cystathionine gamma-synthasegene

Cystathionine gamma-synthase gene-specific primers were designed (FP-5' CCAAACTAGTTCATGGTCCAC 3' and RP-5' TCAAATCCTGCTGATCTTCC 3') using the NCBI primer BLAST tool. The specifically designed primers are synthesized by Eurofins Genomics, Bangalore, India.

2.4. Partial purification of bacteriocin and HPLC analysis

Bacteriocin from goat milk isolate GO3 was partially purified using 60% ammonium sulfate precipitation followed by dialysis with 3 buffer changes. The partially purified sample was subjected to a reversed-phase high-performance liquid chromatography analysis to confirm the presence of bacteriocin. A Shimadzu High-Performance Liquid Chromatography (HPLC) system with a C18 column (4.6 × 250 mm) was used in the study. The mobile phase composed of 0.1% trifluoroacetic acid in water (solvent A) and in acetonitrile (solvent B) was used 25:75 volume/volume ratio with 1mL/min flow rate. The eluted bacteriocin peptide was detected at the wavelength of 250 nm.

2.5. In vitro studies on biotherapeutic applications of isolate GO3

2.5.1. Antibacterial activity by microtiter plate assay

The antibacterial activity of isolate GO3 was performed according to Mezaini et al., 2009 [13]. The partially purified bacteriocin was tested for their antibacterial activity against five food-borne pathogenic bacteria- *Bacillus subtilis, Staphylococcus aureus, Salmonella typhi, Klebsiella pneumoniae* and *Escherichia coli* by microtiter plate assay using 96 wells microtiter plates. All the food-borne pathogens were grown in nutrient broth overnight. 100 µL of partially purified bacteriocin in varying concentrations (50 µL, 100 µL, 150 µL and 200 µL) and 100 µL of the targeted food-borne pathogen (10^4 cells/mL) were pipetted into the wells labeled as S1. 200 µL of the targeted bacteria was used as a positive control (S2). All microtiter plates were incubated at 30°C for 24h. Bacterial growth was monitored at an optical density of 560 nm using a microplate reader. The percentage growth inhibition of targeted bacteria was measured using Equation 1.

$$\%\ \text{of growth inhibition of the targeted bacteria} = \frac{\text{OD S1 (24h)} - \text{OD S1 (0h)}}{\text{OD S2 (0h)}} \times 100 \qquad -\ \mathbf{eq(1)}$$

Where, S1- partially purified bacteriocin and targeted bacteria

S2-targeted bacteria only

2.5.2. Antifungal activity using microtiter plate assay

The partially purified bacteriocin was tested for their antifungal activity against pathogenic fungi- *Fusarium oxysporum*, *Sclerotium rolfsii*, *Rhizoctonia solani* and *Phytophtora infestans* using microtiter plate assay. All the fungal pathogens were grown in potato dextrose broth for 72 h. 100 µL of partially purified bacteriocin in varying concentrations (50 µL, 100 µL, 150 µL and 200 µL) and 100 µL of the fungal pathogen (10^5 conidia/mL) were pipetted into the wells labeled as S1. 200 µL of the targeted fungal pathogen was used as a positive control (S2). All microtiter plates were incubated at 30°C for 72h. Fungal growth was monitored at an optical density of 560 nm using a microplate reader. The percentage growth inhibition of targeted fungal pathogen was measured using Equation 2.

$$\%\ \text{of growth inhibition of the targeted fungal pathogen} = \frac{\text{OD S1 (24h)} - \text{OD S1 (0h)}}{\text{OD S2 (0h)}} \times 100 \quad -\mathbf{eq(2)}$$

Where, S1- partially purified bacteriocin and targeted fungal pathogen

S2-targeted fungal pathogen only

2.5.3. Anti-inflammatory activity

The anti-inflammatory properties of bacteriocin derived from the goat milk isolate GO3 were conducted utilizing the albumin denaturation inhibition method. The reaction mixture comprised partially purified bacteriocin at concentrations ranging from 50 µg/mL to 200 µg/mL and 1% bovine albumin. A negative control with only 1% bovine albumin was kept. The pH of the reaction mixture was adjusted using a small amount of 1N HCl. The reaction mixture was incubated at 37ºC for 20 min and then heated to 51ºC for 20 min. After cooling, the turbidity of the samples was measured at 660nm (UV Visible Spectrophotometer Model 371, Elico India Ltd). The experiment was performed in triplicate. The percentage inhibition of protein denaturation was calculated as Equation 3.

$$\%\ \text{inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100 \quad -\mathbf{eq(3)}$$

2.5.4. Antidiabetic activity using α-amylase inhibition assay

Different concentrations of lyophilized partially purified bacteriocin in citrate buffer (100, 200, 300, 400 and 500µg/mL) were prepared. Standard acarbose was prepared in dimethyl sulfoxide at a concentration of 1 mg/mL. 500 µL of test/standard was added to 500 µL of α-amylase (0.5 mg/mL) and incubated for 10 min at room temperature. 500 µL of 1.0% starch solution was added to the reaction mixture and incubated for another 10 min. On incubation, 1ml of the 3,5 dinitrosalicyclic (DNS) acid was added to the reaction mixture and heated in a boiling water bath for 5 min. The solution was cooled and diluted with 10 ml of distilled water. The absorbance was measured at 540 nm against the reagent blank. A negative control containing only citrate buffer solution was also kept simultaneously. The α-amylase inhibition was expressed as a percentage of inhibition. The percentage inhibition was calculated employing Equation 4.

$$\%\ \text{inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100 \quad -\mathbf{eq(4)}$$

2.5.5. Antioxidant activity using DPPH assay

The radical scavenging activity of bacteriocin was estimated using DPPH assay. 0.5 mL of partially purified bacteriocin in varying concentrations (50µl, 100µl, 150µl, 200µl) and 0.5 ml of 1mM DPPH dissolved in ethanol was added and reaction mixture was made up to 2ml using ethanol. The reaction mixture was incubated at room temperature for 30 minutes. Ethanol was used as the blank and a tube without bacteriocin containing only 0.5 mL DPPH and 1.5 mL ethanol served as the positive control. After 30 minutes of incubation, the discoloration of the purple color was measured at 518 nm in a spectrophotometer. The assay was calculated as Equation 5.

$$\text{Radical scavenging assay} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100 \quad -\mathbf{eq(5)}$$

2.5.6. Anticancer activity using MTT assay

The anticancer activity of partially purified bacteriocin was analyzed using MTT assay. 200 µL of Human colon cancer cell line (HCT116) cell suspension was seeded in a 96-well plate at the required cell density (20,000 cells per well), without the partially purified bacteriocin. Allow the cells to grow for about overnight. Appropriate concentration of partially purified bacteriocin ranging 50 µg/mL to 200 µg/mL was added. The 96 well plate was incubated at 37°C for 48 h in a 5% CO2 atmosphere. On incubation, spent media was removed and MTT reagent was added at a final concentration of 0.5mg/mL of total volume and further incubated for 3 hours. After incubation, the MTT reagent was removed and 100µL of solubilisation solution (DMSO) was added. The plates were read on an ELISA reader at 570nm. The IC50 value was determined by using linear regression equation (Equation 6)

$$Y = Mx + C \quad \text{eq(6)}$$

where, Y = 50, M and C values were derived from the viability graph.

2.6. Statistical analysis

Each experiment was performed in triplicate and the data were presented as mean± standard deviation(SD). Statistical tests were analyzed using the GraphPad Prism version 9.0. Differences among the groups were performed by One-way analysis of variance (ANOVA). P-values below 0.05 indicate statistical significance. The percentage inhibition and growth inhibition data from antimicrobial, antifungal, anti-inflammatory, antidiabetic, antioxidant, and anticancer assays underwent comparison with control group results. The IC50 value for anticancer activity was determined using linear regression analysis.

3. Results

3.1. Screening and identification of bacteriocin-producing bacteria

Among 26 isolates, bacteria isolated from goat milk showed enhanced antimicrobial activity against various pathogens. The isolate was labeled as GO3. Microscopic and biochemical tests revealed the isolate is a Gram-positive rod, non-sporulating and resilient to pH and bile salts, with adaptability to gastric enzymes and surfactants. The isolate GO3 is indole positive, phenylamine deamination positive and methyl red negative. Morphological and biochemical detailed characterization of isolate GO3 were previously published (14). Based on nucleotide homology and 16S RNA phylogenetic analysis, the isolate was identified as *Lactobacillus casei* strain GO3 (GeneBank accession no. ON059683), with maximum evolutionary similarity of 100 % to *Lactobacillus casei* strain NR115322.1. In the 16S rRNA sequence-based maximum likelihood phylogenetic tree (Figure 1), the isolated strain GO3 was separated from its closest *Lactobacillus*-related species relative, with excellent bootstrap support. The strain GO3 showed distinct, separate clusters with adequate phylogenetic distance with various species of the genus *Lactobacillus*.

3.2. Molecular identification of cystathionine gamma-synthase gene

The presence of cystathionine gamma-synthase gene in the isolated GO3 strain was identified by PCR. Approxi-

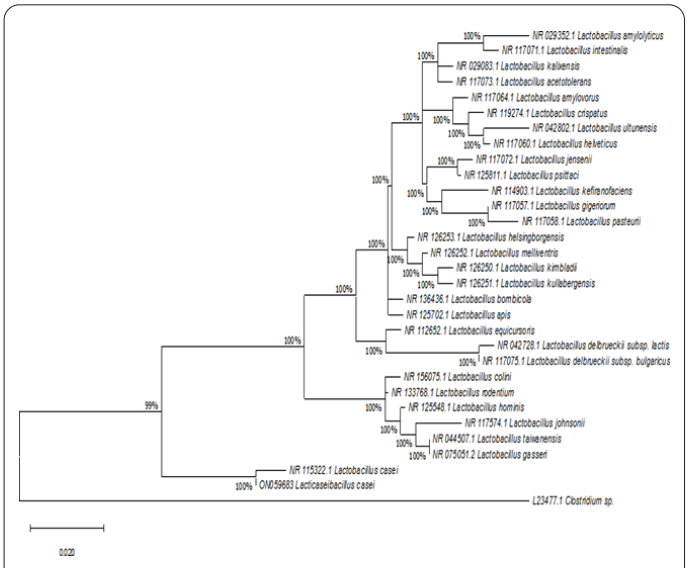

Fig. 1. 16S rRNA gene sequence-based phylogenetic tree, showing the phylogenetic position of GO3 isolates, *Lactobacillus casei* related to members of the genus *Lactobacillus*. Reference sequences and their accession numbers are given in brackets. Bootstrap consensus tree inferred from 1000 replicates.

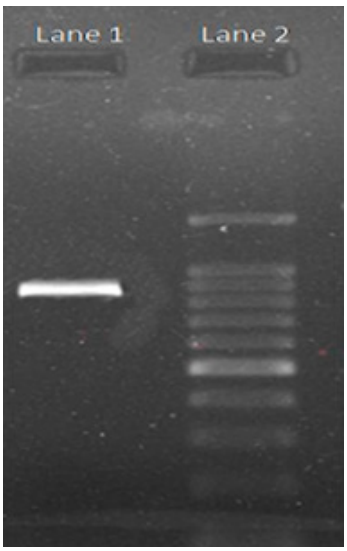

Fig. 2. Molecular identification of cystathionine gamma-synthase gene. Lane1: PCR product of cystathionine gamma-synthase gene; Lane2: DNA marker.

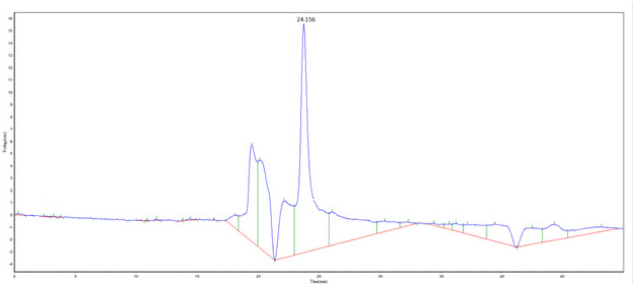

Fig. 3. HPLC chromatogram of partially purified bacteriocin from *Lactobacillus casei* GO3 showing retention time of 24.156.

mately 888 base pair bands were observed on the agarose gel electrophoresis analysis (Figure 2). DNA sequencing followed by the blast analysis of the gene of the isolated GO3 showed 100% identity to cystathionine gamma-synthase gene of *Lacticaseibacillus casei* strain.

3.3. Purity analysis of partially purified bacteriocin using HPLC analysis

Bacteriocin from *Lactobacillus casei* GO3 was partially purified using ammonium sulphate precipitation. The purified bacteriocin exhibited a yield of 2.66% along with a purification fold of 54.9. Using reverse-phase high-performance liquid chromatography, the bacteriocin purity was examined. The compound's primary peak retention duration was found to be 24.156 which correlates with the commercially available standard nisin (Figure 3).

3.4. Anti-bacterial activity

In the present investigation, different bacteriocin concentrations (50, 100, 150 and 200 µl) of *Lactobacillus casei* GO3 were tested against pathogenic bacterial strains such as *Bacillus subtilis, Staphylococcus aureus, Salmonella typhi, Klebsiella pneumoniae* and *Escherichia coli*. Tetracycline (100 µl) was used as standard for antibacterial activity. Antibacterial screening of *L.casei* showed concentration-dependent growth inhibition of pathogenic bacterial strains. *L. casei* showed maximum growth inhibition (76.4 %) against *B. subtilis* and minimum (46.2 %) against *S. typhi* at the maximum concentration (200 µl). Tetracycline showed a consistent growth inhibition against all the pathogens ranging from 65% to 75% (Table 1).

3.5. Anti-fungal activity

In the present investigation, different bacteriocin concentrations of Lactobacillus casei were evaluated for antifungal activity against five pathogenic fungi i.e., *Fusarium oxysporum, Sclerotium rolfsii, Rhizoctonia solani* and *Phytophthora infestans*. Gentamycin (100 µL) was used as standard for antifungal activity. Antifungal screening of L.casei showed concentration-dependent growth inhibition of pathogenic fungal strains. L.casei showed maximum growth inhibition (47.7%) against *Phytophthora infestans* and minimum (42.2%) against Fusarium oxys-

Table 1. Effect of different bacteriocin concentrations of *L. casei* on growth inhibition of pathogenic bacteria.

S.N.	Pathogenic Bacteria	Concentration of Bacteriocin of *Lactobacilluscasei* (µL)				Tetracycline (µL)
		50	100	150	200	100
		Growth inhibition (%)				
1	*Staphylococcus aureus*	44.5	47.5	51.2	53.6	70.3
2	*Bacillus subtilis*	65.4	72.9	74.3	76.4	75.3
3	*Salmonella typhi*	34.6	40.3	42.4	46.2	72.1
4	*Klebsiella pneumonia*	46.6	52.5	54.7	57.8	65.3
5	*Escherichia coli*	59.7	67.3	70.1	73.4	71.8

Table 2. Effect of different bacteriocin concentrations of L. casei on growth inhibition of pathogenic fungi.

S.N.	Pathogenic fungi	Concentration of Bacteriocin of *Lactobacilluscasei* (µL)				Gentamycin(µL)
		50	100	150	200	100
		Growth inhibition (%)				
1	*Fusarium oxysporum*	19.9	32.7	35.8	42.2	67.1
2	*Sclerotium rolfsii*	27.1	35.6	36.4	37.3	72.3
3	*Rhizoctonia solani*	21.3	28.9	38.1	44.8	72.8
4	*Phytophtora infestans*	32.1	36.9	43.2	47.7	76.1

porum at the maximum concentration (200 µL**)** (Table 2).

3.6.Anti-inflammatory activity

In the present study, the *in vitro* anti-inflammatory activity of partially purified bacteriocin from *Lactobacillus casei* was determined by percentage inhibition of protein denaturation method and compared with standard drug Aspirin. *L. casei* showed protein denaturation inhibition 11.99, 24.74, 38.78 and 57.91 % at the concentration of 50, 100, 150 and 200 µg/mL, respectively. The standard drug Diclofenac showed protein denaturation inhibition 77.41 % at the concentration of 100 µg/mL (Table 3).

Table 3. Anti-inflammatory activity of the partially purified bacteriocin.

S.N.	Concentration (µg/ml)	Bovine serum albumin Denaturation Inhibition (%)
1	50	11.99
2	100	24.74
3	150	38.78
4	200	57.91

3.7. Antidiabetic activity

In the present study, different concentration of partially purified bacteriocin of *L. casei* was evaluated for α- amylase inhibition activity. It is a carbohydrate hydrolyzing enzyme and inhibition of activity of theseenzymes aids in decreasing postprandial hyperglycemia or blood glucose levels. Therefore, LAB strains were evaluated as an antidiabetic agent. Lyophilized solution of *L. casei* showed α-amylase inhibition (%) 11.43, 25.87, 40.30, 55.76 and 58.86% at the concentration of 100, 200, 300, 400 and 500µg/ml respectively. On the other hand, α- amylase activity inhibited by Acarbose was 79.47% at the concentration of 500 µg/ml, respectively (Table 4).

Table 4. α-amylase inhibition analysis.

Vol. of supernatant (µl)	% of inhibition
100	11.43
200	25.87
300	40.30
400	55.76
500	58.86

3.8. Antioxidant activity

Results obtained for percentage inhibition of the free radicals showed radical scavenging activity by their electron transfer or hydrogen donating ability. These results showed that bacteriocin inhibited the free radicals by 69% when in comparison with the control drug Aspirin (Table 5).

Table 5. DPPH radical scavenging analysis.

Sl.No	Concentration of Bacteriocin	Percentage ofInhibition
1	50	35.71
2	100	43.57
3	150	58.57
4	200	69.29

3.9. Anticancer activity (MTT assay)

In this study, partially purified bacteriocin was evaluated to analyse the cytotoxicity effect on HCT116 cell lines. The results demonstrated suggested that *L. casei* GO3 showed toxicity in natureafter the treatment period of 48 h with IC50 value of 15% **(Figure 4).**

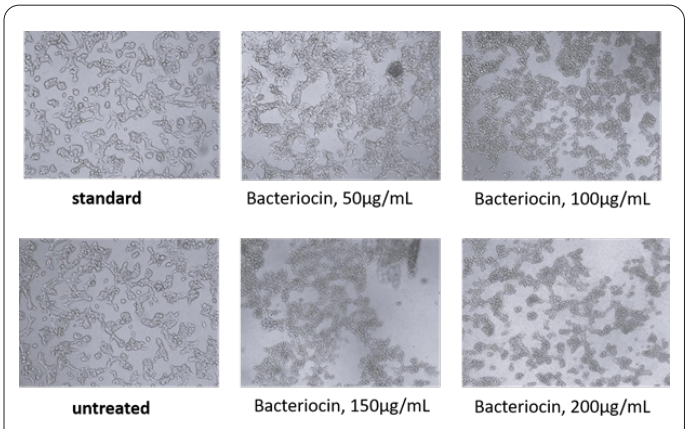

Fig. 4. Cytotoxicity effect of different concentrations of GO3 strain against HCT116 cells.

4. Discussion

According to the Food and Drug Regulation Authority, major foodborne pathogens such as *Campylobacter spp., Salmonella spp., Escherichia coli, Staphylococus aureus, Clostridium species* demonstrated antibiotic resistance and multidrug resistance, presenting a significant threat to public health [15]. Most recent research has focused on studying these bacteriocins because of some limitations on therapeutic antibiotic use in food processing and production, as well as the nontoxicity of therapy with antimicrobial peptides produced from LAB. Given the fact that bacteriocins are cleaved by intestinal proteases, thereby decreasing their *activity in vivo* conditions. Hence, they are considered safe and effective in inhibiting the growth of pathogens [16] . Additionally, bacteriocins are inherently bactericidal or bacteriostatic due to their capacity to harness holes in the cell membrane of susceptible bacteria [17]. In the present study, bacteriocin purified from isolate *Lactobacillus casei* from goat milk was studied.

In the present study, the isolate *Lactobacillus casei* GO3 was able to withstand broad range of pH, and temperature

and was not inhibited in presence of bile salts indicating its probiotic potential. The genetic basis for its metabolic adaptability may be due to presence of the cystathionine gamma-synthase gene (*MetB*) detected in the isolate GO3, which showed high homology to *Lacticaseibacillus casei* strain *MetB* gene [18]. MetB gene is responsible for synthesis of sulfur amino acid cysteine via transsulfuration pathways, It is indicated that this gene is involved in the synthesis of sulfur amino acids, which plays a significant role in growth and survival of organism and in maintaining its redox potential [19,20]

Antimicrobial activity is the chief critical attribute of any viable probiotic species. The LAB strain GO3 evaluated in this investigation has distinct degrees of antagonistic action against the indicator pathogens. The partially purified bacteriocin in this study demonstrated a broad spectrum of antibacterial activity, achieving 76.4% inhibition against gram-positive *B. subtilis* and 46.2% against gram-negative *Salmonella typhi*. Additionally, it exhibited antifungal activity, with maximum growth inhibition of 47.7% against *Phytophthora infestans* and a minimum of 42.2% against *Fusarium oxysporum*. It has been demonstrated that further purification procedures aid in reducing the MIC, suggesting that this may aid in lowering the MIC dose against particular microbes. Depending on the source, the kind of bacteriocin, and the target organism, different studies have shown varying effective ranges of bacteriocin for bacterial and fungal growth inhibition. Consistent with our results, partially purified bacteriocin from various Lactobacillus species showed a strong range of antibacterial and antifungal against various food-borne pathogens [21,22]

In addition to its antimicrobial properties, the bacteriocin exhibited significant anti-inflammatory, antidiabetic, antioxidant, and anticancer effects. Its ability to inhibit protein denaturation underscores its anti-inflammatory potential, comparable to that of conventional medications such as Diclofenac. Results from the bovine serum albumin denaturation assay showed that at 200 µg/mL, the percentage inhibition was 57.97%. The inhibition of inflammation by bacteriocins has therapeutic potential for a range of diseases [23]. Bacteriocin's ability to block alpha-amylase in this study varies with concentration, ranging from 11.7% to 57%. This suggests that it may be useful in controlling postprandial hyperglycemia, which is an important consideration when treating diabetes [24] Additionally, bacteriocins showed antioxidant activity by reducing free radical production by 69%, suggesting they could assist reduce free radicals and provide protection from oxidative stress [25] Further evidence of its potential anticancer effects came from the MTT experiment, which demonstrated cytotoxicity to HCT116 cell lines. Further investigation into its mechanisms of action and its therapeutic applications is required because the IC50 value indicates a high level of cytotoxicity [26]

Antimicrobial compounds that can function as biopreservatives are highly valuable for the feed and food sector, particularly concerning microbiological safety and food security considerations. The findings of this study demonstrated the efficacy of bacteriocin generated by Lactobacillus casei GO3 as an antibacterial and antifungal biomolecule, suggesting potential applications in agriculture and the food industry as a natural bio-controlling agent. The partially purified bacteriocin exhibited significant inhibition of bovine serum albumin, and α-amylase, and demonstrated notable anti-oxidative activity. The findings indicate a favorable outlook regarding the potential benefits of the probiotic LAB derived from goat milk. Future research will focus on the structural characterisation of bacteriocin and its interactions with target microbes.

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Conflict of interest
The authors declare that there is no conflict of interest.

Authors' contribution
All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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none.

Data availability
All data sets generated or analyzed during this study are included in the manuscript.

Ethics statement
This article does not contain any studies on human participants or animals performed by any of the author.

References

1. Gauba A, Rahman KM. (2023) Evaluation of Antibiotic Resistance Mechanisms in Gram-Negative Bacteria. Antibiotics 12:1590.doi:10.3390/antibiotics12111590
2. Simons A, Alhanout K, Duval RE. (2020) Bacteriocins, Antimicrobial Peptides from Bacterial Origin: Overview of Their Biology and Their Impact against Multidrug-Resistant Bacteria. Microorganisms 8:639.doi:10.3390/microorganisms8050639
3. Kumar P, Kizhakkedathu J, Straus S. (2018) Antimicrobial Peptides: Diversity, Mechanism of Action and Strategies to Improve the Activity and Biocompatibility In Vivo. Biomolecule 8:4. doi:10.3390/biom8010004
4. Dicks LMT, Dreyer L, Smith C, van Staden AD.(2018) A Review: The Fate of Bacteriocins in the Human Gastro-Intestinal Tract: Do They Cross the Gut–Blood Barrier?Front Microbiol 9:2297. doi:3389/fmicb.2018.02297
5. Criado R, Diep DB, Aakra Å, Gutiérrez J, Nes IF, Hernández PE, et al. (2006) Complete Sequence of the Enterocin Q-Encoding Plasmid pCIZ2 from the Multiple Bacteriocin Producer *Enterococcus faecium* L50 and Genetic Characterization of Enterocin Q Production and Immunity. Appl Environ Microbiol 72:6653–66. doi:10.1128/AEM.00859-06
6. Zacharof, M. P., & Lovitt, R. W. (2012). Bacteriocins produced by lactic acid bacteria a review article. *Apcbee Procedia*, *2*:50-56. doi:10.1016/j.apcbee.2012.06.010
7. Lahiri D, Nag M, Dutta B, Sarkar T, Pati S, Basu D, et al. (2022) Bacteriocin: A natural approach for food safety and food security. Front Bioeng Biotechnol 10:1005918. doi:10.3389/fbioe.2022.1005918
8. Kierończyk B, Rawski M, Mikołajczak Z, Świątkiewicz S, Józefiak D. (2020) Nisin as a Novel Feed Additive: The Effects on Gut Microbial Modulation and Activity, Histological Parameters, and Growth Performance of Broiler Chickens. Animals 10:101. doi:10.3390/ani10010101

9. Mattick ATR, Hirsch A, Berridge NJ. (1947) Further observations on an inhibitory substance (NISIN) from lactic streptococci.Lancet250:5–8.doi:10.1016/S0140-6736(47)90004-4
10. Mokoena MP. (2017) Lactic Acid Bacteria and Their Bacteriocins: Classification, Biosynthesis and Applications against Uropathogens:A Mini-Review.Molecules 22:1255. doi:10.3390/molecules22081255
11. Kumar A, Kumar D.(2015) Characterization of Lactobacillus isolated from dairy samples for probiotic properties. Anaerobe;33:117–23. doi:10.1016/j.anaerobe.2015.03.001
12. Bhattacharya D, Nanda PK, Pateiro M, Lorenzo JM, Dhar P, Das AK. (2022) Lactic Acid Bacteria and Bacteriocins: Novel Biotechnological Approach for Biopreservation of Meat and Meat Products. Microorganisms10:2058. doi:10.3390/microorganisms10102058
13. Mezaini A, Chihib NE, Dilmi Bouras A, Nedjar-Arroume N, Hornez JP.(2009) Antibacterial Activity of Some Lactic Acid Bacteria Isolated from an Algerian Dairy Product. J Environ Public Health 2009:678495. doi:10.1155/2009/678495
14. Jumaila KP, Chitra AV, Veettil VN.(2024) Isolation and Molecular Characterization of Therapeutic Probiotic Lactobacillus from Different Sources in Ernakulam-Kerala.J Pure Appl Microbiol 18:1752–1767. doi:10.22207/JPAM.18.3.01
15. de Azevedo PO de S, Converti A, Gierus M, Oliveira RP de S.(2019) Antimicrobial activity of bacteriocin-like inhibitory substance produced by Pediococcus pentosaceus: from shake flasks to bioreactor. Mol Biol Rep 46:461–9. doi:10.1007/s11033-018-4495-y
16. Wang Y, Qin Y, Xie Q, Zhang Y, Hu J, Li P. (2018) Purification and Characterization of Plantaricin LPL-1, a Novel Class IIa Bacteriocin Produced by Lactobacillus plantarum LPL-1 Isolated From Fermented Fish. Front Microbiol 9:2276. doi:10.3389/fmicb.2018.02276
17. Aljohani AB, Al-Hejin AM, Shori AB.(2023) Bacteriocins as promising antimicrobial peptides, definition, classification, and their potential applications in cheeses.Food Sci Technol 43:2009. doi:10.1590/fst.2009
18. Irmler S, SchÃ¤fer H, Beisert B, Rauhut D, Berthoud H.(2009) Identification and characterization of a strain-dependent cystathionine Î²/Î³-lyase in *Lactobacillus casei* potentially involved in cysteine biosynthesis.FEMS Microbiol Lett 295:67–76. doi:10.1111/j.1574-6968.2009.01580.x
19. Bogicevic B, Berthoud H, Portmann R, Bavan T, Meile L, Irmler S.(2016) Cysteine biosynthesis in *Lactobacillus casei* : identification and characterization of a serine acetyltransferase.Endo A,editor.FEMS Microbiol Lett 363:fnw012 . doi:10.1093/femsle/fnw012
20. Tikhomirova A, Rahman MM, Kidd SP, Ferrero RL, Roujeinikova A.(2024) Cysteine and resistance to oxidative stress: implications for virulence and antibiotic resistance. Trends Microbiol 32:93–104. doi:10.1016/j.tim.2023.06.007
21. Hassan MU, Nayab H, Rehman TU, Williamson MP, Haq KU, Shafi N, et al.(2020)Characterisation of Bacteriocins Produced by *Lactobacillus* spp. Isolated from the Traditional Pakistani Yoghurt and Their Antimicrobial Activity against Common Foodborne Pathogens. BioMed Res Int 2020:8281623. doi:10.1155/2020/8281623
22. Zhao S, Hao X, Yang F, Wang Y, Fan X, Wang Y.(2022)Antifungal Activity of Lactobacillus plantarum ZZUA493 and Its Application to Extend the Shelf Life of Chinese Steamed Buns. Foods 11:195. doi:10.3390/foods11020195
23. Guryanova SV.(2023) Immunomodulation, Bioavailability and Safety of Bacteriocins. Life 13:1521. doi:10.3390/life13071521
24. Kumari V. B. C, Huligere SS, Shbeer AM, Ageel M, M. K. J, S. JC, et al. (2022) Probiotic Potential Lacticaseibacillus casei and Limosilactobacillus fermentum Strains Isolated from Dosa Batter Inhibit α-Glucosidase and α-Amylase Enzymes. Microorganisms 10:1195.doi:10.3390/microorganisms10061195
25. Vougiouklaki D, Tsironi T, Tsantes AG, Tsakali E, Van Impe JFM, Houhoula D. (2023) Probiotic Properties and Antioxidant Activity In Vitro of Lactic Acid Bacteria. Microorganisms 11:1264. doi:10.3390/microorganisms11051264
26. Wang Y, Wang Y, Sun T, Xu J.(2024) Bacteriocins in Cancer Treatment: Mechanisms and Clinical Potentials. Biomolecules 14:831.doi:10.3390/biom14070831