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Harnessing the Potential of Bacteriocin in the Treatment of Typhoid Fever and Cholera: A Systematic Review

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Abstract

Background: Typhoid fever kills 135,000 to 230,000 people every year and affects an estimated 11–21 million people globally. About 95,000 people die annually from cholera, and an additional 2.86 million get infected. Bacteriocins have been reported to inhibit the growth of various pathogens such as *Salmonella typhi* and *Vibrio cholerae*.
Objective: The aim of the study was to identify bacteriocins having potential in treating typhoid fever and cholera.
Methods: The techniques utilized in this study adhere to the recommended reporting items for systematic reviews and meta-analysis (PRISMA) framework for systematic review. These methods encompass the processes of identification, screening, eligibility, and inclusion.
Results: A total of 13,012 literatures were obtained from google scholar and PubMed. After removal of duplicates, and screening of title, abstract and full-text, a total 34 articles were used for data extraction. Multiple lactic acid bacteria capable of producing highly effective bacteriocins against typhoid and cholera were found. Bacteriocins with potential to combat typhoid include enterocin LD3, bacteriocin DU10, bacteriocin LD4, plantaricin LP 21–2, plantaricin SLG1, enterocin B, enterocin A, plantaricin LD1, plantaricin JLA-9, bacteriocin LJR1, and bacteriocin LB44. Bacteriocins with potential to mitigate cholera include pediocin PA-1, enterocin 12a, and a silver nanoparticle-based bacteriocin. These bacteriocins act against etiological agents of typhoid fever and cholera by pore formation, destruction of cell wall and bactericidal action.
Conclusion Several anti-typhoid and anti-cholera bacteriocins have been identified. Further investigation into their modes action against *Salmonella typhi* and *Vibrio cholerae* is needed. Additionally, in vivo assessment of their potency in treating typhoid fever and cholera is urgently required.

Keywords: Bacteriocin, Salmonella typhi, Vibrio cholerae, Typhoid fever, Cholera

Introduction

The bacteria *Salmonella typhi* causes typhoid fever, an infection that can be fatal. Common vehicles for its transmission include tainted food and drink. Worldwide, typhoid fever affects an estimated 11–21 million people annually, killing between 135,000 and 230,000 people.[1,2] Cholera is caused by the ingestion of fecally contaminated food or water with high concentrations of *Vibrio cholerae*.[3] While the previous six cholera pandemics eventually subsided, the current (seventh) pandemic has endured for more than 50 years.[4] Cholera causes the death of

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approximately 95,000 individuals annually and infects an additional 2.86 million people.[3] A total of 249,793 cases of cholera and 2,137 fatalities were recorded from 25 countries across five WHO regions between 1st January 2024 and 30th June 2024. The highest number of cases was recorded in the Eastern Mediterranean Region, followed by the African Region, the Region of the Americas, the South-East Asia Region, and the European Region.[5]

In January 2023, the World Health Organization (WHO) elevated the worldwide cholera outbreak to the highest category of emergency it recognizes, grade 3. The WHO maintains its assessment of the global risk as very high and classifies the event as a grade 3 emergency due to the number of outbreaks, their geographic growth, and the shortage of vaccines and other resources.[5]

Current therapeutic and preventive approaches for typhoid fever and cholera involve the use of antibiotics, vaccines and good personal hygiene. However, the rapid and global spread of antibiotic resistance, shortage of vaccines, and global economic meltdwon significantly reduce the impact of these approaches.[6] Consequently, the global burden of typhoid fever and cholera continue to rise. This situation clearly underscores the urgent need for alternative therapeutics. Bacteriocins are one of the promising candidates for treating drug-resistant infectious diseases. They are a collection of bacterial peptides that are synthesized by ribosomes.[7]

Overall objective

The overall objective of this study is to identify bacteriocins that have potential in the treatment of typhoid fever and cholera.

Research question

What bacteriocins have antimicrobial activity against *Salmonella typhi* or *Vibrio cholerae*?

Materials and methods

Study protocol

The techniques utilized in this study adhere to the recommended reporting items for systematic reviews and meta-analysis (PRISMA) framework for systematic review. These methods encompass the processes of identification, screening, eligibility, and inclusion.

Data source and Search Strategy

A literature search was performed using the Google Scholar and PubMed databases. The research question was categorized into three distinct concepts: bacteriocin, *Salmonella typhi*, and *Vibrio cholerae* (Table 1). The search utilized a controlled vocabulary (MeSH terms), keywords, and their corresponding synonyms to methodically locate material that could provide information on the research subject.

Study selection

Inclusion criteria

The following studies were included. Studies in which:

1. The antimicrobial compound is a bacteriocin.
2. The bacteriocin has inhibitory activity against *Salmonella typhi* or *Vibrio cholerae*.
3. The bacteriocin producer is known.
4. The bacteriocin was purified.
5. The molecular weight of bacteriocin was determined.

Exclusion criteria

The following studies were excluded. Studies that:

1. Were not written in English language.
2. Did not involve characterization of the bacteriocin.

Screening

Duplicates were removed after which, titles of papers were evaluated to determine their relevance to the study objective. Subsequently, the abstracts were reviewed using specific criteria for inclusion and exclusion. Articles that successfully passed the initial screening based on their abstracts were then further evaluated through a thorough examination of the complete text, employing specific criteria to determine which articles should be included or excluded.

Data Extraction and Analysis

Data on bacteriocin producer, method of bacteriocin purification, molecular weight of bacteriocin, physicochemical properties of bacteriocin, antimicrobial activity of bacteriocin, and mechanism of action of bacteriocin were extracted. Descriptive analysis of data was carried out.

Results

Search Results

Google scholar search yielded a total 12,950 relevant articles. This comprises of 5,280 articles from google

scholar search using “bacteriocin” and "*Salmonella typhi*" and 7,670 articles from google scholar search using “bacteriocin” and "*Vibrio cholerae*" (Table 1). Of the total articles retrieved from google scholar, 59.3% were on bacteriocin in relation to Vibrio cholerae while 40.7% were relevant to bacteriocin in relation to *Salmonella typhi*.

Table 1: Google scholar search strategy and results

Concept	Results
bacteriocin "*Salmonella typhi*"	5,280
bacteriocin "*Vibrio cholerae* "	7,670

Literature in PubMed retrieved a total of 72 articles (Table 2). Further, PubMed search revealed that 55.6% of the relevant articles were on "Vibrio cholerae" while 44.4% were on "Salmonella typhi".

Table 2: PubMed search strategy and results

Concept	Results
((("Bacteriocins"[Mesh]) OR (Bacteriocin*[tiab])) OR (Lantibiotic*[tiab])) AND ((("*Salmonella typhi*"[Mesh]) OR ("*Salmonella typhi*"[tiab])) OR ("*Salmonella enterica serovar* Typhi"[tiab]))	32
((("Bacteriocins"[Mesh]) OR (Bacteriocin*[tiab])) OR (Lantibiotic*[tiab])) AND ((("*Vibrio cholerae*"[Mesh]) OR ("*Vibrio cholerae*"[tiab])) OR ("*Vibrio cholera*"[tiab]))	40

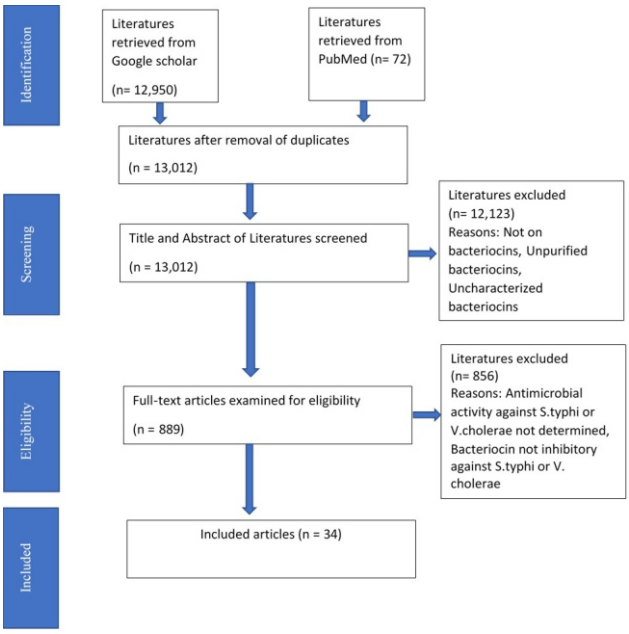

Figure 1: PRISMA flow diagram

A total of 13,012 literatures were obtained from the search strategy (Figure 1). After removal of duplicates, and screening of title, abstract and full-text, a total 34 articles were used for data extraction (Figure 1).

Anti-typhoid and anti-cholera bacteriocins

Enterocin Ld3

Enterocin LD3 is a type of bacteriocin synthesized by *Enterococcus hirae* (Table 3). It was isolated through chromatography. The heat stability of enterocin LD3 was demonstrated at temperatures up to 121°C and within a pH range of 2–6. Mass-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry revealed a resolved m/z value of 4114.6. The mechanism of action involves membrane potential dissipation, depletion of internal ATP, and cell death.[8] A separate investigation noted a rise in absorbance in the cells treated with enterocin LD3, indicating the liberation of nucleic acids and proteins. The elevated infrared absorbance additionally indicated its interaction with the cell membrane and nucleic acids of the targeted bacterium. The binding of bacteriocin to nucleic acids was also verified by gel retardation experiment. Transmission electron microscopy of the cells treated with bacteriocin showed the cell membrane being disrupted and the cytoplasmic contents leaking out.[9]

Bacteriocin Du10

Enterococcus faecalis DU10 produces a bacteriocin called bacteriocin DU10, which was isolated and purified using reverse-phase high-performance liquid chromatography (Table 3). The antibacterial activity of bacteriocin DU10 was found to be correlated with the presence of a band with a molecular weight of 6.3 kDa. MALDI-TOF mass spectrometry confirmed this finding, revealing a distinct peak at 6.313 kDa. The activity of bacteriocin DU10 was decreased by proteinase-K and pepsin, while slightly impacted by trypsin and α-chymotrypsin. The antimicrobial activity of bacteriocin DU10 was found to be resistant to heat treatments within the temperature range of 30 to 90°C for 30 minutes and 121°C for 10 minutes. Bacteriocin production in MRS reached its maximum level after 24 hours, but declined afterwards.[10]

Bacteriocin Ld4

Lactobacillus plantarum LD4 was isolated from Dosa, a fermented food in India. Its bacteriocin

Table 3: Bacteriocins with antimicrobial activity against *Salmonella typhi*

Bacteriocin	Form of bacteriocin	Antimicrobial activity				Mechanism of action	Reference
		ZOI (mm)	Activity (AU/ml)	MIC (µg/ml)	MBC (µg/ml)		
Enterocin LD3	Purified	8.0±0.00	-	-	-	Pore formation	[8,9]
Bacteriocin DU10	Purified	24.0±0.00	10,000	-	-	-	[10]
Bacteriocin LD4	Partially Purified	16.1±0.23	-	-	-	Pore formation	[11]
Plantaricin LP 21–2	Purified	9.3±0.10	-	-	-	Pore formation	[12]
Plantaricin SLG1	Purified	-	-	16.0	32	Pore formation	[13]
Enterocin B	Purified	11.0±1.5	-	-	-	Bactericidal	[14]
Enterocin A	Purified	14.0±0.00	-	-	-	Bactericidal	[14 16]
	Crude	7.0±0.00	-	-	-		
Plantaricin LD1	Crude	15±0.15	-	-	-	Bactericidal	[17]
Plantaricin JLA-9	Purified	-	-	16.0	-	Pore formation	[18]
Bacteriocin LJR1	Purified	-	-	7.8	-	Pore formation	[19]
Bacteriocin LB44	Purified	-	10	-	-	Pore formation	[20]
Nisin + ρ-cymene at 37^{0C}	Purified	-	-	0.3 and 1.5	-	-	[21]
Nisin + ρ-cymene at $4^{0}C$				0.3 and 2.5			
BacZY05-AgNPs	Purified	-	-	28.8	57.5	Bactericidal	[22]
Bacteriocin from *Enterococcus faecalis* CV7	Purified	24.0±0.0	-	-		-	[23]
Bacteriocin from *Lactobacillus acidophilus* TS1	Crude	≤4.0±00	-	-	-	Bactericidal	[24]
Bacteriocin from *Lactococcus lactis* ssp. lactis LL171	Purified	12.0±0.00	-	-	-	-	[25]
Bacteriocin from *Lactobacillus* spp.	Purified	12.0±0.00	-	-	-	-	[26]
Bacteriocin from *Enterococcus faecium* TA0033 and *Enterococcus faecalis* TA102	Crude	≥20	-	-	-	Bactericidal	[27]
Bacteriocin from *Lactobacillus plantarum* 14	Crude	11.5±0.80	-	-	-	-	[28]
Bacteriocin from *Lactobacillus* spp.	Crude	11-12	-	-	-	-	[29]

ZOI: Zone of inhibition, AU: Arbitrary unit, MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration

(bacteriocin LD4) was purified from the cell-free supernatant using ammonium sulphate precipitation, dialysis, and cation-exchange chromatography. Its molecular weight is around 6 kDa. Bacteriocin production starts after 3 hours and peak after 9 hours. Its antimicrobial activity remains unaffected by catalase, lipase, or α-amylase, but diminished by trypsin and protease. The bacteriocin induced efflux of K+ ions in target cells, leading to cell death (Table 3).[11]

Plantaricin LP 21–2

Lactobacillus plantarum SHY 21–2, derived from yak yogurt, has been found to synthesize bacteriocin. The bacteriocin was isolated and purified using reversed-phase high-performance liquid chromatography (RP-HPLC). The bacteriocin, named plantaricin LP 21–2, exhibited heat resistance and retained 96% of its antibacterial activity even after 121°C exposure. It had antibacterial properties throughout the pH range of 2–5 and was susceptible to total inactivation by pepsin, trypsin, and papain. A 48-hour kinetic experiment showed that plantaricin LP 21–2 production occurred after 14 hours of incubation in MRS broth, peaking after 26 hours. Target cells treated with plantaricin LP 21–2 showed wrinkles, poles, shrinkage of cytoplasm, and cavity formation, suggesting intracellular molecules were leakage (Table 3).[12]

Plantaricin SLG1

The bacterium *Lactobacillus plantarum* SLG1, which was obtained from yak cheese, has been found to produce a unique

Table 4: Bacteriocins with antimicrobial activity against Vibrio cholerae

Bacteriocin	Form of bacteriocin	Antimicrobial activity				Mechanism of action	Reference
		ZOI (mm)	Activity (AU/ml)	MIC (µg/ml)	MBC (µg/ml)		
Pediocin PA-1	Crude	1-9	-	-	-	Bactericidal	36
Enterocin 12a	Purified	12.0±0.3	-	-	-	Pore formation	37
BacZY05-AgNPs	Purified	-	-	57.5	115.0	Bactericidal	22
Bacteriocin from *Lactobacillus* spp.	Purified	6.0±0.00	-	-	-	-	26
Bacteriocin from *Lactobacillus ingluviei* ADK10	Purified	21.0±0.00	-	35	70	Cell wall damage	38

ZOI: Zone of inhibition, AU: Arbitrary unit, MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration

bacteriocin called plantaricin SLG. This bacteriocin was purified using a new technique involving magnetic liposome adsorption and reversed-phase high performance liquid chromatography. The bacteriocin's molecular weight is 1083.[25] Da and its amino acid sequence is Tyr-Gly-Asn-Gly-Val-Phe-Ser-Val-Ile-Lys. Production of plantaricin SLG in MRS medium reached maximum level after 24 hours of incubation at 37°C. Scanning electron microscopy revealed that plantaricin SLG1 acts bactericidally, compromising the cell membrane and potentially leading to the pathogen's death.[13]

Enterocin B

Enterococcus faecium TI 36, isolated from Spanish fermented sausage, produced enterocin B (EntB). The bacteriocin was purified using cation-exchange chromatography, hydrophobic interaction chromatography, and reverse-phase chromatography. The molecular weight of EntB is 5479.0 ± 1.2 Da. The gene encoding EntB was determined, resulting in a 71 amino acid peptide with a double-glycine type leader peptide. The mature enterocin B consists of 53 amino acid residues. Enterocin B exhibits heat stability, maintaining 25% of its activity when diluted in MRS. It also exhibits bactericidal activity against its bacterial target (Table 3).[14] Bacteriocinogenic *Enterococcus faecium* MR006 was isolated in a food called miso. Production of enterocin B by *Enterococcus faecium* MR006 reached it maximum after about 10 hours of incubating the producer.[30]

A research team has isolated enterocin B (EntB) from *Enterococcus faecium* BFE 900. The structural gene responsible for EntB production is located on a 2.2-kb HindIII fragment and a 12.0-kb EcoRI chromosomal fragment. EntB's genetic characteristics and production differ from previous findings due to a conserved sequence resembling a regulatory box upstream of the EntB gene. EntB production is constitutive and not regulated. The 2.2-kb chromosomal fragment contains an immunity gene for EntB, oriented in the reverse direction. The 12.0-kb chromosomal fragment lacks typical transport and other genes associated with bacteriocin production, setting EntB apart from other bacteriocin systems.[31] In another study, enterocin B was heterologously expressed in *Escherichi coli*. Purified enterocin B suppressed the growth of *Salmonella typhi*.[32]

Enterocin A

A team isolated *Enterococcus faecium* CTC 492, a bacteriocin producer, from fermented Spanish sausage. The bacterial chromosome was purified using various techniques, and its molecular weight was determined to be 4,829 Da. The structural gene of Enterocin A was sequenced, and it was found that an N-terminal leader sequence of 18 amino acid residues was removed during maturation. This leader is part of a group of double-glycine leaders found in many nonlantibiotic bacteriocins, some lantibiotics, and colicin V. A second open reading frame encoded a potential protein, possibly encoding the immunity factor of Enterocin A.[33] Enterocin A from *Enterococcus faecium* MMRA is heat and chemically stable. Moreover, it retained antimicrobial activity within a pH range of 2-12. Enterocin A was produced during the logarithmic growth phase. It did not attach to the surface of the producing cells and its mode of action was bactericidal (Table 3).[34]

A separate study isolated enterocin A-producing *Enterococcus faecium* from food content contained in the intestines of porcupine. This bacteriocin producer was named *Enterococcus faecium* por1. Enterocin A from *Enterococcus faecium* por1was highly inhibitory to *Salmonella typhi* 15. *Enterococcus durans* F3 was isolated from the gut of fresh water fish, Catla catla. It was ascertained to produce enterocin A. Cell-free supernatant of *Enterococcus durans* F3 culture, containing enterocin A was inhibitory to the growth of *Salmonella typhi*.[16]

Plantaricin Ld1

Plantaricin LD1, a bacteriocin synthesized by *Lactobacillus plantarum*, was studied for its biochemical and antibacterial properties. The

bacteriocin showed thermal stability at elevated temperatures, pH ranges of 2.0-8.0, and presence of organic solvents, surfactants, and detergents. It did not respond to catalase, amylase, and lipase enzymes, but its activity diminished in the presence of pepsin, trypsin, and proteinase K, indicating its proteinaceous nature. The molecular weight of the bacteriocin was around 6.5 kDa, and its antibacterial activity was verified using bioassay. Plantaricin LD1 suppressed the development of *Salmonella typhi*, with a zone of inhibition measuring 15±0.15 mm. The growth process of *Lactobacillus plantarum* LD1 followed a normal sigmoidal pattern, with a lag phase from 0 to 4 hours, late log phase at 8 hours, and stationary phase. Bacteriocin production began at the end of the lag phase and reached its peak at the stationary phase. The growth response was faster, with the highest generation occurring at 8 hours. Plantaricin LD1 suppressed the growth of *Salmonella typhi* in time-killing assay (Table 3).[17]

Plantaricin JLA-9

A novel bacteriocin, plantaricin JLA-9, was isolated from Suan-Tsai, a traditional Chinese fermented cabbage. The molecular mass was determined to be 1044 Da, and its amino acid sequence was confirmed using Edman degradation. Plantaricin JLA-9 showed excellent resistance to heat and maintained stability within a specific pH range. It exhibited sensitivity towards α-chymotrypsin, pepsin, alkaline protease, and papain. Plantaricin JL-9 exerts its action by suppressing oxidative metabolism and affecting the integrity of the cell membrane of its target (Table 3).[18]

Bacteriocin LJR1

Bacteriocinogenic *Pediococcus pentosaceus* LJR1 was isolated from goat rumen fluid and analyzed using a three-step process. The molecular weight of the isolated bacteriocin was determined to be 4.6 kDa. The antibacterial action was no longer present after protease treatment, indicating it is composed of proteins. The bacteriocin showed stability at various temperatures and pH ranges. Analysis using scanning electron microscopy revealed the presence of cell pores, deformation, and shrinkage on the surface of *Salmonella typhi* after treatment with the bacteriocin (Table 3).[19]

Bacteriocin LB44

Bacteriocin LB44, isolated from *Pediococcus pentosaceus* LB44 cell-free supernatant, showed stability at 121°C and pH 2.0-6.0, sensitivity to proteinase K, papain, and trypsin, and maintained full activity even in organic solvents. Its molecular weight is around 6 kDa, with the first ten amino acid residues indicating a novel chemical. The decrease in the number of living cells and the release of potassium ions from the target cells indicated a bactericidal mode of action. The bacteriocin-treated target cells exhibited ruptured cell membranes, as validated by Fourier Transform Infrared (FTIR) analysis (Table 3). This analysis indicated a contact between the bacteriocin and the phospholipids in the cell membrane of the target cells.[20]

Nisin and ρ-cymene

Nisin is a small, cationic, and hydrophobic antimicrobial peptide produced by *Lactococcus lactis* strains. It belongs to the Class I bacteriocins known as lantibiotics and is characterized by five β-methyl lanthionine rings. Eleven genes are involved in its production, organized in a transcriptional arrangement. The synthesis of nisin is controlled by the growth phase, including induction mediated by nisin through the NisRK two-component regulatory system. A study found that nisin and ρ-cymene did not hinder the growth of *Salmonella typhi* at different temperatures. However, when used concurrently, they showed additive antibacterial activity, with synergism being higher at 37°C compared to 4°C (Table 3).[21] Another investigation was carried out to confirm the combined effects of nisin and beta-lactams on clinical isolates of *Salmonella enterica serovar Typhi*. The minimum inhibitory concentrations (MICs) of the chosen b-lactams, EDTA, and nisin were determined using micro and macro broth dilution tests. The interaction between the agents was assessed using the fractional inhibitory concentration (FIC) index (checkerboard test) and time-kill assay in a laboratory setting. All of the combinations that were tested demonstrated synergy against the clinical strains that were tested, with the exception of three combinations. This was determined by analyzing the FIC index (checkerboard test) and doing a time-kill experiment. In particular, the combinations of nisin with ceftriaxone and nisin with cefotaxime showed remarkable synergistic effect.[35]

BacZY05-AgNPs

The study involved extracting and purifying bacteriocin BacZY05 from *Bacillus subtilis* ZY05, which was then mixed with silver nanoparticles to

create BacZY05-AgNPs. Analyzing these nanoparticles, they had an average diameter of 20-60nm and an oval or spherical morphology, revealing their potential in various applications. The antibacterial effectiveness of the BacZY05-AgNPs was assessed against many indicator strains by measuring their zone of inhibition using the agar well diffusion technique (Table 4). The antibacterial activity of bacteriocin-nanoconjugates showed a 1.3-1.5-fold increase compared to bacteriocin alone (ZOI-13 to 20 mm) and AgNPs alone (ZOI- 10-22 mm).[22]

Unnamed Bacteriocins

A bacteriocin from *Enterococus faecalis* CV7 was isolated using chromatography and RP-HPLC C-18 column. The molecular mass of Tricine-SDS PAGE was verified as 4.829 kDa. The bacteriocin showed antibacterial activity against foodborne pathogens like *Salmonella typhi* and was resistant to proteolytic enzymes, temperature variations, pH changes, solvents, and detergents.[23]

Lactobacillus acidophilus TS1 was isolated from fermented milk product dah and purified using RP-HPLC. Its molecular weight is 7.5 kDa and has antimicrobial activity that is rendered inactive by proteinase K and trypsin. The substance has broad thermostability and antibacterial properties across a large pH range. Supplementing with bacteriocin increased its effectiveness against *Salmonella typhi*. Bacteriocin synthesis began 4 hours into the growth phase and reached a maximum concentration of 1600 AU/ml after 9 hours. When bacteriocin was added to the growing culture of the indicator strains, there was a significant decrease in the optical density (OD) of the cultures over time.[24]

Lactococcus lactis ssp. lactis LL171, derived from Tulum Cheese in Turkey, produced a bacteriocin. The bacteriocin was purified using techniques like ammonium sulphate precipitation, dialysis, and gel filtration. Its molecular weight was confirmed to be 3.4 kDa, with a unique amino acid sequence. The bacteriocin showed excellent thermal stability and remained active across a broad pH range from 1 to 11. The bacteriocin exhibited a significant inhibition against *Salmonella typhi*.[25]

Bacteriocin-producing lactobacillus was isolated from water samples obtained from Yamuna river in new Delhi, India. The bacteriocin was purified using ammonium sulphate precipitation and dialysis. Its estimated molecular as determined by SDS-PAGE was 58 kDa. It was discovered that bacteriocin of lactobacillus isolated from polluted river water showed inhibitory activity against Salmonella typhi.[26]

Enterococcus faecium TA0033 and *Enterococcus faecalis* TA102, which produce bacteriocin, were obtained from human milk. The genome of the two isolates had the bacteriocin structural genes, entA and entB. In addition, E. faecalis TA102 also carried the entP and bac31 genes. The culture supernatant fluids of the two isolates had inhibitory effects on the growth of *Salmonella typhi*.[27]

In another study, bacteriocin from fecal Lactobacillus plantarum 14 displayed inhibitory activity against Salmonella typhi. This strain a multibacteriocin producer. Also, co-culture of *Lactobacillus plantarum* 14 with *Salmonella typhi* resulted in upregulation of the following bacteriocin genes, pln J, pln EF, pln NC8, and pln A.[28]

Five lactobacilli (Lb-17, Lb-33, Lb-108, Lb-112, and Lb-N3) that produce bacteriocin were obtained from curd. The cell-free culture supernatants of these five lactobacilli were determined to be thermo-tolerant after being exposed to heat treatment at a temperature of 100°C for a duration of 20 minutes. The antimicrobial activity of the bacteriocin from lactobacilli suppressed the growth of *Salmonella typhi*.[29]

Pediocin PA-1

The bacteriocin pediocin PA-1, produced by Pediococcus acidilactici strain PAC-1.0, is a peptide with a molecular weight of 4629 Da and 44 amino acids. It was purified using various methods, including gel filtration, ion exchange chromatography, dialysis, and HPLC. The amino acid sequence and disulfide linkage arrangement were determined, and the isoelectric point of 10.0 was calculated. Pediocin PA-1 shares similarities with other Gram-positive bacteriocins.[39]

Pediocin PA-1 produced by Pediococcus acidilactici QC38 isolated from Cotija cheese displayed good thermal and pH stability. Kinetics study revealed that pediocin PA-1 production started in the early exponential phase (10 hours) and peaked in the latter stage of the same phase (around 18 hours). Crude pedion PA-1 displayed inhibitory activity against Vibrio cholerae 36. Pediocin PA-1 functions by attaching to cytoplasmic membranes, incorporating bacteriocin molecules into the membranes, and creating the poration complex. This process ultimately results in cellular demise, which can happen with or without cellular lysis.[40]

Enterocin 12a

Enterocin 12a, produced by Vaginal *Enterococcus faecium* 12a, was purified using ammonium sulphate precipitation, cation-exchange chromatography, and RP-HPLC. It showed antibacterial activity at 60 and 80°C but inactivates at 100°C for over 30 minutes. It maintains effectiveness within pH ranges 2 to 10, with its highest activity at pH 4. Enterocin 12a was found to permeabilize cell membranes of multidrug resistant Vibrio cholerae. In addition, it was observed that enterocin 12a did not have a noteworthy impact on normal human peripheral blood mononuclear cells and red blood cells, thus demonstrating its safety.[37]

A *Lactobacillus ingluviei* ADK10 fermentation of whey resulted in the production of an antimicrobial peptide weighing 4892.26 Da. An investigation was conducted to determine the efficacy of this peptide against multidrug resistant Vibrio cholerae strains isolated from hospitals. The results showed that the peptide had a zone of inhibition of 21 mm, a minimum inhibitory concentration (MIC) of 35 μg/ml, and a minimum bactericidal concentration (MBC) or lethal dose (LD50) of 70 μg/ml. In addition, safety assessments such as Hemolytic assay and In vitro cytotoxicity assay were conducted. The tested peptide did not have significant membrane-damaging effects on red blood cells. Scanning electron microscopy demonstrated that the peptide specifically targeted the damage to the bacterial cell wall and caused the aggregation of bacterial cells.[38]

Discussion

Search Results

Typhoid fever and cholera are major contributors to global morbidity and mortalities.[1,3] Hence, they are receiving widespread interest by the research community as shown by the results of literature search. The outcomes of literature search on google scholar and PubMed revealed that majority of articles relevant to the research question were on Vibrio cholerae. This may be due to the WHO's declaration in 2023, which classifies cholera as a grade 3 disease, implying that it requires the highest level of attention 5. It is worth noting that despite the potential of bacteriocins in treating typhoid fever and cholera, which are more prevalent in Africa than other parts the world, Africa has not invested much in exploring the potential of these highly valuable antimicrobial agents.

Purification, biophysical characteristics and production kinetics of anti-typhoid and anti-cholera bacteriocins

The purification of anti-typhoid and anti-cholera bacteriocins involved centrifugation, ammonium sulphate precipitation, gel filtration, and chromatography, typically using cation-exchange chromatography, RP-HPLC, hydrophobic interaction chromatography, and LC-MS/MS. These methods have also been used successfully used in the purification of other bacteriocins[41]. Other rapid methods of bacteriocin purification should be explored to save time and cost. These methods include aqueous micellar two-phase system,[42] macroporous polyacrylamide monoliths,[43] and expanded bed adsorption chromatography.[44]

Molecular weights of the purified anti-typhoid and anti-cholera bacteriocins were determined via SDS-PAGE and MALDI-TOF mass spectrometry. These techniques have also been utilized to determine molecular weight of other bacteriocins.[45,46]

Bacteriocin production followed a sigmoidal curve, comprising of lag, log, stationary phase. Detectable level of bacteriocin occurred in the early log phase and peaked in the late log phase to early stationary phase. In a few of the bacteriocins degradation of the bacteriocin was observed after the stationary phase. These patterns were also observed for acidophilin 801.[47]

Antimicrobial activity of bacteriocins with anti-typhoid potential

Bacteriocins that display inhibitory activity against *Salmonella typhi* include enterocin LD3, bacteriocin DU10, bacteriocin LD4, plantaricin LP 21–2, plantaricin SLG1, enterocin B, enterocin A, plantaricin LD1, plantaricin JLA-9, bacteriocin LJR1, bacteriocin LB44, Nisin + ρ-cymene, BacZY05-AgNPs, and some unnamed bacteriocins (bacteriocins from *Enterococcus faecalis* CV7, *Lactobacillus acidophilus* TS1, *Lactococcus lactis* ssp. lactis LL171, *Lactobacillus* spp., *Enterococcus faecium* TA0033, Enterococcus faecalis TA102, *Lactobacillus plantarum* 14, *Lactobacillus* spp.). It is worth mentioning that all producers of these bacteriocins are lactic acid bacteria. This finding shows that lactic acid bacteria are a rich source anti-typhoid bacteriocins. Several studies have reported that lactic acid bacteria are producers of bacteriocins.[48,49] Majority of the anti-typhoid bacteriocins potentially acts against *Salmonella typhi* by inducing pores in the cell membrane, leading to loss of intracellular molecules and cell death. These

pore-forming bacteriocins include enterocin LD3, bacteriocin LD4, plantaricin LP 21–2, plantaricin SLG1, plantaricin JLA-9, bacteriocin LJR1, and bacteriocin LB44. Pore formation has also been reported to be a mechanism of action of bacteriocins against several bacteria.[50,51] The other potential mechanism of action of the anti-typhoid bacteriocins observed in this study is the bactericidal effect. This involves eradicating the bacteria by dismantling their cellular structures, impairing vital functions, or impeding their capacity to survive. This mode of action was displayed by enterocin B, enterocin A, plantaricin LD1, nisin + ρ-cymene, bacteriocin from *Lactobacillus acidophilus* TS1, bacteriocin from *Enterococcus faecium* TA0033 and *Enterococcus faecalis* TA102. Several bacteriocins have been reported to exert their antimicrobial action through bactericidal effect.[52] Mode of action of bacteriocin DU10, nisin + ρ-cymene, bacteriocins from *Enterococcus faecalis* CV7, *Lactococcus lactis* ssp. lactis LL171, *Lactobacillus* spp. *Lactobacillus plantarum* 14 have not been determined. It is worth noting that for most of the studies used in this systematic review, modes of action of the bacteriocin were investigated using other bacteria. Hence, it is important to confirm the proposed mechanisms of action using *Salmonella typhi*. Additionally, minimum inhibitory concentration and minimum bactericidal concentrations of the anti-typhoid bacteriocins should be determined.

Antimicrobial activity of bacteriocins with anti-cholera potential

Only a few characterized bacteriocins have antimicrobial activity against *Vibrio cholerae*. They are as follows, pediocin PA-1, enterocin 12a, BacZY05-AgNPs, and some unnamed bacteriocins (bacteriocins from Lactobacillus spp., *Lactobacillus ingluviei* ADK10). All producers of these bacteriocins are lactic acid bacteria. Bacteriocinogenic lactic acid bacteria have been widely reported 49. Proposed mechanisms of action of these bacteriocins include, pore formation (enterocin 12a), damage of cell wall (bacteriocin from *Lactobacillus ingluviei* ADK10) and bactericidal effect (pediocin PA-1 and BacZY05-AgNPs). Pore formation[53], inhibition of cell wall synthesis[54] and bactericidal action[55] has been observed other bacteriocins. Mode of action of bacteriocin from *Lactobacillus* spp. has not been ascertained. Moreover, mechanisms of action of the anti-cholera bacteriocins should also be carried out using *Vibrio cholerae*.

Conclusion

Several bacteriocinogenic lactic bacteria have been identified to produce potent anti-typhoid and anti-cholera bacteriocins. Those having anti-typhoid potential include enterocin LD3, bacteriocin DU10, bacteriocin LD4, plantaricin LP 21–2, plantaricin SLG1, enterocin B, enterocin A, plantaricin LD1, plantaricin JLA-9, bacteriocin LJR1, bacteriocin LB44, while those with anti-cholera potency include pediocin PA-1, and enterocin 12a and a silver nanoparticle-based bacteriocin (BacZY05-AgNPs). These bacteriocins exert their inhibitory effect against etiological agents of typhoid fever and cholera using mechanisms such as pore formation, destruction of cell wall and bactericidal action. Further investigation into the modes action of these bacteriocins against *Salmonella typhi* and *Vibrio cholerae* is needed. Additionally, *in vivo* assessment of their potency in treating typhoid fever and cholera is urgently required. Moreover, enhancement of their potency and yield using bioengineering could prove highly valuable in facilitating their clinical applications.

References

1. WHO. Typhoid. *World Health Organization*. 2023.https://www.who.int/news-room/fact-sheets/detail/typhoid (accessed 11 Aug2024).
2 CDC. Typhoid & Paratyphoid Fever CDC Yellow Book 2024. *Centers for Disease Control and Prevention*. 2024.https://wwwnc.cdc.gov/travel/yellowbook/2024/infections-diseases/typhoid-and-paratyphoid-fever#:~:text=Epidemiology,an%20estimated%20135%2C000%E2%80%93230%2C000%20deaths. (accessed 13 Aug2024).
3 Legros D. Global Cholera Epidemiology: Opportunities to Reduce the Burden of Cholera by 2030. *J Infect Dis* 2018; 218: S137–S140.
4 Hegde ST, Khan AI, Perez-Saez J, Khan II, Hulse JD, Islam MT et al. Clinical surveillance systems obscure the true cholera infection burden in an endemic region. *Nat Med* 2024; 30: 888–895.
5 WHO. Multi-country outbreak of cholera, External situation report #16 - 18 July 2024. *World Health Organization*. 2024.https://www.who.int/publications/m/item/multi-country-outbreak-of-cholera--external-situation-report--16---18-july-2024 (accessed 11 Aug2024).

6 Lawal L, Amosu OP, Lawal AO, Wada YH, Abdulkareem AO, Shuaib AK et al. The surging cholera epidemic in Africa: a review of the current epidemiology, challenges and strategies for control. International Journal of Surgery: *Global Health* 2024; 7. doi:10.1097/GH9.0000000000000440.

7 Ovchinnikov K V., Kranjec C, Thorstensen T, Carlsen H, Diep DB. Successful Development of Bacteriocins into Therapeutic Formulation for Treatment of MRSA Skin Infection in a Murine Model. *Antimicrob Agents Chemother* 2020; 64. doi:10.1128/AAC.00829-20/SUPPL_FILE/AAC.00829-20-S0001.PDF.

8 Gupta A, Tiwari SK, Netrebov V, Chikindas ML. Biochemical Properties and Mechanism of Action of Enterocin LD3 Purified from Enterococcus hirae LD3. *Probiotics Antimicrob Proteins* 2016; 8: 161–169.

9 Sheoran P, Tiwari S. Enterocin LD3 from Enterococcus hirae LD3 causing efflux of intracellular ions and UV-absorbing materials in Gram-negative bacteria. *J Appl Microbiol* 2019; 126: 1059–1069.

10 Perumal V, Repally A, Dasari A, Venkatesan A. Partial purification and characterization of bacteriocin produced by Enterococcus faecalis DU10 and its probiotic attributes. *Prep Biochem Biotechnol* 2016; 46: 686–694.

11 Kumar V, Sheoran P, Gupta A, Yadav JP, Tiwari SK. Antibacterial property of bacteriocin produced by Lactobacillus plantarum LD4 isolated from a fermented food. *Ann Microbiol* 2016; 66: 1431–1440.

12 Peng S, Song J, Zeng W, Wang H, Zhang Y, LWT JX- et al. A broad-spectrum novel bacteriocin produced by Lactobacillus plantarum SHY 21–2 from yak yogurt: Purification, antimicrobial characteristics and antibacterial. *LWT* 2021; 142.https://www.sciencedirect.com/science/article/pii/S0023643821001080 (accessed 3 Aug2024).

13 Pei J, Li X, Han H, Tao Y. Purification and characterization of plantaricin SLG1, a novel bacteriocin produced by Lb. plantarum isolated from yak cheese. *Food Control* 2018; 84: 111–117.

14 Casaus P, Nilsen T, Cintas LM, Nes IF, Hernández PE, Holo H. Enterocin B, a new bacteriocin from enterococcus faecium T136 which can act synergistically with enterocin A. *Microbiology (N Y)* 1997; 143: 2287–2294.

15 Ankaiah D, Esakkiraj P, Perumal V, Ayyanna R, Venkatesan A. Probiotic characterization of Enterococcus faecium por1: Cloning, over expression of Enterocin-A and evaluation of antibacterial, anti-cancer properties. *J Funct Foods* 2017; 38: 280–292.

16 Alshammari E, Patel M, Sachidanandan M, Kumar P, Adnan M. Potential Evaluation and Health Fostering Intrinsic Traits of Novel Probiotic Strain Enterococcus durans F3 Isolated from the Gut of Fresh Water Fish Catla catla. *Food Sci Anim Resour* 2019; 39: 844.

17 Gupta A, Tiwari SK. Plantaricin LD1: a bacteriocin produced by food isolate of Lactobacillus plantarum LD1. *Appl Biochem Biotechnol* 2014; 172: 3354–3362.

18 Zhao S, Han J, Bie X, Lu Z, Zhang C, Lv F. Purification and Characterization of Plantaricin JLA-9: A Novel Bacteriocin against Bacillus spp. Produced by Lactobacillus plantarum JLA-9 from Suan-Tsai, a Traditional Chinese Fermented Cabbage. *J Agric Food Chem* 2016; 64: 2754–2764.

19 Ladha G, Jeevaratnam K. Characterization of purified antimicrobial peptide produced by Pediococcus pentosaceus LJR1, and its application in preservation of white leg shrimp. *World J Microbiol Biotechnol* 2020; 36. doi:10.1007/S11274-020-02847-W.

20 Kaur R, Tiwari K. Membrane-acting bacteriocin purified from a soil isolate Pediococcus pentosaceus LB44 shows broad host-range. *Biochem Biophys Res Commun* 2018; 498: 810–816.

21 Rattanachaikunsopony P, Phumkhachorn P. Synergistic antimicrobial effect of nisin and p-cymene on Salmonella enterica serovar Typhi in vitro and on ready-to-eat food. *Biosci Biotechnol Biochem* 2010; 74: 520–524.

22 Chandrika K, Sachan A. Biosynthesis of bacteriocin BacZY05-silver nanoconjugates and evaluation of their antibacterial properties. *World J Microbiol Biotechnol* 2024; 40. doi:10.1007/S11274-024-04093-W.

23 Perumal V, Venkatesan A. Antimicrobial, cytotoxic effect and purification of bacteriocin from vancomycin susceptible Enterococcus faecalis and its safety evaluation for probiotization. *Food Science and Technology* 2017.https://www.sciencedirect.com/science/arti

cle/pii/S0023643816308337 (accessed 28 Jul2024).
24 Maqsood S, Hasan F, Masud T, Imran M. Preliminary characterisation of bacteriocin produced by Lactobacillus acidophilus TS1 isolated from traditional dahi. *Ann Microbiol* 2008; 58: 617–622.
25 Kumari A, Akkoç N, Akçelik M. Purification and partial characterization of bacteriocin produced by Lactococcus lactis ssp. lactis LL171. *World J Microbiol Biotechnol* 2012; 28: 1647–1655.
26 Bonhi R, Imran S. Study of Bacteriocin Production and Partial Characterization from Lactobacillus spp. Isolated from Polluted River Water. *International Journal of Science and Engineering* 2015; 3: 100–103.
27 Khalkhali S, Mojgani N. Bacteriocinogenic potential and virulence traits of Enterococcus faecium and E. faecalis isolated from human milk. *Iran J Microbiol* 2017; 9: 224–233.
28 Darbandi A, Ghanavati R, Asadi A. Prevalence of bacteriocin genes in Lactobacillus strains isolated from fecal samples of healthy individuals and their inhibitory effect against foodborne. *Iran J Basic Med Sci* 2021; 24: 1117–1125.
29 Negi YK, Pandey C, Saxena N, Sharma S, Garg FC, Garg SK. Isolation of antibacterial protein from Lactobacillus spp. and preparation of probiotic curd. *J Food Sci Technol* 2018; 55: 2011–2020.
30 Takumi O, Fujitoshi Y, Masao T, Takashi S, Koki Y. Enterococcus faecium MR006 Produces Enterocin B, Isolated from Miso. *Food Preservation Science* 2003; 29: 105–109.
31 Franz CMAP, Worobo RW, Quadri LEN, Schillinger U, Holzapfel WH, Vederas JC et al. Atypical genetic locus associated with constitutive production of enterocin B by Enterococcus faecium BFE 900. *Appl Environ Microbiol* 1999; 65: 2170–2178.
32 Ankaiah D, Esakkiraj P, Palanichamy E, Bharathi Antonyraj C, Ayyanna R, Ibrahim Basheer Ahamed S et al. Cloning, overexpression, purification of bacteriocin enterocin-B and structural analysis, interaction determination of enterocin-A, B against pathogenic bacteria and. *Int J Biol Macromol* 2018. doi:10.1016/j.ijbiomac.2018.05.002.
33 Aymerich T, Holo H, Håvarstein LS, Hugas M, Garriga M, Nes IF. Biochemical and genetic characterization of enterocin A from Enterococcus faecium, a new antilisterial bacteriocin in the pediocin family of bacteriocins. *Appl Environ Microbiol* 1996; 62: 1676–1682.
34 Rehaiem A, Martínez B, Manai M, Rodríguez A. Production of enterocin A by Enterococcus faecium MMRA isolated from 'Rayeb', a traditional Tunisian dairy beverage. *J Appl Microbiol* 2010; 108: 1685–1693.
35 Rishi P, Preet Singh A, Garg N, Rishi M. Evaluation of nisin-β-lactam antibiotics against clinical strains of Salmonella enterica serovar Typhi. *J Antibiot (Tokyo)* 2014; 67: 807–811.
36 Morales-Estrada AI, López-Merino A, Gutierrez-Mendez N, Ruiz EA, Contreras-Rodríguez A. Partial Characterization of Bacteriocin Produced by Halotolerant Pediococcus acidilactici Strain QC38 Isolated from Traditional Cotija Cheese. *Pol J Microbiol* 2016; 65: 279–285.
37 Sharma P, Kaur S, Chadha BS, Kaur R, Kaur M, Kaur S. Anticancer and antimicrobial potential of enterocin 12a from Enterococcus faecium. *BMC Microbiol* 2021; 21. doi:10.1186/S12866-021-02086-5.
38 Mandal A, Paul T, Patra A, Samanta A, Ch Mondal K, Kumar Nandi D. A Novel Antibacterial Peptide from Whey-Based Fermented Product of Lactobacillus Ingluviei: Valorization of Whey Waste. *Indian Journal of Biological Sciences* 2018; 24: 22–30.
39 Henderson JT, Chopko AL, van Wassenaar PD. Purification and primary structure of pediocin PA-1 produced by Pediococcus acidilactici PAC-1.0. *Arch Biochem Biophys* 1992; 295: 5–12.
40 Rodríguez M, Martínez M, Kok J. Pediocin PA-1, a wide-spectrum bacteriocin from lactic acid bacteria. *Crit Rev Food Sci Nutr* 2002; 42: 91–121.
41 Jamaluddin N, Stuckey DC, Ariff AB, Faizal Wong FW. Novel approaches to purifying bacteriocin: A review. *Crit Rev Food Sci Nutr* 2018; 58: 2453–2465.
42 Jozala AF, Lopes AM, Mazzola PG, Magalhães PO, Vessoni Penna TC, Pessoa A. Liquid–liquid extraction of commercial and biosynthesized nisin by aqueous two-phase micellar systems. *Enzyme Microb Technol* 2008; 42: 107–112.
43 Deraz S, Plieva FM, Galaev IY, Karlsson EN, Mattiasson B. Capture of bacteriocins directly from non-clarified fermentation broth using macroporous monolithic cryogels with phenyl ligands. *Enzyme Microb Technol* 2007; 40:

786–793.
44 Moreno MRF, Callewaert R, De Vuyst L. Isolation of bacteriocins through expanded bed adsorption using a hydrophobic interaction medium. *Bioseparation* 2001; 10: 45–50.
45 Dey BC, Rai N, Das S, Mandal S, Mandal V. Partial purification, characterization and mode of action of bacteriocins produced by three strains of Pediococcus sp. *J Food Sci Technol* 2019; 56: 2594–2604.
46 Milioni C, Martínez B, Degl'Innocenti S, Turchi B, Fratini F, Cerri D et al. A novel bacteriocin produced by Lactobacillus plantarum LpU4 as a valuable candidate for biopreservation in artisanal raw milk cheese. *Dairy Sci Technol* 2015; 95: 479–494.
47 Zamfir M, Callewaert R, Cornea PC, Vuyst L De. Production kinetics of acidophilin 801, a bacteriocin produced by Lactobacillus acidophilus IBB 801. *FEMS Microbiol Lett* 2000; 190: 305–308.
48 Perez RH, Zendo T, Sonomoto K. Multiple bacteriocin production in lactic acid bacteria. *J Biosci Bioeng* 2022; 134: 277–287.
49 Daba GM, Elkhateeb WA. Ribosomally synthesized bacteriocins of lactic acid bacteria: Simplicity yet having wide potentials – A review. *Int J Biol Macromol* 2024; 256: 128325.
50 Arbulu S, Kjos M. Revisiting the Multifaceted Roles of Bacteriocins : The Multifaceted Roles of Bacteriocins. *Microb Ecol* 2024; 87. doi:10.1007/S00248-024-02357-4.
51 Ladjouzi R, Dussert E, Teiar R, Belguesmia Y, Drider D. A Review on Enterocin DD14, the Leaderless Two-Peptide Bacteriocin with Multiple Biological Functions and Unusual Transport Pathway. *Antibiotics* 2023; 12. doi:10.3390/ANTIBIOTICS12071188.
52 Choi GH, Holzapfel WH, Todorov SD. Diversity of the bacteriocins, their classification and potential applications in combat of antibiotic resistant and clinically relevant pathogens. *Crit Rev Microbiol* 2023; 49: 578–597.
53 Negash AW, Tsehai BA. Current Applications of Bacteriocin. *Int J Microbiol* 2020; 2020. doi:10.1155/2020/4374891.
54 Teng K, Huang F, Liu Y, Wang Y, Xia T, Yun F et al. Food and gut originated bacteriocins involved in gut microbe-host interactions. *Crit Rev Microbiol* 2023; 49: 515–527.
55 Timothy B, Iliyasu AH, Anvikar AR. Bacteriocins of Lactic Acid Bacteria and Their Industrial Application. *Current Topics in Lactic Acid Bacteria and Probiotics* 2021; 7: 1–13.