

Characterization, Bio-preservative Potential and Functional Applications of Bacteriocin-Like Inhibitory Substance Produced by *Lactobacillus kefir* Isolated from Kefir

Shalini Priyadarishini C, Priya Iyer*

Department of Biotechnology, Women's Christian College (Affiliated to University of Madras), Chennai, Tamil Nadu, INDIA.

ABSTRACT

Background: *Lactobacillus* species are known to produce various inhibitory substances that combat pathogenic microorganisms. Kefir, a fermented milk product, is a rich source of these beneficial bacteria. **Objectives:** This study aimed to isolate, identify, and characterize a Bacteriocin-Like Inhibitory Substance (BLIS) produced by *Lactobacillus kefir* and to evaluate its potential applications in food preservation and biotechnology. **Materials and Methods:** *Lactobacillus kefir* was identified using morphological, biochemical, and molecular techniques. BLIS was produced in MRS broth under anaerobic conditions. Purification was achieved through a multi-step process involving ammonium sulphate precipitation, dialysis, ultrafiltration, and gel filtration chromatography. The substance was characterized using HPLC and MALDI-TOF mass spectrometry. Stability was assessed across various temperatures, pH levels, UV light exposure, and enzymatic treatments. Additionally, the antioxidant, cytotoxic, anticancer, and food preservative activities of the BLIS were evaluated. **Results:** The purified fractions demonstrated significant antimicrobial activity against *E. coli*. The proteinaceous nature of the substance was confirmed by the loss of activity following proteolytic enzyme treatment. The BLIS remained stable under varying physical and chemical conditions. Biological assays revealed potent antioxidant, cytotoxic, and anticancer properties. In food preservation trials, the BLIS effectively extended the shelf life of apple, carrot, cheese, and meat samples at both room temperature and 4°C. **Conclusion:** The partially purified BLIS from *L. kefir* exhibits strong antimicrobial, antioxidant, and anticancer activities, alongside excellent stability. These findings suggest that it is a promising bioactive compound for use as a natural preservative and therapeutic agent in the food and biotechnological industries.

Keywords: Anticancer Activity, Antioxidant Activity, BLIS, Food Preservation, *Lactobacillus kefir*.

Correspondence:

Priya Iyer

Associate Professor and Head, PG and Research, Department of Biotechnology, Women's Christian College (Affiliated to University of Madras), Chennai, Tamil Nadu, INDIA.
Email: brajuraj@yahoo.com

Received: 09-06-2026;

Revised: 19-08-2026;

Accepted: 27-10-2026.

INTRODUCTION

There is an increase in demand for safe and naturally preserved foods (Behera *et al.*, 2018). This requires the use of natural bioactive compounds instead of synthetic preservatives (Behera *et al.*, 2018). Among the many antimicrobial compounds, Bacteriocin-Like Inhibitory Substances (BLIS) produced by probiotic bacteria have shown considerable efficiency due to their effectiveness against food-borne pathogens and biodegradability (Todorov, 2009; Das and Goyal, 2014). BLIS also possess antioxidant and anticancer properties making these effective in food, and biotechnology applications (Yap *et al.*, 2022).

Kefir is a traditional fermented dairy product dominated by lactic acid bacteria and yeast (Leite *et al.*, 2013). *Lactobacillus kefir* has probiotic potential and the ability to produce bioactive compounds (Carasi *et al.*, 2014a). Previous studies have reported probiotic properties and antimicrobial activity (Bourrie *et al.*, 2016). Studies integrating purification, characterization and functional applications of BLIS from *L. kefir* are limited (Carasi *et al.*, 2022). Information on the antioxidant, anticancer, cytotoxic activity and food preservation potential of BLIS from *L. kefir* in a single study is limited (Bessa *et al.*, 2024).

With a growing interest in natural preservatives, comprehensive characterization of the *L. kefir* produced BLIS is necessary (Yap *et al.*, 2022; Carasi *et al.*, 2014a). The present study primarily aimed to isolate and identify *Lactobacillus kefir* from kefir, study the probiotic potential, produce and partially purify BLIS, characterize the BLIS, evaluate its functional properties and focus on the preservative potential of the BLIS. The results of this study are expected to contribute to the development of BLIS, as a natural



DOI: 10.5530/ajbls.20260213

Copyright Information :

Copyright Author (s) 2026 Distributed under
Creative Commons CC-BY 4.0

Publishing Partner : Manuscript Technomedia. [www.mstechnomedia.com]

preservative and also as a functional bioactive compound for biomedical and biotechnological applications (Yap *et al.*, 2022).

MATERIALS AND METHODS

Isolation and Identification of the BLIS- producing *Lactobacillus* strain

The sample, kefir was serially diluted using sterile distilled water and 100 µL was spread plated on MRS (de Man, Rogosa and Sharpe) agar plates (De Man *et al.*, 1960). The plates were anaerobically incubated in an anaerobic chamber with a GasPak system at 37°C for 24 hr. Morphologically distinct colonies were repeatedly streaked to obtain pure cultures. Colony morphology, Gram's staining and biochemical properties were studied. These were performed using standard microbiological procedures (Sharma, 2007). Molecular identification of the *Lactobacillus* strain was done by 16S rRNA gene sequencing.

Evaluation of Probiotic Characteristics

Autoaggregation, cell-surface hydrophobicity, tolerance to acidic conditions, salt concentration variation, bile salt concentration variation and phenol resistance were evaluated (Meira *et al.*, 2012). Autoaggregation and cell surface hydrophobicity were measured at 600 nm. Acid tolerance was tested at pH 2, 4, 6, 8 and 10, while salt tolerance was tested using MRS broth supplemented with 2-10% NaCl. 0.2-0.4% sodium taurodeoxycholate and 1-4 % phenol was used to check the bile tolerance (Singhal *et al.*, 2021) and phenol resistance. Haemolysis was assessed on blood agar incubated at 37°C for 24 hr. Antibiotic sensitivity was assessed by Kirby-Bauer disc diffusion method using commercial antibiotic discs on Mueller- Hinton agar plates (Charteris *et al.*, 1998).

Production and Partial Purification of Cell- Free Supernatant

A single *L. kefir* colony was inoculated into MRS broth and incubated anaerobically at 37°C for 24 hr. After incubation, the culture was centrifuged to obtain Cell- Free Supernatant (CFS) which was considered as crude BLIS preparation (Lowry *et al.*, 1951). BLIS was precipitated by gradual addition of ammonium sulphate to 80% saturation under constant stirring (Wu *et al.*, 2022). The proteins were then recovered by dialysis using a 3.5 kDa MWCO membrane against phosphate buffer (pH 7.0±0.25) and concentrated by ultrafiltration using a Vivaspin® 20 centrifugal filter (50 kDa MWCO).

Characterization of Partially Purified BLIS

The concentrated BLIS was subjected to gel filtration chromatography using Sephadex G-25 column (Brand-Williams *et al.*, 1995) and ten fractions were collected. The active fraction was selected for further characterization by selecting the fraction with both highest antimicrobial activity against *E. coli* (*E. coli* cultured procured from NCCS, Pune, India) and highest protein

concentration determined using Lowry method with Bovine Serum Albumin (BSA) as standard (Mosmann, 1983). Stability of the partially purified BLIS was evaluated under different pH conditions, temperatures, UV exposure, SDS treatment and trypsin digestion. The peptide profile of the active fraction was analysed by High Performance Liquid Chromatography (HPLC) at the National Agro Foundation, Chennai, using a Shimadzu HPLC system equipped with a C18 column and a Photodiode Array (PDA) detector (Brand-Williams *et al.*, 1995). The chromatograms were recorded at 220 nm. Matrix- Assisted Laser Desorption/ionization time- of- flight mass spectrometry (MALDI-TOF MS) analysis was performed at the IIT, Madras using a Bruker Daltonics MALDI-TOF mass spectrometer. The matrix used was α-cyano-4-Hydroxycinnamic Acid (HCCA), and the spectra were processed using Bruker flex analysis software (Brand-Williams *et al.*, 1995).

Functional evaluation of the partially purified BLIS

Cytotoxicity, anticancer and antioxidant activity assays were carried out at Life Tech Research Centre, Chennai. VERO cells and COLO 320 DM human colon cancer cells were purchased from National Centre for Cell Science, Pune. DPPH free radical scavenging assay was used to determine the antioxidant activity of the partially purified BLIS at different concentrations (Chuah *et al.*, 2019). Cytotoxicity against VERO cells and anticancer activity against COLO 320 DM human colon cancer cells were assessed using MTT assay (Chuah *et al.*, 2024; Kairey *et al.*, 2023). The food preservation potential was tested by treating sterile apple, carrot, cheese and meat samples with the partially purified BLIS (Carasi *et al.*, 2014b), followed by storage at room temperature and under refrigeration at 4°C. The treated samples were monitored for qualitative characters such as changes in colour, texture, visible fungal growth and overall spoilage during the storage period.

RESULTS

Isolation and Identification of *Lactobacillus kefir*

The bacterial isolate obtained from kefir using MRS agar produced medium- sized, white, circular colonies with entire margins, smooth surface, convex elevation, creamy consistency and an opaque appearance. Biochemical tests showed negative results for catalase, oxidase, MR-VP, indole, citrate utilization, gelatin liquefaction and TSI. Starch hydrolysis and milk coagulation was observed. Gram staining showed gram-positive rod- shaped cells. The biochemical profile was consistent with the characteristics of the genus *Lactobacillus* (Table 1). The *Lactobacillus* strain showed high similarity with *Lactobacillus kefir* based on nucleotide homology and phylogenetic analysis.

Probiotic Characteristics

Autoaggregation of the *L. kefir* increased throughout the incubation period, reaching 88.78% after 24 hr. This indicates

that the isolate has a strong ability to aggregate over time. Cell surface hydrophobicity was 61.22% showing favourable adhesion properties of the isolate. Maximum growth was observed at pH 6 and reduced growth at highly acidic (pH 2) and alkaline pH (pH 8- 10). Although growth decreased with increasing salt concentration, *L. kefir* strain showed tolerance up to 10% NaCl. Growth was also observed under different concentrations of bile salt (0.2%-0.4% sodium taurodeoxycholate) and phenol (1-4%). This shows the resistance of the strain to simulated gastrointestinal stress. No haemolytic activity was detected and this confirms the safety of the *Lactobacillus kefir*. Antibiotic sensitivity results showed sensitivity to doxycycline, tetracycline, azithromycin, ciprofloxacin and clindamycin, whereas resistance was observed against methicillin, cefuroxime and ceftriaxone.

Production, partial purification and characterization of BLIS

The cell-free supernatant obtained from *L. kefir* was subjected to ammonium sulphate precipitation, dialysis, ultrafiltration and gel filtration chromatography to obtain partially purified BLIS. Ten fractions were collected. Among the fractions obtained, F1, F6, F9 and F10 exhibited antimicrobial activity against *E. coli*. F6 produced the largest zone of inhibition (10 mm) and contained the highest protein concentration (1.320 mg/mL). Hence, F6 fraction was selected as active fraction for further characterization (Table 2).

HPLC analysis of the active fraction showed multiple chromatographic peaks indicating the presence of several peptides in the partially purified preparation (Figure 1). Similarly, MALDI-TOF mass spectrometric analysis revealed multiple peptide ion peaks distributed over different molecular masses (Figure 2). This confirms that the BLIS consists of more than one peptide component.

The partially purified BLIS was subjected to different physicochemical conditions to evaluate its stability. The BLIS retained antimicrobial activity at 30°C but lost activity at 4°C, 80°C and 121°C. The BLIS showed activity over the pH range of

2-10 with maximum activity at pH 6. Activity reduced with the increasing time of UV exposure. Trypsin treatment completely inhibited the antimicrobial activity. SDS- treated BLIS retained inhibitory activity against *E. coli*. These results indicate that the antimicrobial compound is proteinaceous in nature and exhibits physicochemical stability (Table 2).

Functional Applications of Partially Purified BLIS

The partially purified BLIS exhibited concentration- dependent increase in antioxidant activity in DPPH assay with radical scavenging activity increasing to 27.35% at the highest

Table 1: Morphological, Microscopic and Biochemical Characteristics of *Lactobacillus kefir*.

Characteristic	Observation
Colony size	Medium- sized
Colony shape	Circular
Colony colour	Creamy white
Opacity	Opaque
Margin	Smooth
Elevation	Convex
Gram's staining	Gram-positive
Cell morphology	Rod-shaped
Biochemical test	
Catalase	-
Oxidase	-
Methyl red	-
Voges-Proskauer	-
Indole production	-
Triple Sugar Iron (TSI)	-
Gelatin liquefaction	-
Citrate utilization	-
Starch hydrolysis	+
Milk coagulation	+
Haemolysis	-

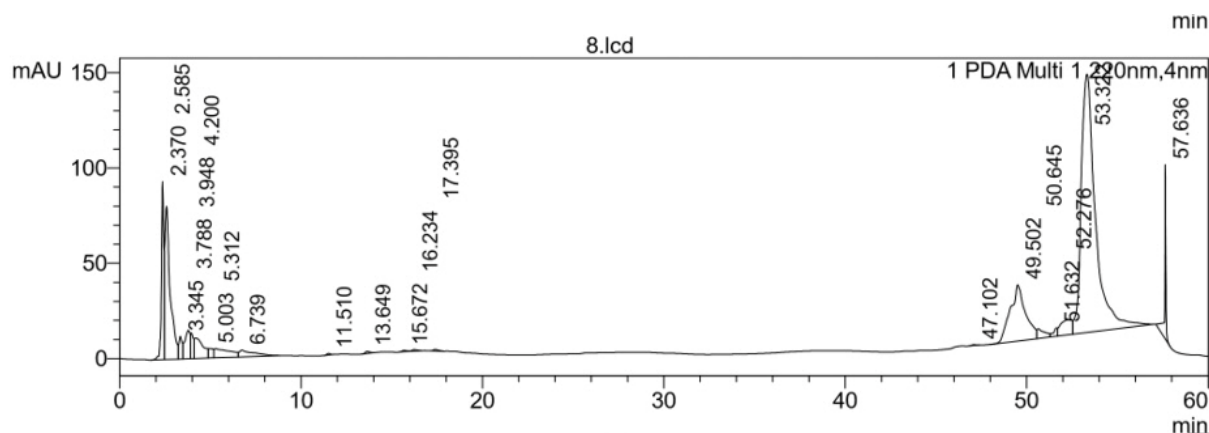


Figure 1: HPLC chromatogram of Partially purified BLIS produced by *Lactobacillus kefir*, showing distinct peptide peaks.

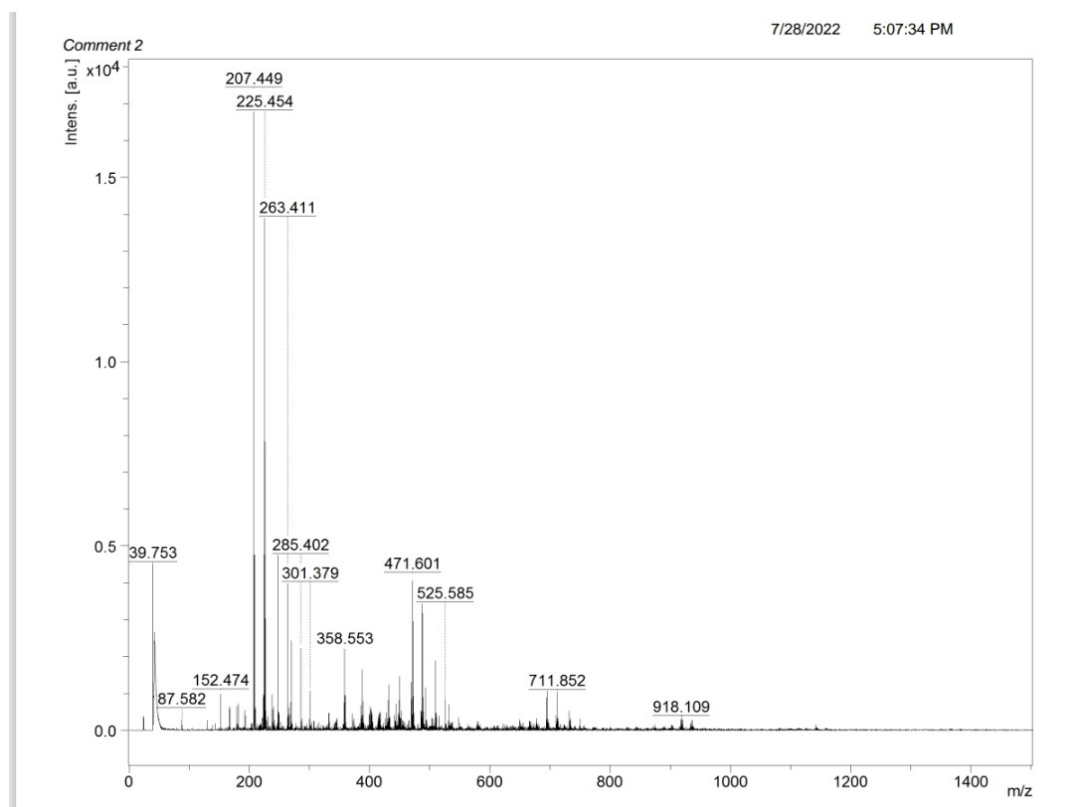


Figure 2: MALDI-TOF Mass Spectrum of the Partially Purified BLIS produced by *Lactobacillus kefir*, confirming the presence of peptide molecules with different molecular masses.

concentration. Cytotoxicity studies on VERO cell demonstrated high cell viability across all concentrations, indicating low toxicity towards normal mammalian cells. BLIS reduced the viability of COLO 320 DM human colon cancer cells in a concentration-dependent manner with an estimated IC_{50} of 125 $\mu\text{g/mL}$ (Table 3).

The food preservation study demonstrated that the efficiency of the partially purified BLIS was influenced by storage temperatures. Refrigeration significantly improved the food preservation efficiency of BLIS-treated samples. There were no visible changes in the colour and texture of carrot, cheese and meat samples throughout the observation period of 30 days. Only apple exhibited browning on day 2 and delayed fungal growth after day 24 (Figure 3). Under room temperature, spoilage progressed rapidly in apple with browning on day 2 and visible fungal growth from day 10 onwards. There was complete fungal colonization by day 14. There was sliminess observed in cheese and meat samples from day 7 onwards which led to complete deterioration of the samples by day 21. Watery exudate and colour change was observed in carrot by day 21 (Figure 3). This emphasizes that BLIS-treated samples were more stable under refrigeration than at room temperature thereby extending the shelf life of the treated food samples.

DISCUSSION

Lactobacillus kefir isolated from kefir exhibits probiotic characteristics with the ability to produce active BLIS (Carasi *et al.*, 2014a). Strong autoaggregation, cell surface hydrophobicity, tolerance to acid, bile salts, sodium chloride and phenol with the absence of haemolytic activity indicate that the *L. kefir* strain is capable of surviving in the conditions similar to gastrointestinal tract (Meira *et al.*, 2012; Singhal *et al.*, 2021). These observations are similar to previous studies that have reported on *Lactobacillus* species isolated from dairy and fermented products exhibited strong adhesion, aggregation and tolerance to gastrointestinal stress conditions (Bourrie *et al.*, 2016; Carasi, Malamud, and Serradell, 2022). This is an important probiotic character to promote intestinal survival and colonization.

The purification methods used in this study yielded a partially purified yet active fraction with inhibitory effect against *E. coli* (Das and Goyal, 2014; Yap *et al.*, 2022). Loss of antimicrobial activity after trypsin treatment confirms the proteinaceous nature of the BLIS (Das and Goyal, 2014). In addition, HPLC and MALDI-TOF analysis showed that the BLIS contains multiple peptide components (Wu *et al.*, 2022). The presence of multiple peptide components observed in this study is comparable to previous studies on partially purified BLIS (Wu *et al.*, 2022; Yap *et al.*, 2022). The retention of activity over different pH and temperatures and chemical conditions exhibit that the partially

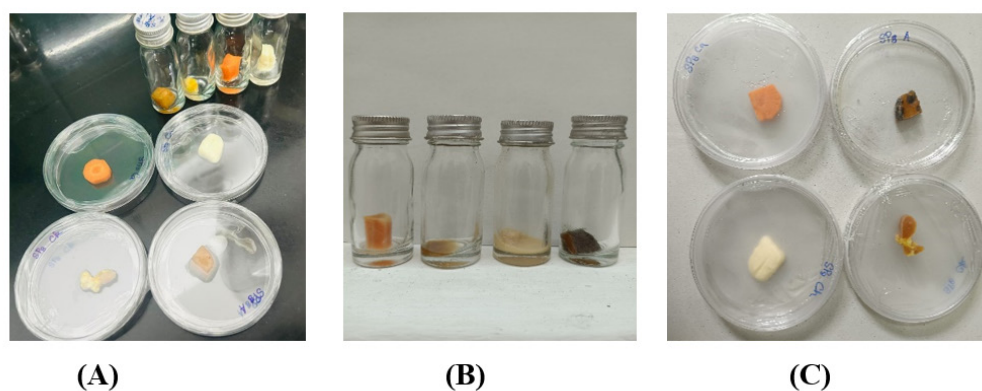


Figure 3: Food preservation potential of Partially purified BLIS produced by *Lactobacillus kefir*; (A) BLIS-treated food sample on the day of treatment (day 0); (B) BLIS- treated food samples kept at room temperature after 21 days; (C) BLIS- treated food sample kept under refrigeration after 30 days.

Table 2: Antimicrobial activity, Protein Estimation and Characterization of partially purified BLIS.

Fractions	Zone of inhibition (mm)	Protein Concentration (mg/mL)
F1	8	1096
F2	-	-
F3	-	-
F4	-	-
F5	-	-
F6	10	1320
F7	-	-
F8	-	-
F9	-	-
F10	8	1020
Stability Treatment of BLIS	Condition	Zone of inhibition (mm)
Temperature	4°C	-
	30°C	9
	80°C	-
	121°C	-
pH	2	7
	4	8
	6	11
	8	9
	10	7
UV exposure	15 min	13
	30 min	8
	45 min	-
	60 min	-
Surfactant	1% SDS	10
Proteolysis	Trypsin	-

purified BLIS has the potential to be used in food preservation where stability is important for practical use (Yap *et al.*, 2022).

The partially purified BLIS demonstrated antioxidant activity, low cytotoxicity against VERO cells as well as antiproliferative activity against COLO 320 DM cells (Das and Goyal, 2014; Kairey *et al.*, 2023). Similar antioxidant and anticancer activities have been reported for bacteriocins and BLIS produced by probiotic *Lactobacillus* strains. These findings indicate that the partially purified BLIS is not only an effective antimicrobial agent but also has the ability to be used in food and biomedical applications (Yap *et al.*, 2022).

The food preservation study emphasized the practical importance of the partially purified BLIS (Carasi *et al.*, 2014b). BLIS- treated food samples under refrigeration remained stable longer than those kept at room temperature (Carasi *et al.*, 2014b). This shows that BLIS treatment combined with low temperature can delay spoilage of food effectively (Leite *et al.*, 2013). The present findings emphasize the potential of *Lactobacillus kefir*- derived BLIS as natural and multifunctional biopreservatives (Behera *et al.*, 2018; Yap *et al.*, 2022). Further purification and peptide sequencing can facilitate its transition into practical applications.

CONCLUSION

The present study demonstrated that *Lactobacillus kefir* isolated from kefir is capable of producing Bacteriocin-Like Inhibitory Substances (BLIS) with probiotic properties and has the potential for food and biotechnological applications. The probiotic characteristics along with physicochemical stability and biological activity make the BLIS a natural bioactive compound. The partially purified BLIS showed effective antimicrobial activity against *E. coli*, antioxidant potential, antiproliferative activity against COLO 320 DM cells and low cytotoxicity against VERO cells. This indicates that the BLIS can be considered an alternative to synthetic preservatives. HPLC and MALDI-TOF confirmed the presence of multiple peptide components. Food preservation

Table 3: Cytotoxicity, Anticancer and Antioxidant Activity of Partially Purified BLIS.

BLIS Concentration (µg/mL)	Dilutions	Cytotoxicity Absorbance (OD: 570 nm)	VERO Cell viability (%)
1000	Neat	0.415	68.82
500	1:1	0.461	76.45
250	1:2	0.506	83.91
125	1:4	0.552	91.54
62.5	1:8	0.599	99.33
31.2	1:16	0.645	106.96
15.6	1:32	0.682	113.10
7.8	1:64	0.728	120.72
Cell control	-	0.603	100
Concentration (µg/mL)	Dilutions	Anticancer Absorbance (OD: 570 nm)	Cancer Cell viability (%)
1000	Neat	0.193	31.43
500	1:1	0.232	37.78
250	1:2	0.272	44.29
125	1:4	0.311	50.65
62.5	1:8	0.350	57.00
31.2	1:16	0.388	63.19
15.6	1:32	0.427	69.54
7.8	1:64	0.465	75.73
Cell control	-	0.614	100
Concentration (µg/mL)	BLIS OD (517 nm)	BLIS DPPH activity (%)	
200	0.090	15.09	
400	0.087	17.92	
600	0.084	20.75	
800	0.081	23.58	
1000	0.077	27.35	

studies demonstrated stability of BLIS- treated samples under refrigeration. Overall, these results strengthen the application of *Lactobacillus kefir*- derived BLIS as a natural food preservative and a bioactive compound in biological applications. Further purification and structural characterization of the peptides are needed to facilitate future industrial and therapeutic applications.

ACKNOWLEDGEMENT

The authors sincerely acknowledge National Agro Foundation, Research and Development Centre Chennai, for providing the facilities to perform HPLC analysis. The authors are grateful to the Indian Institute of Technology Madras (IIT Madras), Guindy, Chennai, for providing the MALDI-TOF analysis facilities. The authors also express their sincere thanks to Life Tech Research Centre, Chennai, for providing laboratory facilities and technical support for the antioxidant, cytotoxicity and anticancer studies.

ABBREVIATIONS

BLIS: Bacteriocin-Like Inhibitory Substance; **MRS:** de Man, Rogosa and Sharpe (agar/broth); **CFS:** Cell-Free Supernatant; **MWCO:** Molecular Weight Cut-Off; **HPLC:** High-Performance Liquid Chromatography; **PDA:** Photodiode Array; **MALDI-TOF MS:** Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry; **HCCA:** α -Cyano-4-hydroxycinnamic Acid; **SDS:** Sodium Dodecyl Sulfate; **MTT:** 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide; **DPPH:** 2,2-Diphenyl-1-Picrylhydrazyl; **IC₅₀:** Half-Maximal Inhibitory Concentration; **TSI:** Triple Sugar Iron; **BSA:** Bovine Serum Albumin; **NCCS:** National Centre for Cell Science.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTION

The first author conceived and designed the study, performed the experiments, collected and analyzed the data, interpreted the results, and prepared the original draft of the manuscript. The corresponding author supervised the research work, provided scientific guidance, critically reviewed the manuscript, and approved the final version of the manuscript. All authors have read and approved the final manuscript.

REFERENCES

- Behera, S. S., Ray, R. C., & Zdolec, N. (2018). *Lactobacillus plantarum* with functional properties: An approach to increase safety and shelf-life of fermented foods. *BioMed Research International*, 2018(1), 9361614. <https://doi.org/10.1155/2018/9361614>
- Bessa, M. K., Bessa, G. R., & Bonamigo, R. R. (2024). Kefir as a therapeutic agent in clinical research: A scoping review. *Nutrition Research Reviews*, 37(1), 79-95. <https://doi.org/10.1017/S0954422423000070>
- Bourrie, B. C. T., Willing, B. P., & Cotter, P. D. (2016). The microbiota and health promoting characteristics of the fermented beverage kefir. *Frontiers in Microbiology*, 7, 647. <https://doi.org/10.3389/fmicb.2016.00647>
- Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, 28(1), 25-30.
- Carasi, P., Ambrosio, N. M., De Antoni, G. L., Bressollier, P., Urdaci, M. C., & Serradell, M. A. (2014b). Adhesion properties of potentially probiotic *Lactobacillus kefir* to gastrointestinal mucus. *Journal of Dairy Research*, 81(1), 16-23. <https://doi.org/10.1017/S0022029913000526>
- Carasi, P., Díaz, M., Racedo, S. M., De Antoni, G. L., Urdaci, M. C., & Serradell, M. A. (2014a). Safety characterization and antimicrobial properties of kefir-isolated *Lactobacillus kefir*. *BioMed Research International*, 2014(1), 208974. <https://doi.org/10.1155/2014/208974>
- Carasi, P., Malamud, M., & Serradell, M. A. (2022). Potentiality of food-isolated *Lactobacillus kefir* strains as probiotics: State-of-the-art and perspectives. *Current Microbiology*, 79(1), 21. <https://doi.org/10.1007/s00284-021-02728-x>
- Charteris, W. P., Kelly, P. M., Morelli, L., & Collins, J. K. (1998). Antibiotic susceptibility of potentially probiotic *Lactobacillus* species. *Journal of Food Protection*, 61(12), 1636-1643.
- Chuah, L. O., Foo, H. L., Loh, T. C., Mohammed Alitheen, N. B., Yeap, S. K., Abdul Mutalib, N. E., et al. (2019). Postbiotic metabolites produced by *Lactobacillus plantarum* strains exert selective cytotoxicity effects on cancer cells. *BMC Complementary and Alternative Medicine*, 19(1), 114.
- Chuah, W. W., Tan, J. S., Oslan, S. N. H., & Raja, P. B. (2024). Enhancing food preservation with postbiotic metabolites γ -aminobutyric acid (GABA) and bacteriocin-like inhibitory substances (BLIS) produced by *Lactobacillus brevis* C23 co-cultures in plant-based medium. *Preparative Biochemistry & Biotechnology*, 54(5), 514-525.
- Das, D., & Goyal, A. (2014). Characterization of a noncytotoxic bacteriocin from probiotic *Lactobacillus plantarum* DM5 with potential as a food preservative. *Food & Function*, 5(10), 2453-2462.
- De Man, J. C., Rogosa, M., & Sharpe, M. E. (1960). A medium for the cultivation of lactobacilli. *Journal of Applied Bacteriology*, 23(1), 130-135.
- Kaibre, L., Leech, B., El-Assaad, F., Bugarcic, A., Dawson, D., & Lauche, R. (2023). The effects of kefir consumption on human health: A systematic review of randomized controlled trials. *Nutrition Reviews*, 81(3), 267-286.
- Leite, A. M. O., Miguel, M. A. L., Peixoto, R. S., Rosado, A. S., Silva, J. T., & Paschoalin, V. M. F. (2013). Microbiological, technological and therapeutic properties of kefir: A natural probiotic beverage. *Brazilian Journal of Microbiology*, 44(2), 341-349. <https://doi.org/10.1590/S1517-83822013000200001>
- Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, 193(1), 265-275.
- Meira, S. M. M., Helfer, V. E., Velho, R. V., Lopes, F. C., & Brandelli, A. (2012). Probiotic potential of *Lactobacillus* spp. isolated from Brazilian regional ovine cheese. *Journal of Dairy Research*, 79(1), 119-127.
- Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, 65(1-2), 55-63. [https://doi.org/10.1016/0022-1759\(83\)90303-4](https://doi.org/10.1016/0022-1759(83)90303-4)
- Sharma, K. (2007). *Manual of microbiology: Tools & techniques* (2nd ed.). Ane Books Pvt. Ltd.,
- Singhal, N., Singh, N. S., Mohanty, S., Kumar, M., & Viridi, J. S. (2021). Rhizospheric *Lactiplantibacillus plantarum* strains exhibit bile salt hydrolysis, hypocholesterolaemia and probiotic capabilities *in vitro*. *Scientific Reports*, 11(1), 15288.
- Todorov, S. D. (2009). Bacteriocins from *Lactobacillus plantarum*: Production, genetic organization and mode of action. *Brazilian Journal of Microbiology*, 40(2), 209-221.
- Wu, D., Dai, M., Shi, Y., Zhou, Q., Li, P., & Gu, Q. (2022). Purification and characterization of bacteriocin produced by a strain of *Lactocaseibacillus rhamnosus* ZFM216. *Frontiers in Microbiology*, 13, 1050807. <https://doi.org/10.3389/fmicb.2022.1050807>
- Yap, P. G., Lai, Z. W., & Tan, J. S. (2022). Bacteriocins from lactic acid bacteria: Purification strategies and applications in food and medical industries: A review. *Beni-Suef University Journal of Basic and Applied Sciences*, 11(1), 51.

Cite this article: Priyadarishini CS, Iyer P. Characterization, Bio-preservative Potential and Functional Applications of Bacteriocin-Like Inhibitory Substance Produced by *Lactobacillus kefir* Isolated from Kefir. *Asian J Biol Life Sci.* 2026;15(3):731-7.