# Characterization and Production of Novel ACE-Inhibitory Bioactive Peptides derived from Fermented Goat Milk using potent Lactobacillus cultures

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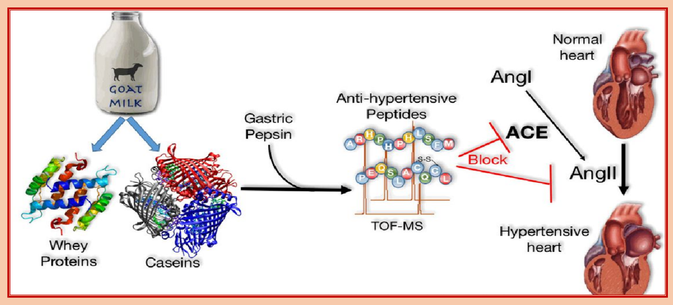
## Introduction

- ✓ Goat milk is superior to cow milk in terms of nutritional value.
- ✓ Fermented goat milk may reduce the risk of cardiovascular disease by anti-oxidative, anti-atherogenic and anti-thrombotic effects.
- ✓ Biologically active peptides are generated during milk fermentation by proteolytic Lactic cultures.
- ✓ ACE-inhibitory peptides produced by fermentation of milk are reported to reduce hypertension without any side effects.
- ✓ Present study was designed to purify and separate the novel ACE-inhibitory peptides from fermented goat milk (*Capra aegagrus hircus*) using potent proteolytic *lactobacillus* cultures.

## Objectives

- ✓ To evaluate the proteolytic activity, di and tripeptidase activity and ACE-inhibitory activity of lactobacillus cultures.
- ✓ To optimise the growth conditions for the production of peptides.
- ✓ To determine the relative proteolytic activity of lactobacillus cultures.
- ✓ To purify and separate the novel ACE-inhibitory peptides from fermented goat milk.




## Methods

- ✓ The LAB cultures used in the study i.e. *Lactobacillus casei* (KR732325) (NK9) and *Lactobacillus fermentum* (TDS030603) (MTCC 25067) (LF) were obtained from the Culture Collection of Dairy Microbiology Department, SMC College of Dairy Science, Anand, India.
- ✓ The proteolytic activity of the selected *Lactobacillus* cultures was expressed as the absorbance of free amino groups measured at 340 nm. The peptide content was expressed as mg/ml.
- ✓ Di and Tipeptidase activity was also determined (Donkor *et al.* 2007).
- ✓ Growth conditions (i.e., inoculation rate and incubation periods) for the production of peptides were optimized according to o-phthaldialdehyde (OPA) method (Donkor et al. 2007).
- ✓ ACE-inhibitory was determined according to Hati *et al.* (2015) and Solanki *et al.* (2017).
- ✓ Relative proteolytic activity was carried out following Vasiljevic and Jelen (2002).
- ✓ Identification and Characterization of Purified ACE-Inhibitory Peptides Derived from Fermented Goat Milk Through RPLC/MS analysis (Solanki *et al.* 2017).

## Results

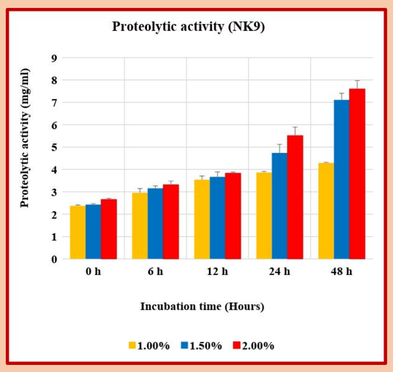



Figure 1 Effect of inoculation rates and incubation periods on proteolytic activity of NK9 in goat milk

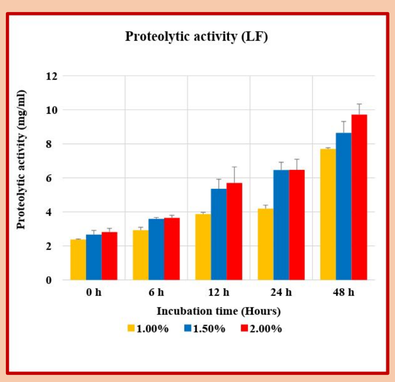



Figure 2 Effect of inoculation rates and incubation periods on proteolytic activity of LF in goat milk

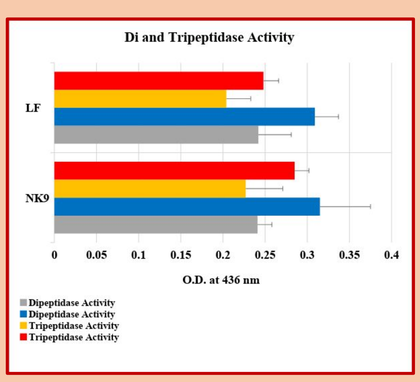



Figure 3 Dipeptidase and tripeptidase activity of Lactobacillus cultures under optimized growth conditions

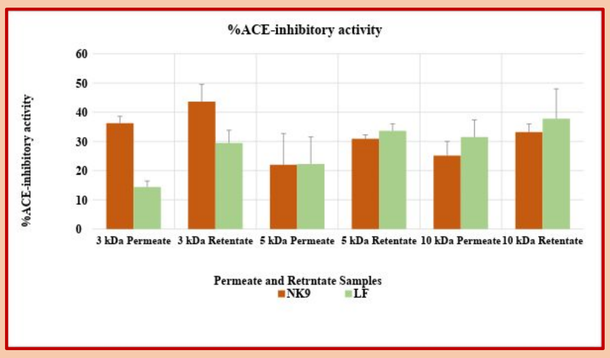



Figure 4 ACE-inhibitory activity of 3, 5 and 10 kDa permeates and retentates produced by the Lactobacillus cultures

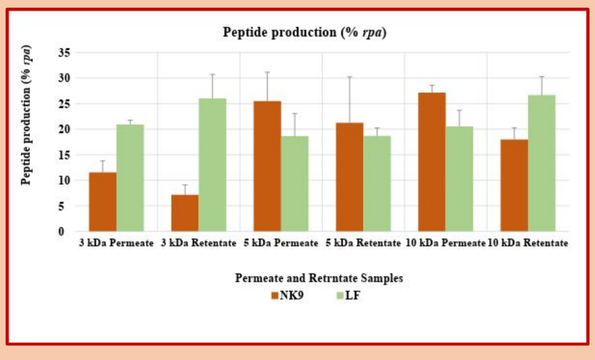



Figure 5 Peptide production (% rpa) of 3, 5 and 10 kDa permeates and retentates produced by the Lactobacillus cultures

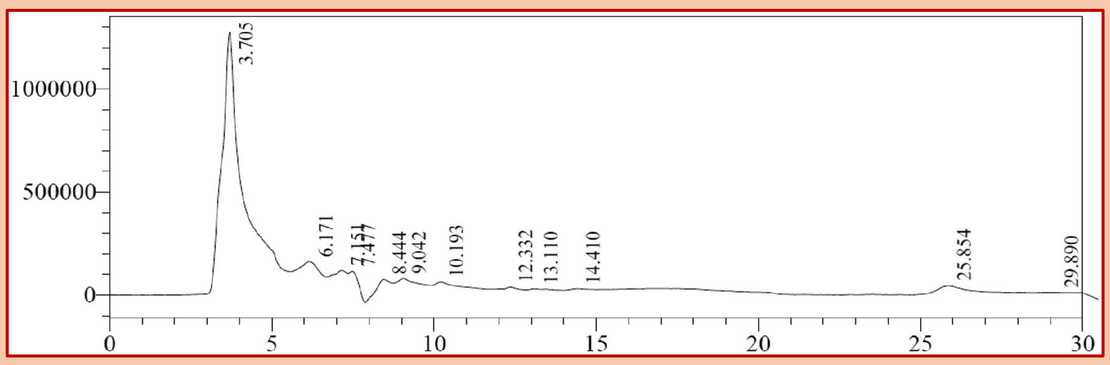



Figure 6 RP-HPLC chromatogram of unfermented goat milk

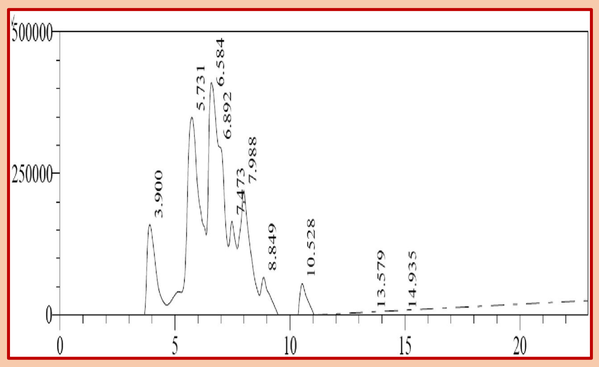



Figure 7 RP-HPLC chromatogram of fermented goat milk by NK9

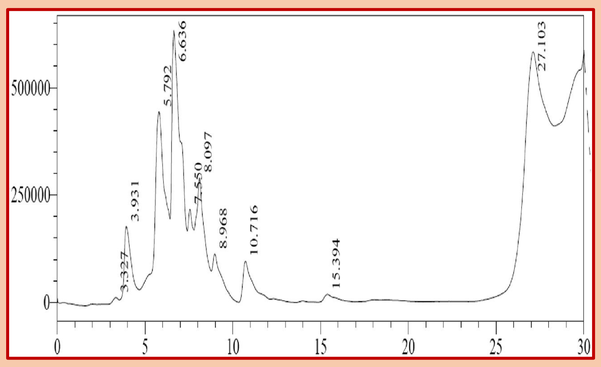



Figure 8 RP-HPLC chromatogram of fermented goat milk by LF

## Results

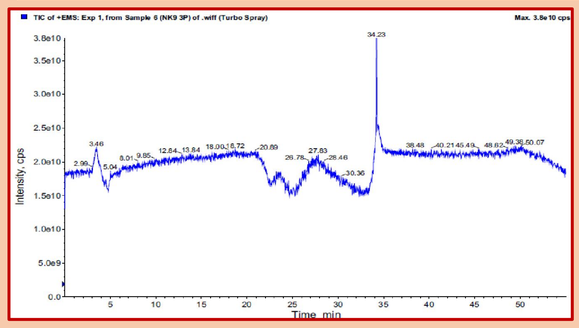



Figure 9 The Total ion chromatogram of *Lactobacillus* culture NK9 (3 kDa permeate) generated by EMS to EPI scan in LC–MS.

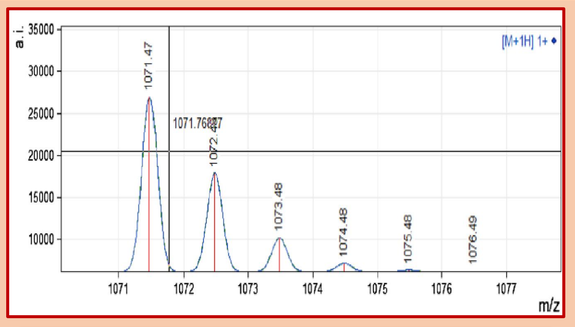



Figure 10 M/Z spectrum of NK9 (3 kDa permeate) inspected in mMass peptide processing and deconvolution software. Identified as DERFFDDK with expected molar mass 1071.47.

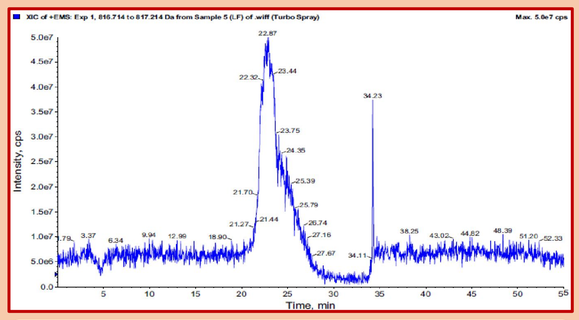



Figure 11 The Total ion chromatogram of *Lactobacillus* culture LF (3 kDa permeate) generated by EMS to EPI scan in LC–MS.

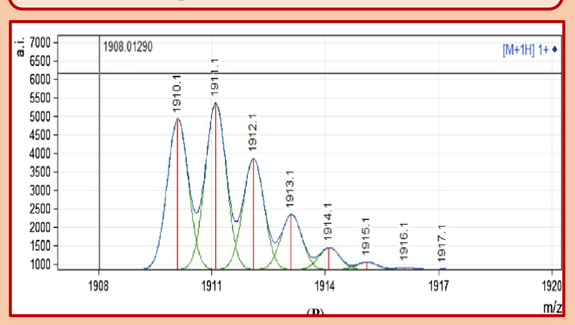



Figure 12 M/Z spectrum of LF (3 kDa permeate) inspected in mMass peptide processing and deconvolution software. Identified as MMKSFFLVVTILALTLP with expected molar mass 1911.1.

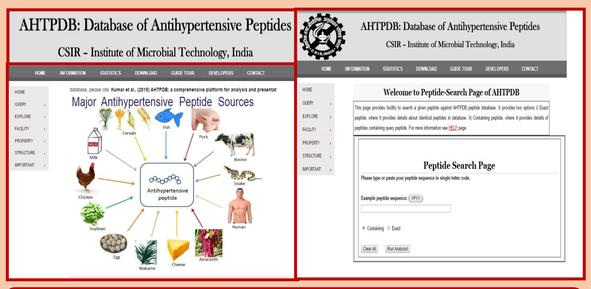



Figure 13 Results indicating that peptides belong from goat milk proteins having ACE-inhibitory activity by databases of AHTPDB.

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 5532 | 5532_link | VVAPFPEVF | 9 | 1004.19 | ND | ND | Milk (Goat milk protein) | ND | ND | / |
| 5533 | 5533_link | FVVAPFPEVF | 10 | 1151.37 | ND | ND | Milk (Goat milk protein) | ND | ND | ✓ |
| 5534 | 5534_link | FFVAPFPEVFGK | 12 | 1384.64 | ND | ND | Milk (Goat milk protein) | ND | ND | ✓ |
| 5535 | 5535_link | FVAPFPEVF | 9 | 1052.24 | ND | ND | Cheese (Probiotic Cheddar cheeses with Lb. Casei ) | RP-HPLC | ND | ✓ |
| 5536 | 5536_link | FVAPFPEVF | 9 | 1052.24 | ND | ND | Cheese (Probiotic Cheddar cheeses with Lb. | RP-HPLC | ND | ✓ |

Figure 14 Results indicating that peptides belong from goat milk proteins having ACE-inhibitory activity by databases of AHTPDB.

## Conclusion

- ✓ NK9 and LF showed good proteolytic, ACE-inhibitory activity and di-tri peptidase activity during the fermentation of goat milk.
- ✓ Various antihypertensive bioactive peptides were characterized and their similarity with different goat milk proteins were confirmed against goat milk protein databases of AHTPDB.
- ✓ Validation of ACE inhibitory activity need to be conducted through clinical trials for health claim of fermented goat milk.

## Key Message

- ✓ Fermented goat milk could be a novel source of ACE-inhibitory peptides with these two potential *Lactobacillus* cultures.