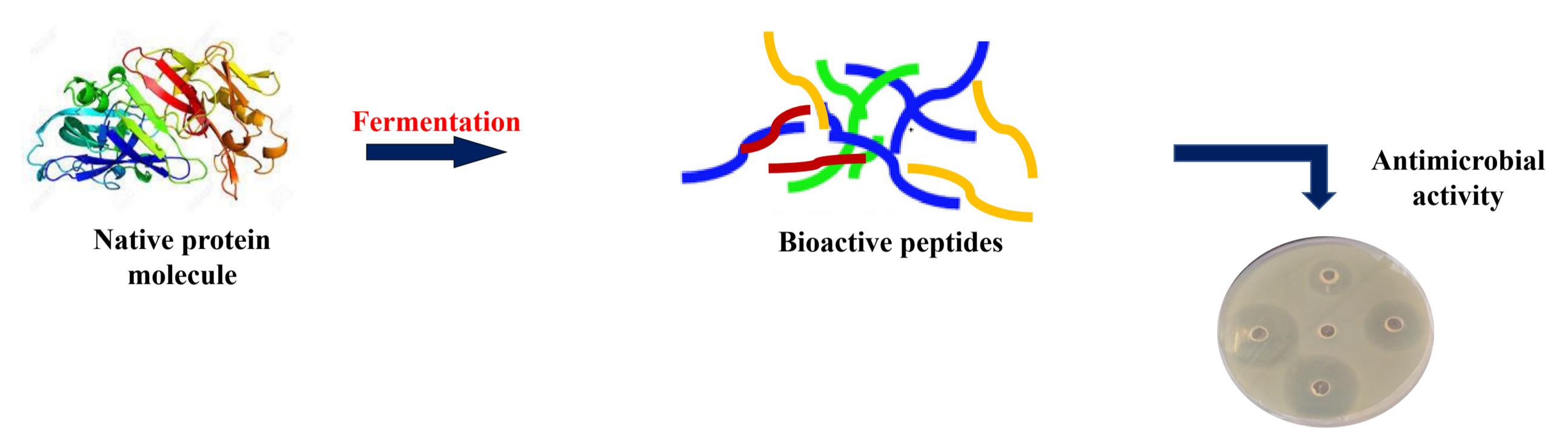


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Introduction

- Bioactive peptides have been defined as specific protein fragments that are nascent or encrypted in the primary sequences of proteins and have a positive impact on body functions or conditions and may ultimately influence health. They are considered to promote diverse activities, including, antioxidant, immunomodulatory, antihypertensive, antimicrobial, opiate-like, mineral binding and antithrombotic actions.
- They can be released through gastrointestinal digestion or food processing or fermentation from plant and animal proteins such as milk, soy or fish proteins and usually include 2-20 amino acid residues per molecule
- Antimicrobial bioactive peptides (AMPs) interact specifically with bacterial membrane and kill the cell by causing leakage of its content.



Objective: Purification and identification of antimicrobial bioactive peptide derived from Camel and Goat milk by microbial fermentation

Methods

Preparation of 50 kDa, 10kDa, 5kDa and 3kDa fractions from fermented camel milk

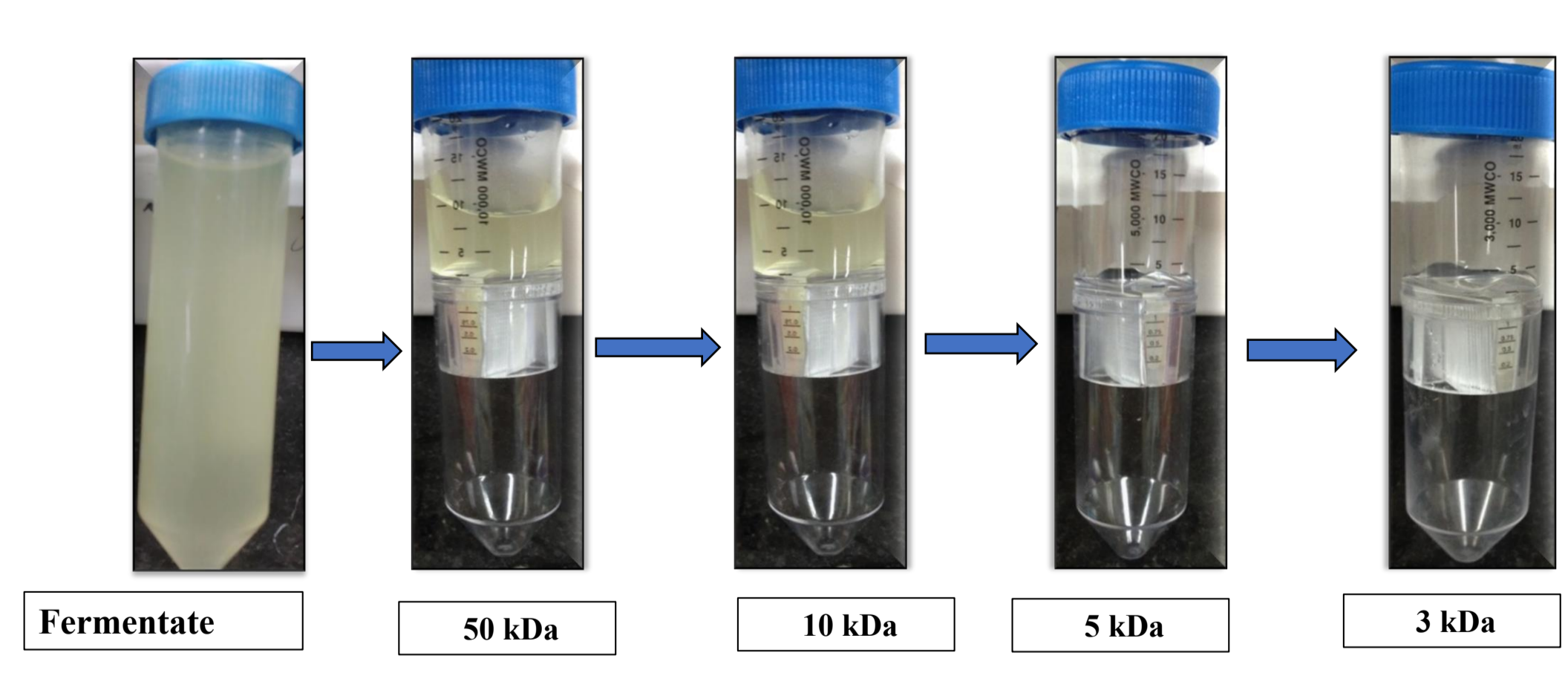
Camel and Goat Milk + *Lactobacillus* culture (*Lb. rhamnosus* C25 @ 2%)

Fermentation for 48h at 37°C

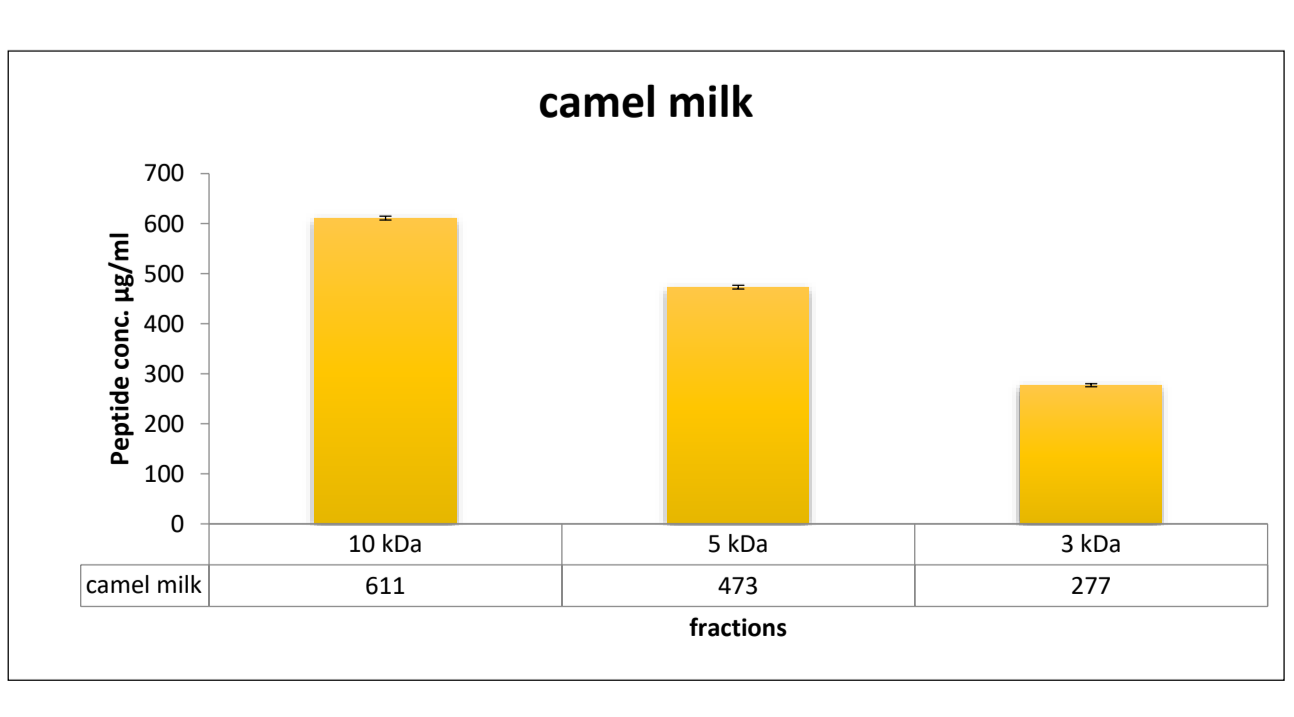
Centrifugation @ 8000rpm for 15 min / 4°C

supernatant passed through Millipore filter (0.42µm)

Filtered through different molecular weight cut-off membranes (50 kDa, 10kDa; 5kDa; 3kDa Millipore VIVASPIN). @3200rpm for 15 min / 5°C



Peptide quantification by OPA method

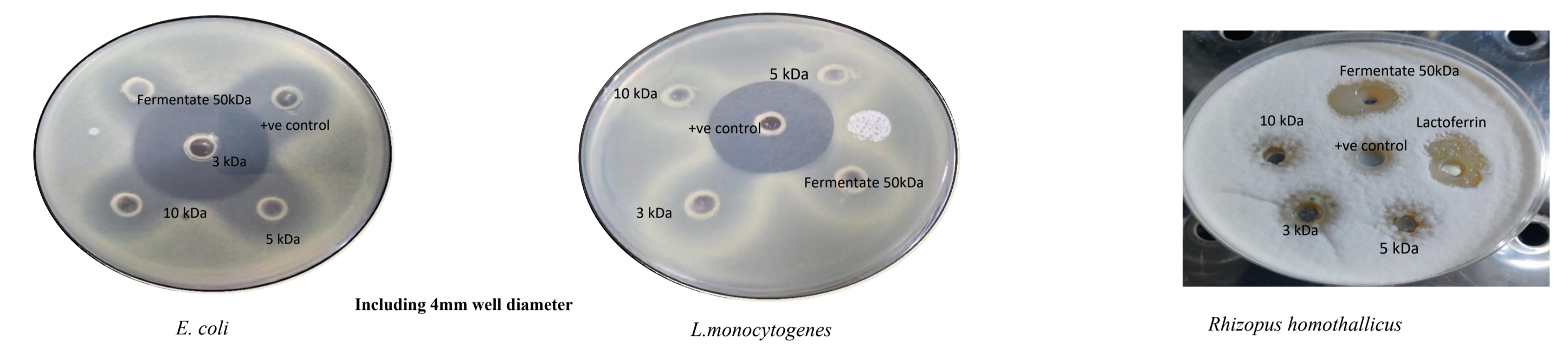
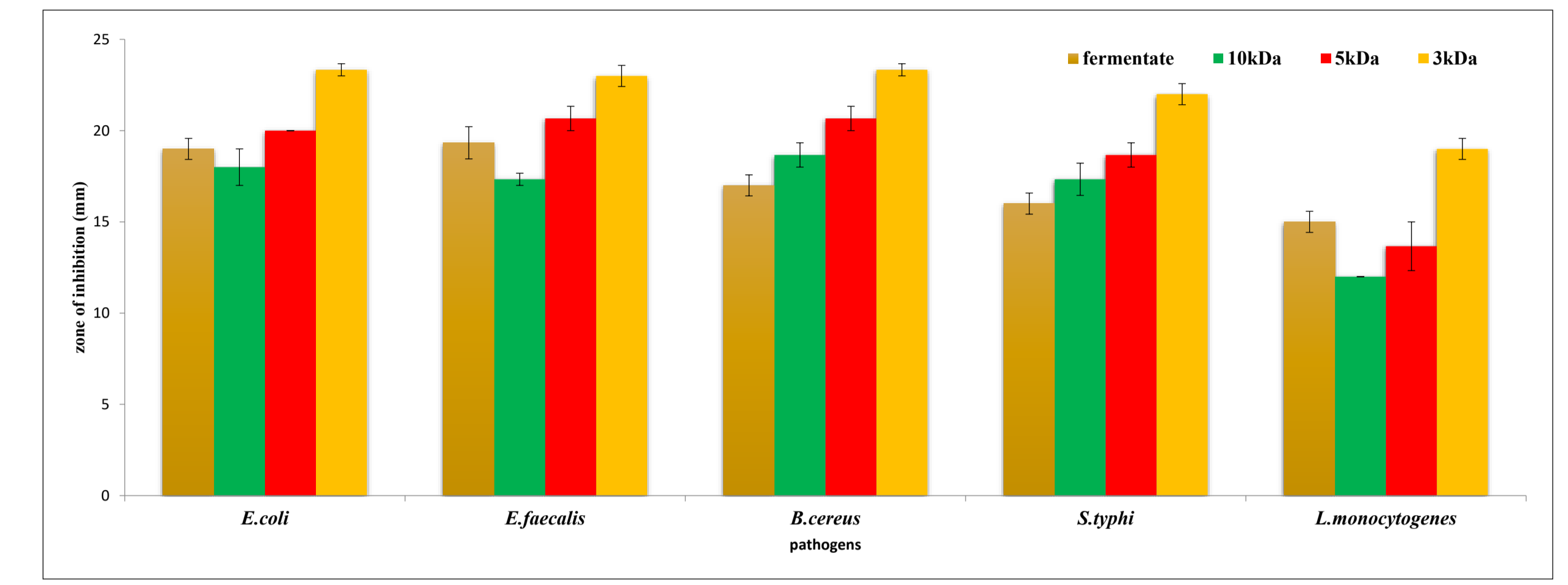


Minimal inhibitory Concentration

Pathogens	3 kDa camel milk (µg/ml)
<i>E. coli</i>	24.96
<i>E. faecalis</i>	31.2
<i>B. cereus</i>	34.44
<i>S. typhi</i>	36.2
<i>L. monocytogenes</i>	36.5
<i>Rhizopus oryzae</i> (50 kDa)	24.96
<i>Rhizopus homothallicus</i> (50 kDa)	31.2

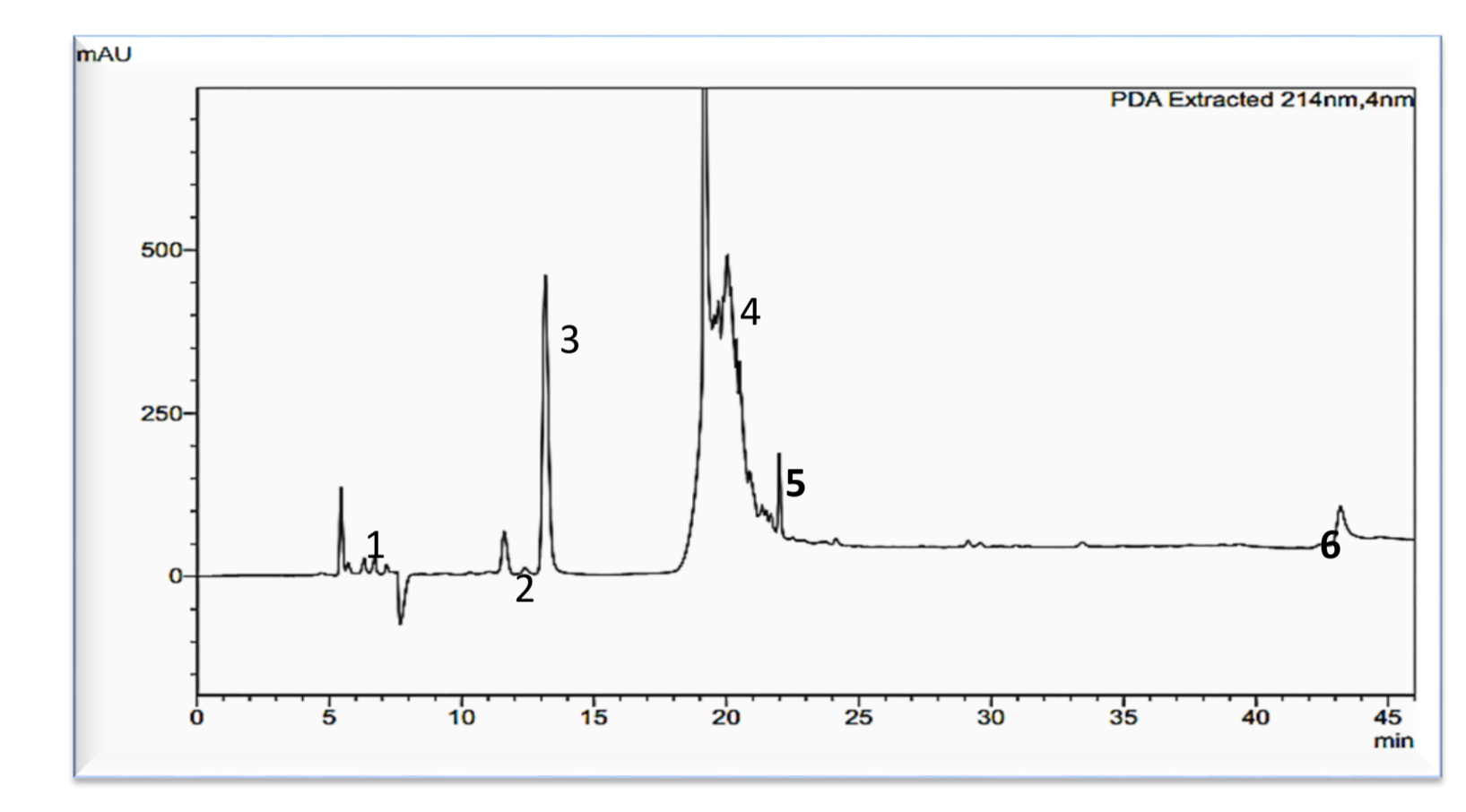
Results

Antimicrobial activity of camel milk fermentates 50 kDa, 10 kDa, 5kDa & 3kDa fractions



RP-HPLC analysis of camel milk bioactive peptides

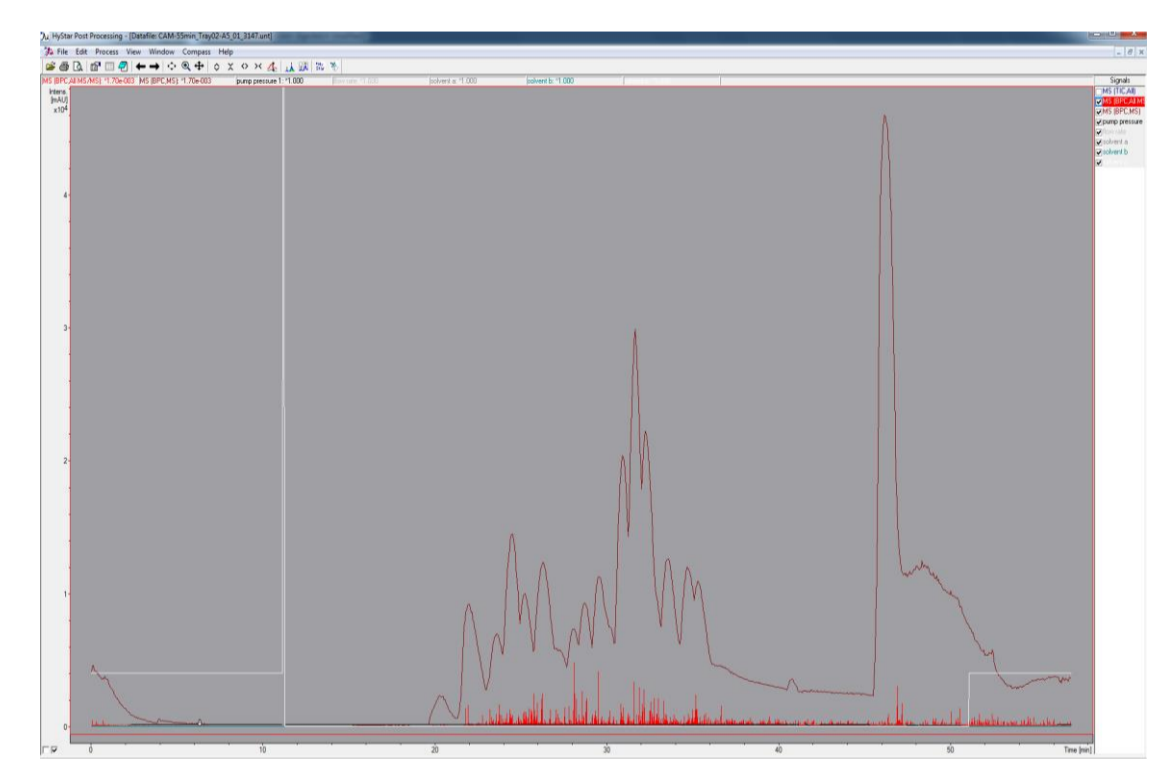
- HPLC peak of 3kDa fraction from Camel Milk-
- Bioactive peptides of 3kDa for camel milk fractionated and collected by Reverse phase-HPLC.
- HPLC peaks from 3kDa camel milk showed highest antimicrobial activity in peak number 1 & 3 against *E. coli*.



LC-MS/MS analysis of camel and goat milk bioactive peptides

- One mL of sample of 3 kDa fractions of fermented camel has been outsourced
- Obtained Raw Files were searched in Mascot engine using *Bos taurus* database

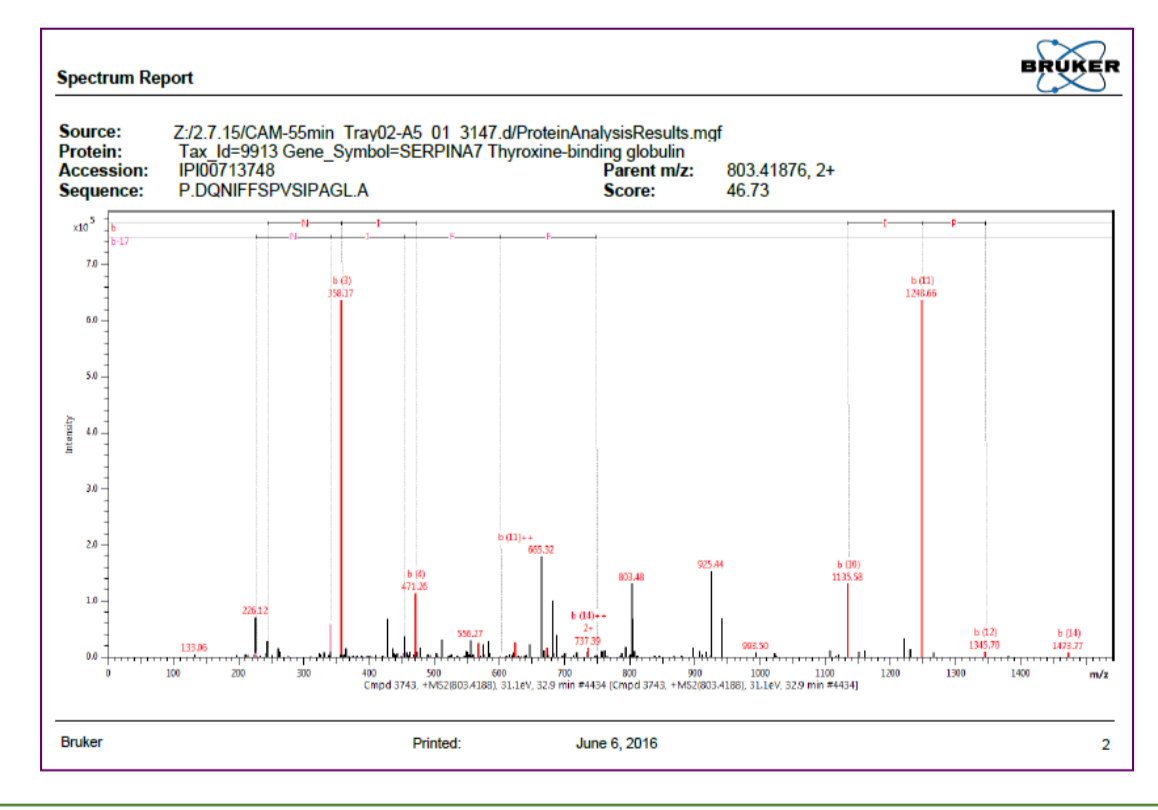
LC-MS/MS peaks of 3kDa Camel milk peptide



Camel milk protein analysis results

Search Result	Location	Search Engine	Database	Ident. Compounds
Protein 1	Tax_Id=9913 Gene_Symbol=Uncharacterized protein	Mascot 2.5.3	IP_20094	500000
Protein 2	Tax_Id=9913 Gene_Symbol=Uncharacterized protein	Mascot 2.5.3	IP_20094	500000

Camel milk protein analysis spectrum report



Identified Proteins using peptides generated through camel milk

Accession	Protein	MW [kDa]	pI	#Peptides	Scores
IP100721027	Tax_Id=9913 Gene_Symbol=ZCHC6 zinc finger, CCHC domain containing 6	171.2	6.8	3	55.4 (M:55.4)
IP100838436	Tax_Id=9913 Gene_Symbol=CASC5 hypothetical protein	268.3	5.5	3	55.0 (M:55.0)
IP100713748	Tax_Id=9913 Gene_Symbol=SERPINA7 Thyroxine-binding globulin	46.0	5.5	2	62.0 (M:62.0)
IP100825160	Tax_Id=9913 Gene_Symbol=LOC789339 myosin light chain 2a-like, partial	12.3	4.4	2	49.7 (M:49.7)
IP100692457	Tax_Id=9913 Gene_Symbol=GPD2 Uncharacterized protein	81.0	6.6	2	47.9 (M:47.9)
IP101003719	Tax_Id=9913 Gene_Symbol=FRAS1 related extracellular matrix 3	239.1	5.0	2	47.1 (M:47.1)
IP100728345	Tax_Id=9913 Gene_Symbol=BAZ2A bromodomain adjacent to zinc finger domain protein 2A	210.0	6.3	2	46.9 (M:46.9)
IP100843435	Tax_Id=9913 Gene_Symbol=LRRC29 leucine rich repeat containing 29-like	66.2	6.6	2	46.7 (M:46.7)
IP101002995	Tax_Id=9913 Gene_Symbol=LOC100337431 solute carrier family 12, member 7-like	117.8	8.7	2	46.3 (M:46.3)
IP100963807	Tax_Id=9913 Gene_Symbol=SLC12A4 solute carrier family 12 (potassium/chloride transporters), member 4	120.5	6.2	2	44.3 (M:44.3)
IP101028244	Tax_Id=9913 Gene_Symbol=SETDB1 histone-lysine N-methyltransferase SETDB1	142.9	5.8	2	43.6 (M:43.6)
IP100907863	Tax_Id=9913 Gene_Symbol= Uncharacterized protein (Fragment)	35.1	6.3	2	43.1 (M:43.1)

Conclusion

- This work has evaluated the presence of antimicrobial bioactive peptide in camel milk fermented by *Lactobacillus* strain of dairy origin.
- 34 peptides were identified by LC-MS/MS in 3kDa peptide fractions of camel milk fermented with *L. rhamnosus* C25.
- Consequently, it may be concluded that camel and goat milk proteins underwent an extensive degradation process during fermentation and generated a large number of bioactive peptides.
- The identified peptide sequences from camel milk were as follows: QIITYRDYLPVLVGLREAMR, HGVTFGYEGQKPLFK, PAVKAHLFAAEPPGR, QKAVPYPQRD and goat milk were GQPQVVPVEGSR, DPFLPKQILVLPQR, AKYIPIQVLSR, VLLFMVDFEK, ESKEVSPSLPQSPVK, REEPRVPLK.
- Moreover, use of fermented camel and goat milk and their bioactive peptides for nutraceuticals and functional food or inclusion in therapeutic diet for patients with diseases linked to immunity system could be functional.