

Characteristic of bacteriocin like substance and cholesterol removal of lactic acid bacteria Isolated from Ewes Milk and traditional sour buttermilk in Iran

M. Iranmanesh^{1*}, N. Mojjani², H. Ezzatpanah³, M. A. K. Torshizi⁴

¹PH. D Research Student of the Department of Food Science and Technology, Science and Research Branch, Islamic Azad University, Tehran, Iran

²Biotechnology Department, Razi Vaccine and Serum Research Institute, Karaj, IR Iran

³Department of Food Science and Technology, Science and Research Branch, Islamic Azad University, Tehran, Iran

⁴Department of Animal Science, College of Agriculture, Tarbiat Modarres University, Tehran, IR Iran

Email: mi_manseh@yahoo.com



Introduction:

Spontaneous milk fermentation has a long history in Iran. Buttermilk is by-product which produce by churning of yoghurt in butter manufacture processing. The product is spontaneously fermented from raw sheep milk. Production of traditional buttermilk was occurred by use of mashk . This product has a lot of bacteria and yeast.

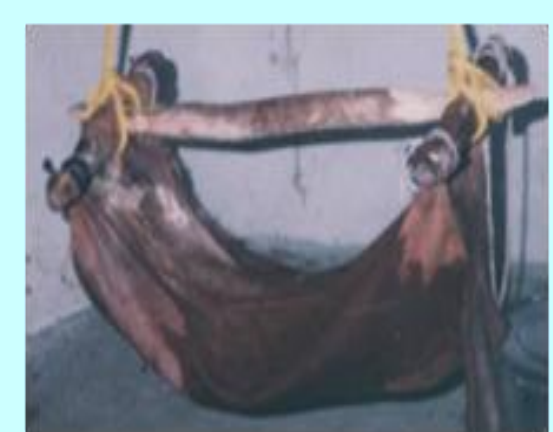
Objective:

Present study was done to determine:

- ✓ The presence of Lactic acid bacteria
- ✓ Characteristic of their bacteriocin
- ✓ Their cholesterol removal

Materials and Methods:

60 samples sheep milk from Myaneh and Hashtrood



Milk Yogurt Buttermilk

Isolation LAB: gram stain, catalase test

Antagonistic activity against *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella enteritidis*

select 10 best bacteria with higher antimicrobial activity



Identification of bacteria: API 50CHL kit (Biomerieux, France)



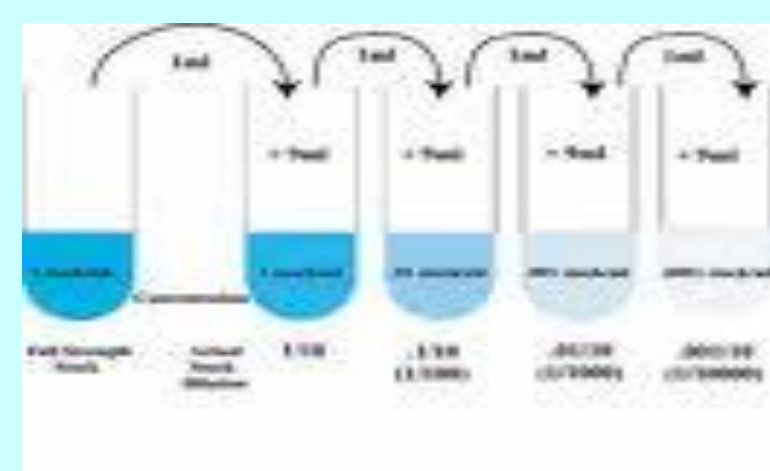
Sensitivity of antimicrobial activity against *Staphylococcus aureus* to pH

Sensitivity of antimicrobial activity to enzymes

catalase
lysozyme
trypsin
proteinase K
pronase E

Kinetics of bacteriocin-like production

Molecular size estimation of bacteriocin-like substances
By ultrafiltration with 5, 10 and 30 KDa



Bacteriocin titration

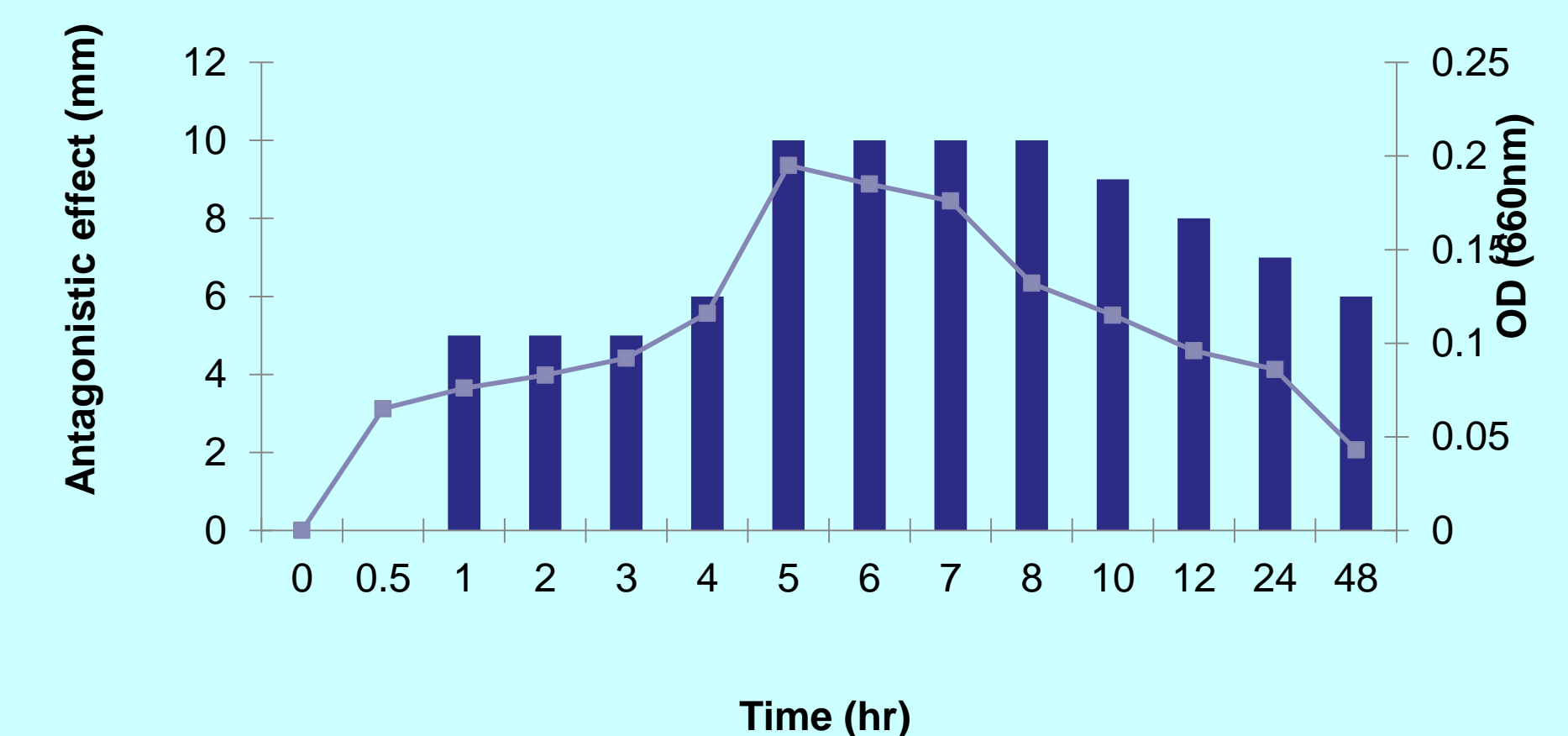
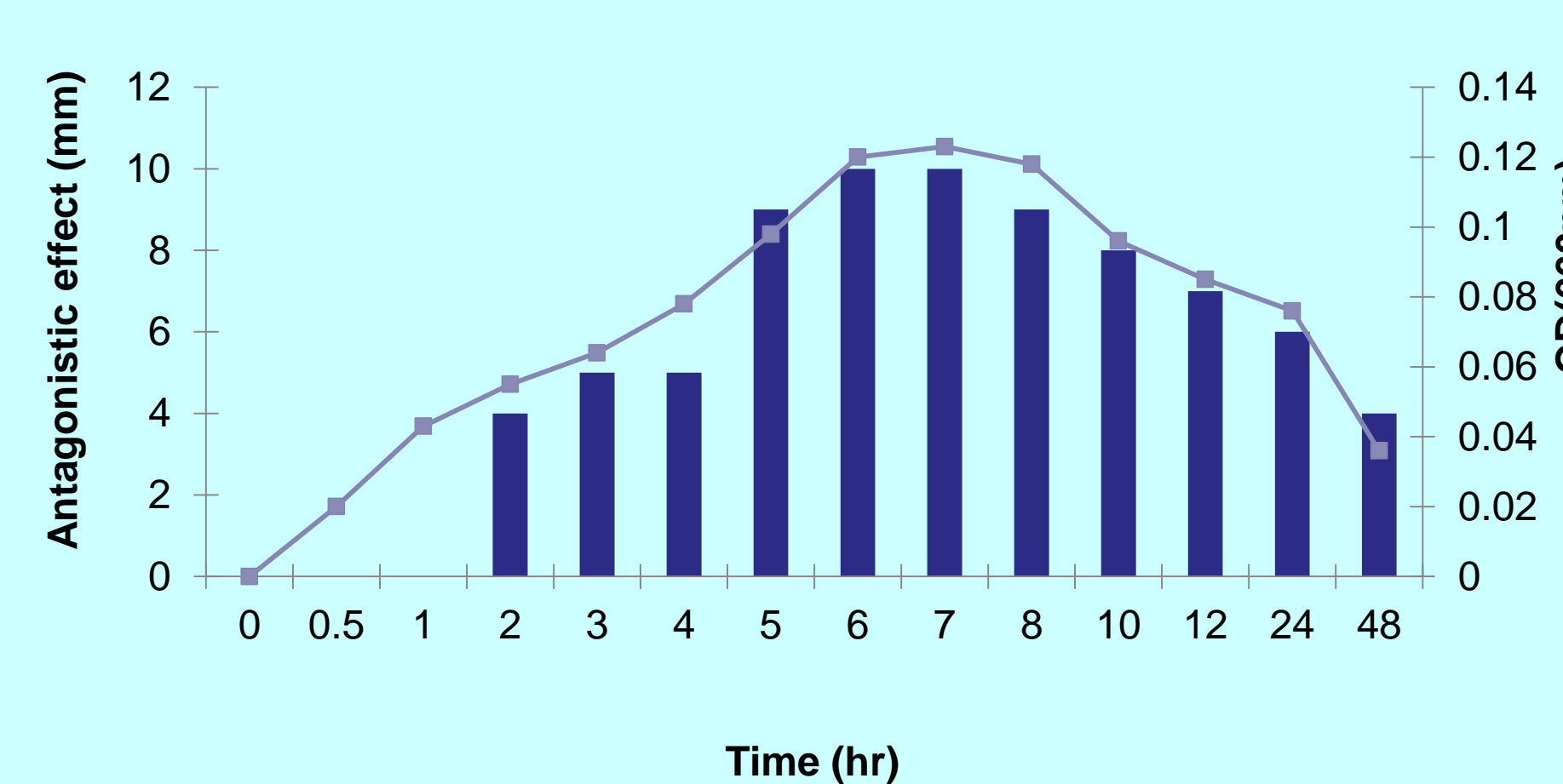
Cholesterol removal: 0.3% bile+ MRS broth+ Cholesterol solution (40 mg/ml in 96% ethanol alcohol)

Results and Discussion:

A total of 77 lactic acid bacteria were isolated from samples. Then 10 of them identified as 4 strains of *Pediococcus acidilactici* , 2 strains of *Lactobacillus paracasei* , 2 strains of *Lactococcus lactis* , one strain of *Lactobacillus pentosus* and one strain of *Lactobacillus brevis*.

✓ Inhibitory activity of selected LAB isolates against *Staphylococcus aureus* after pH neutralization and enzyme treatments

LAB isolates	pH	Catalase	Lysozyme	Pronase E	Pronase K	Trypsin
<i>L.B pentosus</i>	+	+	-	-	-	+
<i>L.B brevis</i>	+	+	-	-	-	+
<i>LC. Lacti 1</i>	-	Acid	Acid	Acid	Acid	Acid
<i>LC. Lacti 2</i>	+	+	+	-	-	+
<i>L.B paracasei 1</i>	-	Acid	Acid	Acid	Acid	Acid
<i>L.B paracasei 2</i>	+	+	+	-	-	+
<i>Ped. Acidilactici 1</i>	-	Acid	Acid	Acid	Acid	Acid
<i>Ped. Acidilactici 2</i>	-	Acid	Acid	Acid	Acid	Acid
<i>Ped. Acidilactici 3</i>	-	Acid	Acid	Acid	Acid	Acid
<i>Ped. Acidilactici 4</i>	+	+	-	-	-	+



✓ Antimicrobial activity of *lactobacillus paracasei* and *pediococcus acidilactici* against *staphylococcus aureus* respectively.

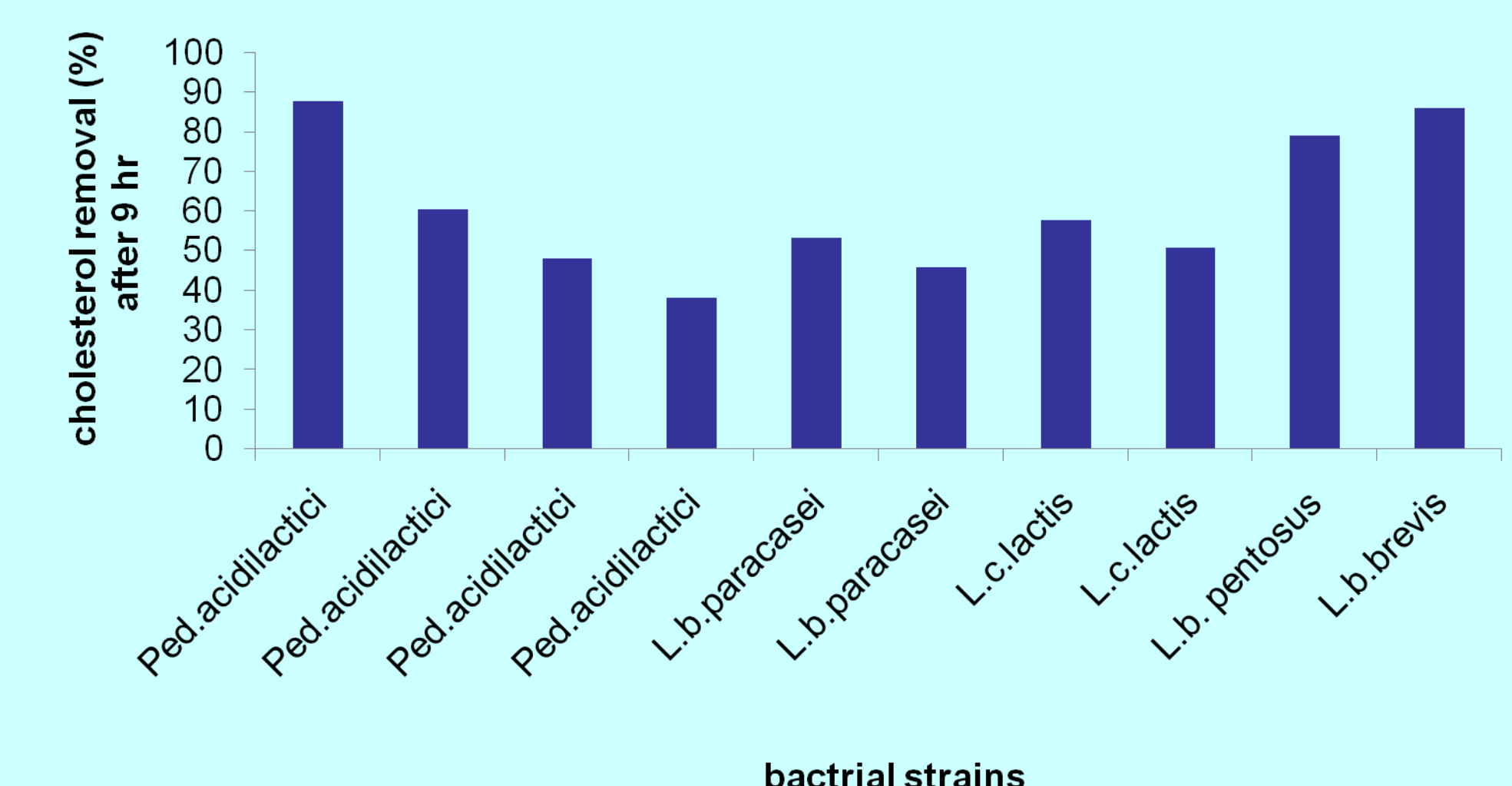
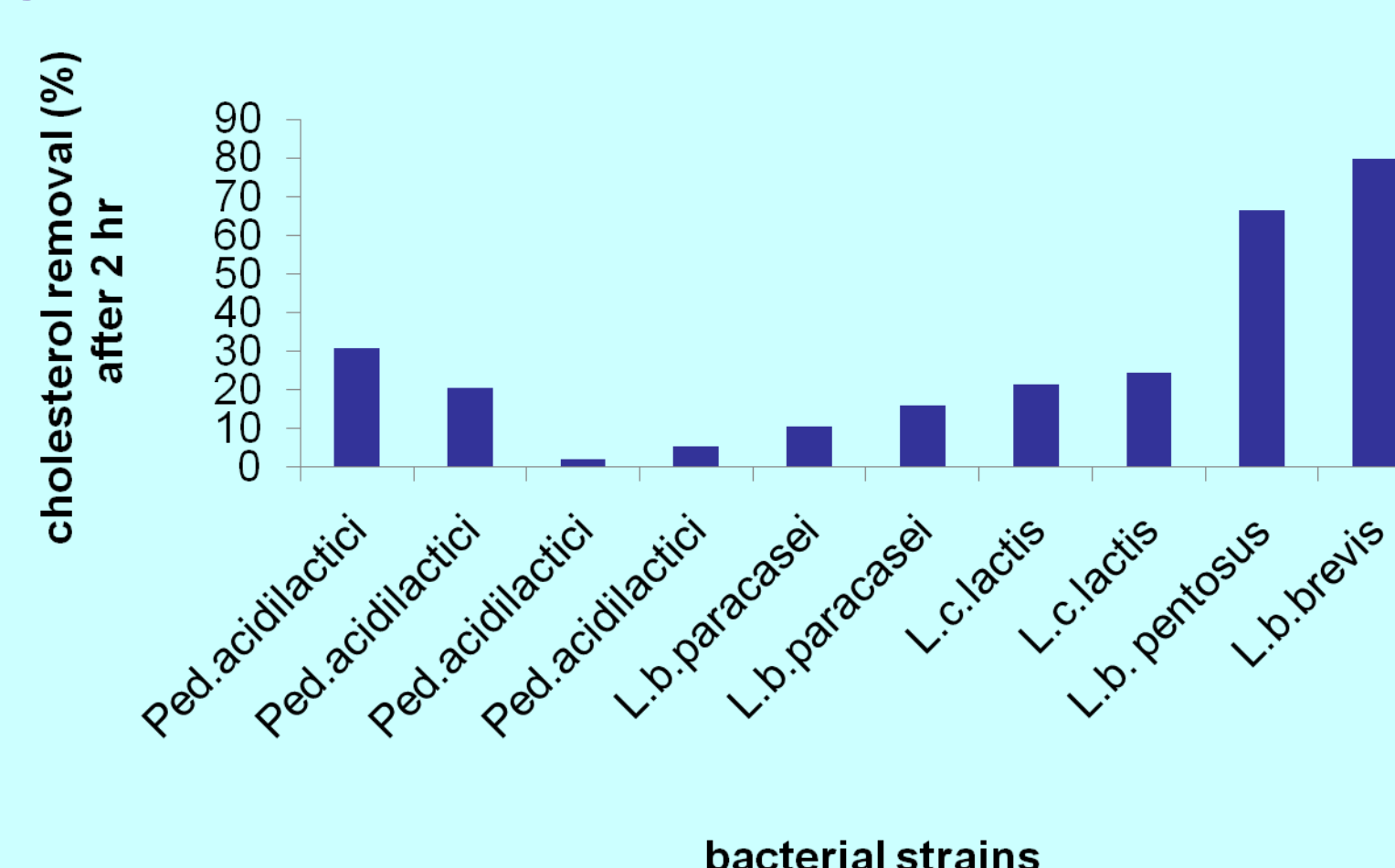
✓ Bacteriocin titration

The highest titration of bacteriocin of *Lactobacillus pentosus* (LP2), *Lactobacillus paracasei* (LP2) and *Pediococcus acidilactici* (PA4) was 1600 AU/ml though in *Lactobacillus brevis* was 400 AU/ml.

✓ Molecular size estimation of bacteriocin-like substances

The fractions of the bacteriocins produced by *Lactobacillus pentosus* (LP2), *Pediococcus* and *Lactobacillus brevis* showing antibacterial activity corresponded to peptide molecules in the range of 5-10 KDa while *Lactobacillus Paracasei* (LP2) and *Pediococcus acidilactici* (PA4) range of 10-30 KDa which was subsequently confirmed by subjecting the fractions to ultra filtration using filtron membranes with 5, 10 and 30 Kda molecular weight cut off.

✓ Cholesterol removal:



Conclusion:

Lactic acid bacteria isolated from traditional product, have strong characteristic like producing bacteriocin-like substrate and remove cholesterol from the culture medium, therefore were regarded as a candidate probiotic .