Potential of probiotics and their glycopeptides in treatment of bacterial mastitis in dairy cows

Gundega Gulbe[1]  **Anda Valdovska**[2] 

[1] Latvia University of Agriculture, Research Institute of Biotechnology and Veterinary Medicine "Sigra", Latvia. e-mail: gundega.gulbe85@gmail.com
[2] Latvia University of Agriculture, Faculty of Veterinary Medicine, Latvia. e-mail: Anda.Valdovska@llu.lv

INTRODUCTION

Probiotic therapy is substantial alternative to antibiotics for a treatment of bacterial diseases. Glycopeptides are an active part of probiotic bacteria with antagonistic activity to variety of pathogens. This study demonstrates that *L. helveticus* glycopeptides have a great potential for the treatment of bacterial mastitis in dairy cows.

OBJECTIVE

An objective of this study is to examine some probiotics and their glycopeptides for the inhibition of causative agents of bovine mastitis and to test haemolytic activity of glycopeptides.

MATERIAL

Lactobacillus reuteri, Pediococcus pentosaceus, glycopeptides of *Lactobacillus helveticus* 1% (GP1), 2% (GP2), 1% with ß-glucans (GP3), glycopeptides 0.5% (GP4) and <0.5% (GP5) were tested in vitro to examine their for the inhibition of isolated bacteria from bovine mastitis: *Serratia spp., Staphylococcus aureus, Staphylococcus saprophyticus, Streptococcus agalactiae, Streptococcus uberi, Enterococcus faecium, Escherichia coli, Kocuria kristinae* and mixed culture of above mentioned bacteria.

METHODS

- Inhibitory activity of glycopeptides and probiotic lactobacilli was investigated by **well diffusion assays** (see Figure 1). An 18 h old cultures of bacterial strains were prepared. 0.1 mL of bacterial culture peptone saline dilution of $1.5x10^8$ cfu mL^{-1} (0.5 Mc Farland) and $0.75x10^8$ cfu mL^{-1} (0.25 Mc Farland) were inoculated by streaking over an entire triptic soy agar medium surface. Wells (6 mm) were cut into the agar plate and 30 µl of each test culture was placed into each well.
- Antibiotic suspension "Pen Strep" and amoxicillin with clavulanic acid were used as control.
- The plates were incubated for 24 h at 37°C and inhibition of growth was examined by clear zone surrounding each well.
- Haemolytic activity of glycopeptides was tested using blood agar medium with purpose to detect presence of haemolysis.
- The data were analysed using the IBM SPSS Statistics 20 software package, data are presented as mean ± standard deviation.

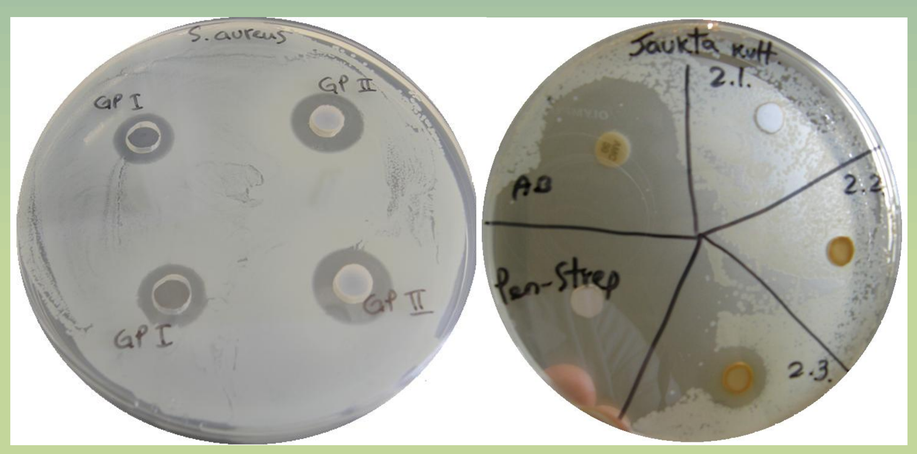

Fig. 1. Well and disk diffusion assay on tryptic soy agar medium. Bacterial clearance is present.

Acknowledgments. Isolation of glycopeptides was supported by ERAF project Nr.2DP/2.1.1.1.0/10/APIA/VIAA/125, but experiment – by Nr.2010/0226/2DP/2.1.1.1.0/10/APIA/VIAA/099 foundations.

RESULTS

1. GP1, GP4 and GP5 have the greatest inhibitory effect.

Table 1. Antimicrobial activity of glycopeptides

Pathogens	Zone of inhibition in diameter (mm)							
	Concentration 20 mg mL^{-1}					Concentration 20 mg 0.5mL^{-1}		
	GP1	GP2	GP3	GP4	GP5	GP1	GP2	GP3
S. aureus	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	11.50 ±1.22	13.38 ±0.75	14.17 ±0.29	0.00 ±0.00	0.00 ±0.00
S. saprophyticus	6.33 ±5.51	6.67 ±5.77	0.00 ±0.00	-	-	12.17 ±0.29	0.00 ±0.00	0.00 ±0.00
S. uberis	6.33 ±5.51	10.67 ±0.58	6.33 ±5.51	-	-	22.17 ±0.29	8.00 ±0.00	7.83 ±1.26
K. kristinae	9.67 ±0.58	9.33 ±0.58	9.33 ±0.58	-	-	20.00 ±0.50	0.00 ±0.00	10.00 ±0.00
E. coli	-	-	-	-	-	11.00 ±0.00	8.00 ±0.00	0.00 ±0.00
S. agalactiae	-	-	-	12.88 ±0.85	14.25 ±1.04	-	-	-
Serratia spp.	-	-	-	14.25 ±2.63	15.25 ±1.19	-	-	-
E. faecium	-	-	-	11.63 ±1.38	11.13 ±0.25	-	-	-
Mixed culture	13.00 ±0.50	0.00 ±0.00	7.00 ±0.00	-	-	13.00 ±0.50	0.00 ±0.00	7.00 ±0.00
Average	8.83 ±3.02	6.67 ±1.73	5.67 ±1.52	12.56 ±1.52	13.50 ±0.81	15.42 ±0.31	2.67 ±0.00	4.14 ±0.21

2. All bacterial strains are resistant to probiotics, but antibiotics show pronounced antagonistic activity.

Table 2. Antimicrobial activity of antibiotics and probiotics

Pathogens	Zone of inhibition in diameter (mm)			Antibiotics	
	Concentration 20 mg 0.5mL^{-1}				
	LR	PP	LR + PP	Pen Strep	AMC30
S. aureus	0.00±0.00			59.67 ±0.29	29.67 ±0.29
S. saprophyticus				40.12 ±0.29	21.67 ±0.29
S. uberis				54.17 ±0.29	26.83 ±0.29
K. kristinae				54.00 ±1.00	32.00 ±0.00
E. coli				31.83 ±0.29	0.00 ±0.00
Mixed culture				49.00 ±1.00	27.00 ±0.50
Average	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	48.13 ±0.53	22.86 ±0.23

CONCLUSIONS

- ✓ Investigated glycopeptides demonstrate inhibitory effect against bacterial cultures, but GP1, GP4 and GP5 had a greatest action (12.56-15.42 mm zone of clearance).
- ✓ All bacterial strains were resistant to test solutions containing probiotics *L. reuteri* and *P. pentosaceus*.
- ✓ Glycopeptides displayed no haemolysis when tested with sheep blood indicating that it is non-pathogenic.