# Microbial analysis of ovine cheese by next generation sequencing

***Vladimir Kmet*** **and** ***Dobroslava Bujnakova***

*Institute of Animal Physiology, Slovak Academy of Sciences, Soltesovej 4, 040 01 Kosice, Slovakia, Email: kmetv@saske.sk*

## INTRODUCTION

**The most common genera of lactic acid bacteria in ovine milk include *Lactococcus*, *Lactobacillus*, *Streptococcus*, and *Enterococcus* (Chebenova-Turcovska et al., 2011; Pangallo et al., 2014). Strains identified in ovine cheese and bryndza cheese by MALDI-TOF analysis belonged to 10 species of non-enterococcal lactic acid bacteria, i.e. *Lactobacillus casei/Lactobacillus paracasei*, *Lactobacillus* plantarum, Lactobacillus rhamnosus, Lactobacillus helveticus, Lactobacillus *delbrueckii, Lactobacillus fermentum, Lactobacillus brevis, Lactococcus lactis*, *Pediococcus pentosaceus*, and *Pediococcus acidilactici* (Kmet and Drugdova, 2012). Moreover Bunesova et al (2014) isolated *Bifidobacterium crudilactis* and *B. animalis* subsp. *lactis* in ovine cheese.**

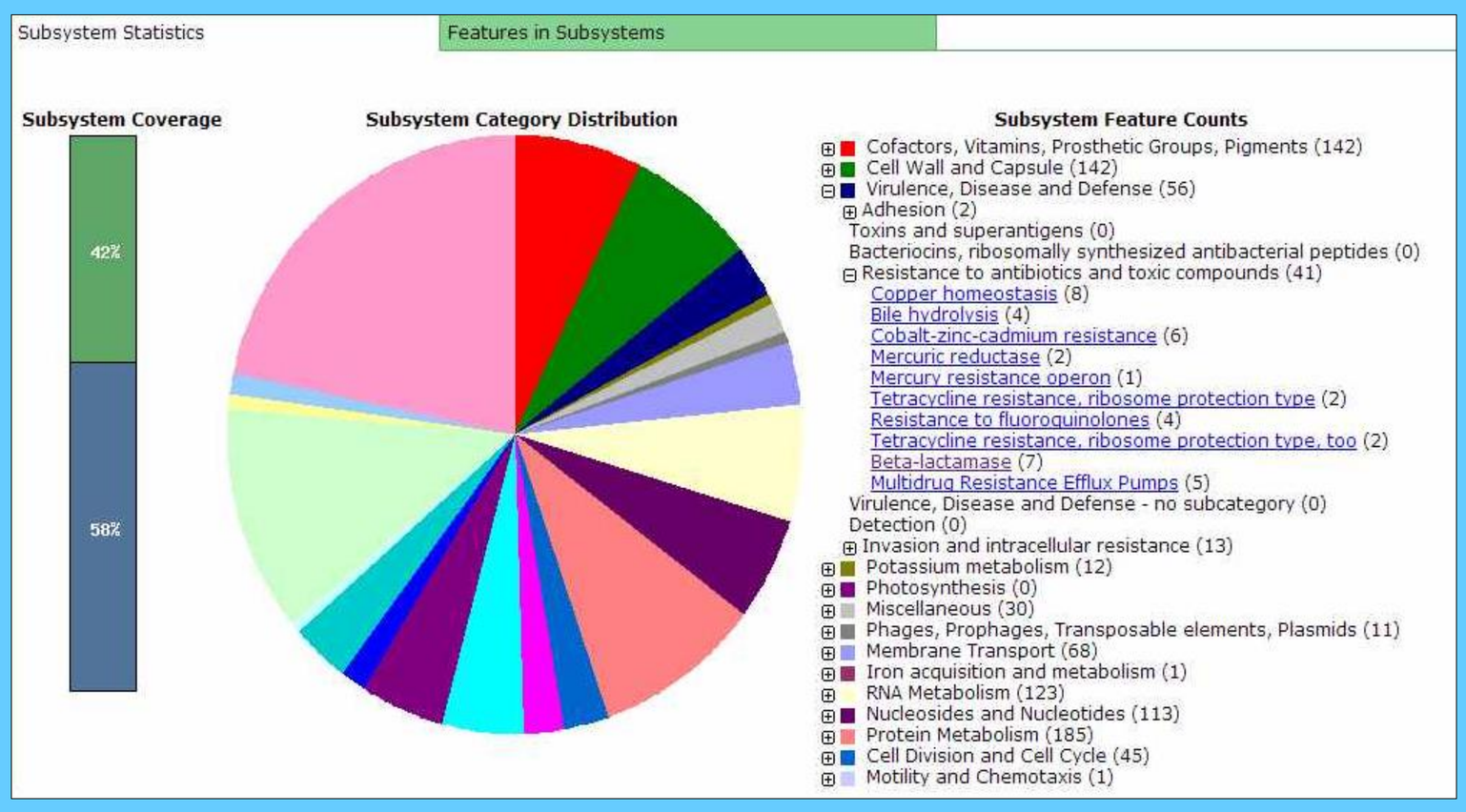
## MATERIAL and METHODS

**This study aimed to analyse microbiota of the ovine cheese „Slovak Bryndza“ and to investigate the presence of *Lactobacillus* antibiotic resistance, virulence or probiotic genes by pyrosequencing. The 16S rRNA PCR products from ovine cheese and genomes of lactobacilli ( *L. plantarum, L. brevis, L. paracasei*) were sequenced using a Roche 454 GS FLX Titanium sequencer platform.**

**Fig.1 *Lactobacillus* proteins related to antibiotic resistance**

| protID | protDesc | pf.rol |
|---|---|---|
| PF00905 | Penicillin binding protein transpeptidase domain | Serine beta-lactamase-like superfamily |
| PF03965 | Penicillinase repressor | Helix-turn-helix clan |
| PF00144 | **Beta-lactamase** | Serine beta-lactamase-like superfamily |
| PF00753 | **Metallo-beta-lactamase superfamily** | **NA ??????** |
| PF00204 | DNA gyrase B | NA |
| PF00440 | Bacterial regulatory proteins | tetR family |
| PF01610 | Transposase | Ribonuclease H-like superfamily |

**Fig. 2. Annotation of *L. plantarum* 19L3 genome in RAST**




**This study was supported by Slovak grant VEGA 2/0014/13**

## RESULTS

**The V1-V3 regions of the 16S ribosomal DNA were amplified from different ovine cheeses using PCR. In all samples, the microbial populations consisted of *Lactobacillus helveticus*, *Lb. acidophilus*, *Lb. plantarum*, *Lb. rhamnosus*, *Lb. brevis*; *Lactococcus lactis*, *L. raffinolactis*, *L. garviae*; *Enterococcus italicus* and *E. cameliae*; *Streptococcus salivarius*, *St. thermophilus*, *St. caballi* and *St. ferus*.**

**Laboratory evaluation showed that *L. plantarum* 19L3 was susceptible to antibiotics, according to the ISO 10932 standard**

**Furthermore, the genomes of selected *Lb. plantarum*, *Lb. brevis*, *Lb. paracasei* were pyrosequenced. The assembly of *L. plantarum* resulted in 203 contigs longer than 1,000 bp (D'Auria *et al*., 2014).**

**There were identified probiotic proteins as an alpha amylase (protein ID PF00128), peptidase (PF01433), catalase (PF00199), heat shock protein 33 (PF01430). Nevertheless, there was discrepancy between *Lb. plantarum* ampicillin sensitivity and the presence of serine beta-lactamase like superfamily (Fig. 1). No virulence factors were detected. RAST results (Fig.2) indicated new properties of lactobacilli, which were not occurred by phenotyping testing. EFSA ( 2012) recommended that probiotic LAB strains should lack acquired antimicrobial resistance and virulence genes detected by PCR or by next generation sequencing.**

## CONCLUSIONS

**Unusual data (antibiotic resistance) from NGS is necessary to verify by PCR. New *Lactobacillus* probiotic proteins were detected.**