

Fecal microbiota composition of Krškopolje pig breed raised in two different production systems

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Background & Objectives

- Krškopolje breed is the only preserved Slovenian autochthonous pig breed known for its robustness and meat quality
- This study aimed to evaluate the fecal microbiome of Krškopolje breed reared on organic farms, which has not yet been investigated

Material & Methods

- 18 pigs kept on two different farms and fed with organic commercial feed were included into the study:
 - pigs kept on pasture together with small ruminants (group L1; Fig. 1) → farm 1
 - pigs kept indoors without habitation with other farm animals and fed only commercial feed (group L2; Fig. 2A) → farm 2
 - pigs kept on pasture with cattle (group L3, Fig. 2B) → farm 2
- Fecal samples were collected rectally in all four seasons of 2022, from grower to finisher phase
- 18×4=72 DNA samples underwent 16S rRNA gene (V3–V4 region) sequencing using Illumina paired-end (2×250 bp) technology
- Operational taxonomic unit (OTU)-based microbiome analysis was performed using QIIME 1.9.1

Results

- Alpha diversity was comparable between the study groups; the average number of observed OTUs was 1746
- The two predominant bacterial phylum in all three groups was *Bacteroidota*, followed by *Firmicutes* (Fig. 3)
- Significant seasonal changes in microbiome composition were observed between all seasons (nonparametric MANOVA, $p < 0.008$). The microbiome composition differed significantly between study groups L1 and L2–L3, as shown by principal coordinate analysis of weighted UniFrac distances (Fig. 4) and nonparametric MANOVA ($p = 0.001$), whereas groups L2 and L3 did not differ significantly ($p = 0.2$)
- Linear discriminant analysis Effect Size (LefSe) identified several bacterial taxa that were most differentially abundant between the different study groups and the different seasons



Fig. 1 (left). Pigs from group L1 were co-habiting with small ruminants on farm 1.



Fig. 2. Pigs on farm 2 were either (A) indoor-raised with no co-habitation with other animals (group L2) or (B) pasture-raised and co-habiting with cattle (group L3), respectively. Both groups were reared on the same organic farm and maintained by the same livestock workers, who differed from those on farm 1.

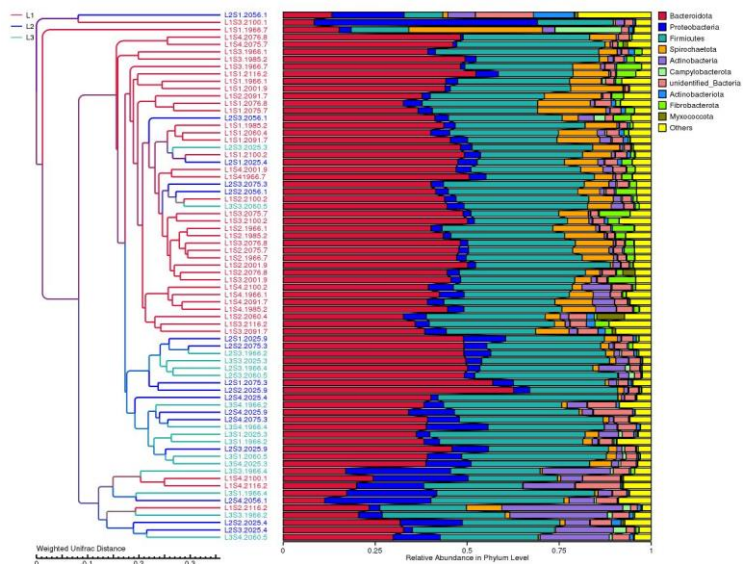


Fig. 3. UPGMA cluster tree at the phylum level based on weighted UniFrac distances. Samples on the tree are colored by study group.

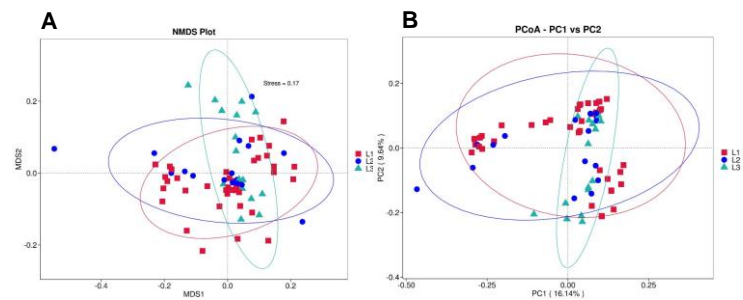


Fig. 4. Beta diversity of Krškopolje pig. (A) NMDS plot, (B) PCoA plot. Both plots are colored according to the study group. The microbiota composition of group L1 differed significantly from groups L2–L3, as shown by nonparametric PERMANOVA and MRPP analysis.

Discussion & Conclusions

- Pig microbiota composition is not stable over time and is influenced by rearing-related factors, but appears to be robust throughout the grower-finisher phase
- Fecal microbiota composition of Krškopolje pig appears to be most similar to that of Iberian pig, an autochthonous Spanish breed (Sebastià C et al., 2024, doi: 10.1128/msystems.01049-23)
- In this study, the production system or co-rearing with other animal species had no significant influence on the pig microbiome composition
- Rather, the main observed differences in microbiota composition could be explained by the farm of origin and livestock workers

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