71st ICoMST
GIRONA - CATALONIA - SPAIN
3rd-8th August - 2025

SUCCESSION OF ACTIVE SPECIES IN BROILER SPOILAGE MICROBIOME AT +4°C

Julia A. Manninen[1*], Elio Nushi[1], Per Johansson[1], Johanna Björkroth[1]

[1] University of Helsinki, Helsinki, Finland.

*Corresponding author email: julia.manninen@helsinki.fi

I. INTRODUCTION

Transcriptomic analysis is based on RNA-seq data, which has many advantages compared to DNA-based methods such as metagenomics, since only living and active species are detected. Transcriptomics allows the detection of active microbial species in samples, and coupled with longitudinal studies shows the succession of active species during the spoilage of a sample. Our group has previously performed metatranscriptomic analysis of beef spoilage microbiome [1], but the active broiler spoilage microbiome has not yet been studied over time. The aim of this research was to investigate the succession of active bacterial species in the broiler over the course of time and to identify the most prominent species at different timepoints.

II. MATERIALS AND METHODS

RNA samples were isolated from modified atmosphere packaged broiler chicken stored in +4°C every other day from day 5 after packaging to day 15 after packaging, which was also 3 days after the best-by date. RNA was sequenced by the Institute of Biotechnology. Ribosomal RNA, both 16S and 18S, were removed using SortMeRNA [2] and host DNA by aligning to *Gallus gallus* genome GCA_016699485.1 using Bowtie2 [3]. FastQC [4] and MultiQC [5] were used to quality control the data.The processed RNA sequences were analysed with Kraken2 [6] and Bracken [7] for abundance of active bacterial species in the samples. Kraken2 database was built with the bacterial database and six species of interest added to the database: *Dellaglioa algida* (GCF_030127605.1), *Dellaglioa carnosa* (GCF_026948345.1), *Lactococcus carnosus* (GCF_006770265.1), *Photobacterium carnosum* (GCF_021236965.1), *Vagococcus proximus* (GCF_029143285.1) and *Vagococcus fessus* (GCF_003987565.1). The results were visualised with Krona [8] for easy identification of changes in the active spoilage microbiome.

III. RESULTS AND DISCUSSION

The percentage of RNA reads aligning to bacteria rose from less than a percent in the first samples to on average 71 percent in the last samples. Lactic acid bacteria were the most abundant order of bacteria detected in the samples during the whole 15-day experiment. Most prevalent bacterial species during the longitudinal study were *Carnobacterium divergens* and *maltaromaticum*, *Vagococcus proximus* and *Leuconostoc gelidum*.
Bacterial diversity was highest in the first samples, with the aforementioned species slowly growing more abundant in the samples until the best-by date. The percentage of active gram-negative bacteria decreased over time. *V. proximus* was not abundant in the early samples, becoming prevalent around the best-by date and peaking in the samples taken on day 15.

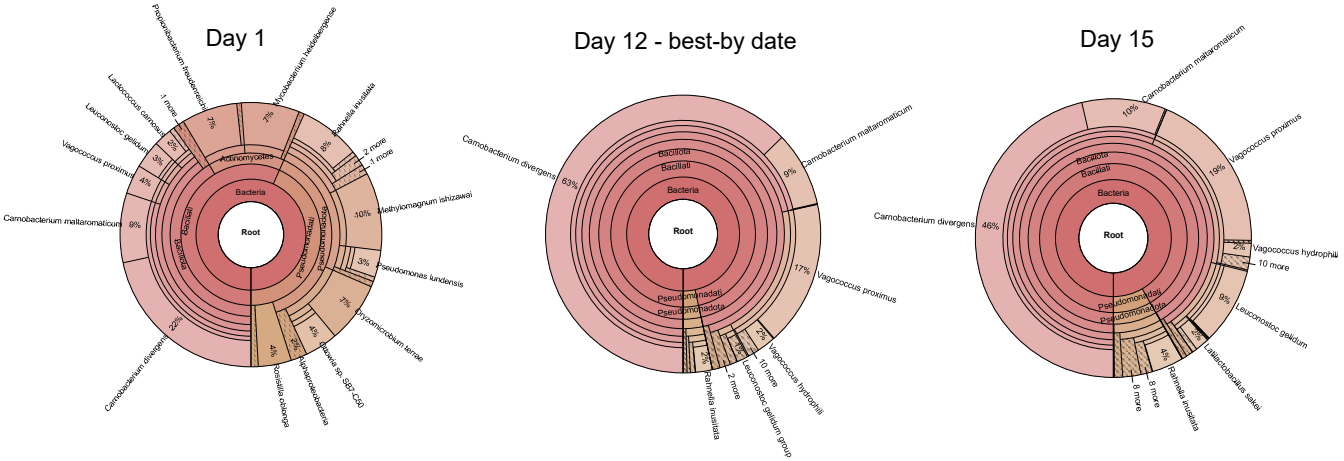


Figure 1. Compositions of active spoilage bacteria in broilers samples obtained from Bracken and visualized with Krona.

IV. CONCLUSION

In this experiment we performed the first longitudinal transcriptomic analysis of broiler spoilage microbiome. Few species were found to compose the majority of the broiler spoilage microbiome in +4°C. Lactic acid bacteria were the most prevalent spoilers, with gram-negative bacteria composing only a small part of the spoilage microbiome. We will continue this research with broiler in +6°C to investigate the effect of temperature on spoilage microbiome over time, as well as investigating the active metabolic pathways to find RNA markers associated with spoilage.

ACKNOWLEDGEMENTS

We would like to acknowledge Henna Niinivirta for her skillful work in extracting the RNA. This work was supported by Novo Nordisk distinguished investigator grant of Professor Björkroth (NNF20OC0061239).

REFERENCES

1. Hultman, J., Johansson, P. & Björkroth, J. (2020) Longitudinal Metatranscriptomic Analysis of a Meat Spoilage Microbiome Detects Abundant Continued Fermentation and Environmental Stress Responses during Shelf Life and Beyond. Applied and Environmental Microbiology 86: e01575-20.
2. Kopylova, E., Noé, L. & Touzet, H. (2012) SortMeRNA: fast and accurate filtering of ribosomal RNAs in metatranscriptomic data. Bioinformatics 28: 3211–3217.
3. Langmead, B. & Salzberg, S. L. (2012) Fast gapped-read alignment with Bowtie 2. Nature Methods 9: 357–359.
4. Andrews, S. (2010) FastQC: A Quality Control Tool for High Throughput Sequence Data. Babraham Bioinformatics. https://www.bioinformatics.babraham.ac.uk/projects/fastqc/
5. Ewels, P., Magnusson, M., Lundin, S. & Käller, M. (2016) MultiQC: summarize analysis results for multiple tools and samples in a single report. Bioinformatics 32: 3047–3048.
6. Wood, D. E., Lu, J. & Langmead, B. (2019) Improved metagenomic analysis with Kraken 2. Genome Biology 20: 257.
7. Lu, J., Breitwieser, F. P., Thielen, P. & Salzberg, S. L. (2017) Bracken: estimating species abundance in metagenomics data. PeerJ Computer Science 3: e104.
8. Ondov, B. D., Bergman, N. H. & Phillippy, A. M. (2011) Interactive metagenomic visualization in a Web browser. BMC Bioinformatics 12:385.