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Buschhardt, Tasja; Aabo, Søren; Hansen, Tina Beck

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DTU Food
National Food Institute

Analysis of dynamic changes in the meat microbiota during varied temperature exposures - a novel method to estimate temperature history and pathogen growth in meat

Tasja Buschhardt, PhD student

Supervisors: Søren Aabo and Tina Beck Hansen, Microbial Food Safety, Division of Food Microbiology

Summary The overall objective of this project is to develop a method that enables an estimation of the temperature exposure of meat from slaughter to any chosen point in the meat chain. This temperature estimate will preferably be given as s standardised time temperature measure (index) which will allow for an estimation of pathogen growth (e.g. *Salmonella*). The temperature index will be developed based on an analysis of the meat microbiota during varied temperature exposures. A nested 16S rRNA pyrosequencing protocol will be used as a novel approach to investigate temperature dependent changes in the meat microbiota.

Purpose Fresh meat handling is often performed in ambient temperatures, without temperature monitoring. This could permit pathogen growth and compromise the consumer safety. The presented research project will presumably change the understanding of how temperature exposures of meat affect food safety. Further, it will potentially provide a new tool for meat safety control system in production lines.

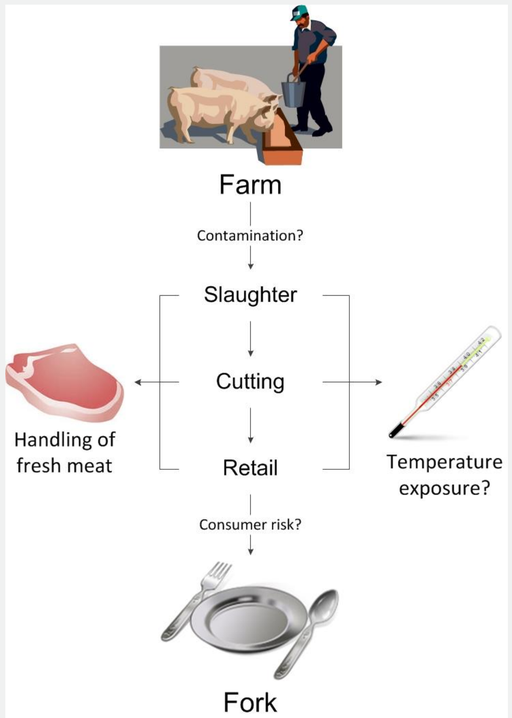

Fig. 1: Schematic meat chain “from farm to fork”. The spread of pathogens in the meat chain represents a major food safety concern. Enteropathogens from the environment and the intestines of animals are introduced to the slaughter line and contaminate process equipment and carcass surfaces as well as fresh meat during processing.

Hypotheses We hypothesize that the composition of the microbiota in pork will reflect the time-/temperature exposure (temperature history) of meat and allow for an estimation of pathogen growth.

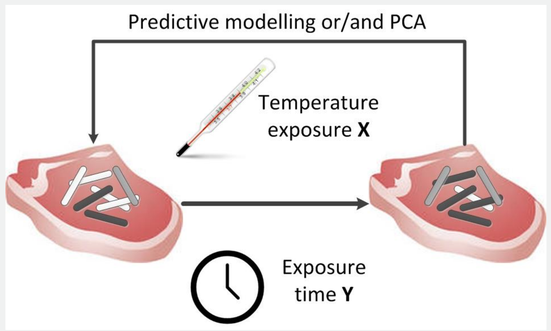

Fig. 2: Schematic drawing of the time and temperature depended change in the meat microbiota. Reverse predictive modelling and/or principal component analysis will be used with the attempt to describe the initial microbiota after a defined temperature exposure.

Objectives

1. To develop a refined 16S rRNA gene pyrosequencing protocol.
2. To investigate the development of a pork meat microbiota during varied temperature exposures.
3. To develop a **method that enables an estimation of the temperature exposure (temperature history) of meat** from slaughter to any chosen point in the meat chain based on the composition of the meat flora.

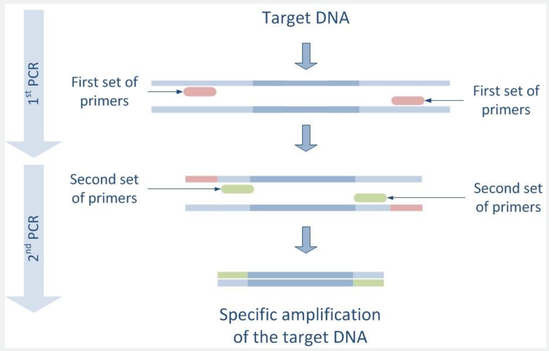

Fig. 3: The nested PCR principle. An Enterobacteriaceae-specific primer is introduced during a first PCR run, while a universal primer is employed during a second run. The barcode-tagging is not presented in this schematic drawing.

Methods It is intended to **employ a nested pyrosequencing protocol as a novel approach to analyse microbial subpopulations in meat** by using phylotype-specific primers targeting 16S rRNA variable regions.

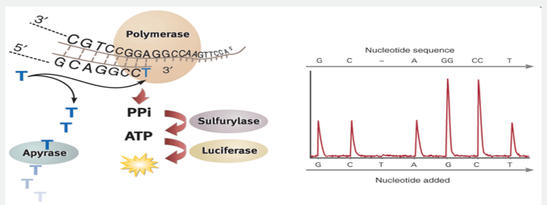

Fig. 4: The Principle of pyrosequencing and the output pyrogram™. Double peak height indicate the incorporation of two nucleotides in a row (Source: Engand and Petterson (2005): *Pyro Q-CpG™: quantitative analysis of methylation in multiple CpG sites by Pyrosequencing®*, Nature Methods, *2)*. Pyrosequencing allows for massively parallel sequencing with low error rates. Different samples can be analysed simultaneously due to sample-specific barcode-tagging. Sequence alignments are used to describe the abundance and diversity of bacterial phylotypes in a given sample.

Results & Conclusions An Entero-bacteriaceae-specific primer pair was developed and successfully used to amplify the 16S gene of eight target genera during the first PCR run (fig. 5). The differentiation of eight focus genera with a universal primer targeting the V4 variable 16S gene region during the second PCR run was so far not successful (fig. 6). An *in silico* analysis showed that a differentiation based on the V3 variable region is more suitable.

Fig. 5: PCR product bands from pure cultures of eight target genera (*Citrobacter*, *Enterobacter*, *Escherichia*, *Hafnia*, *Klebsiella*, *Salmonella*, *Serratia*, and *Yersinia*) on a 2% agarose gel with SYBR® Green amplified with a newly developed Enterobacteriaceae-specific primers.

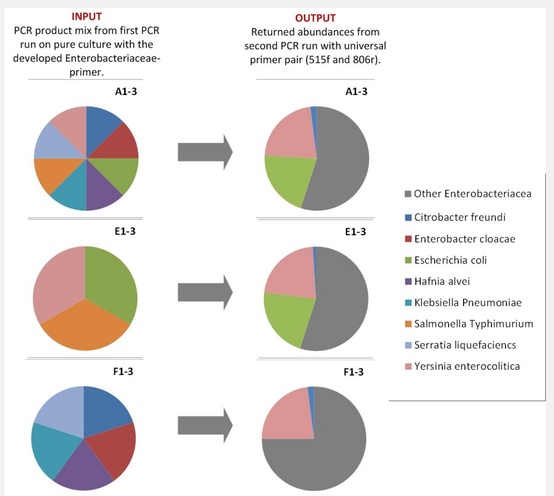

Fig. 6: Comparison of the pyrosequencing input (PCR product mixes) with the output (results obtained). The differentiation of the eight selected Enterobacteriacea genera was not successful. Consequently, the shown abundances of each genera are incorrect as well.

Contact information: Tasja Buschhardt
busch@food.dtu.dk