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16S rRNA gene sequencing as a tool to study microbial populations in foods and process environments – limitations and opportunities

Tasja Buschhardt*, Tina Beck Hansen, Martin Iain Bahl, Waleed Abu Al-Soud, Martin Asser Hansen, Søren Aabo

Summary In this study we compared the influence of variable regions of the 16S rRNA gene, DNA extraction methods, and polymerases on the microbial diversity in whole communities and subpopulations detected by 16S gene sequencing. Taxonomic assignments and abundances of sequences were affected by all tested variables. However, using the same method for each sample community structures were highly reproducible, allowing quantitative estimations of subpopulations using culturing of specific bacterial groups as anchor points. This opens for the use of sequencing data in predictive models to study microbial community dynamics.

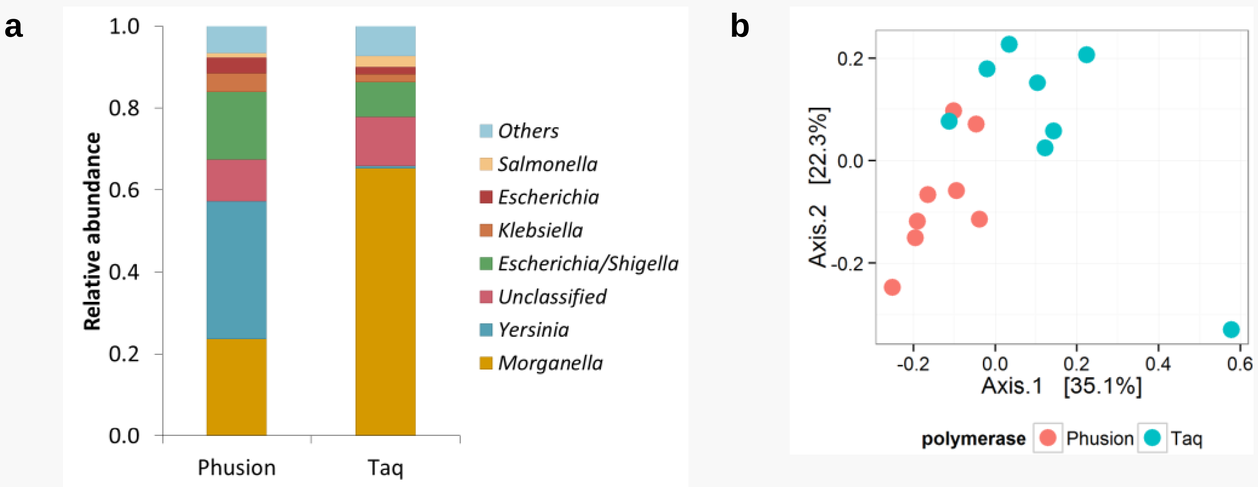

Fig. 3. (a) Stacked column plot (100%) showing the abundance of *Enterobacteriaceae* genera in Phusion and Taq amplified samples. Genera with an average of less than 100 reads per sample were aggregated in the artificial group *Others*; (b) Two-dimensional PCoA plot based on Bray-Curtis distance. Phusion (red) and Taq amplified samples (blue) are presented in single points and the explained diversity by each axis are shown.

Introduction Traditional cultivation methods leave the true microbiological diversity of food and process environments unexplored. While a few microorganisms can be identified and enumerated by standardized methods the majority of the population cannot be cultured or isolated. 16S-RNA gene sequencing has the potential to provide a more in depth understanding of how the ecology of microbial populations in meat may influence pathogens and the safety and quality of food.

Materials and Methods Pig carcass swab samples and PCR product mixes from pure cultures were analyzed by nested PCR combined with MiSeq® Illumina® 16S DNA sequencing and standardized culture methods as cross reference.

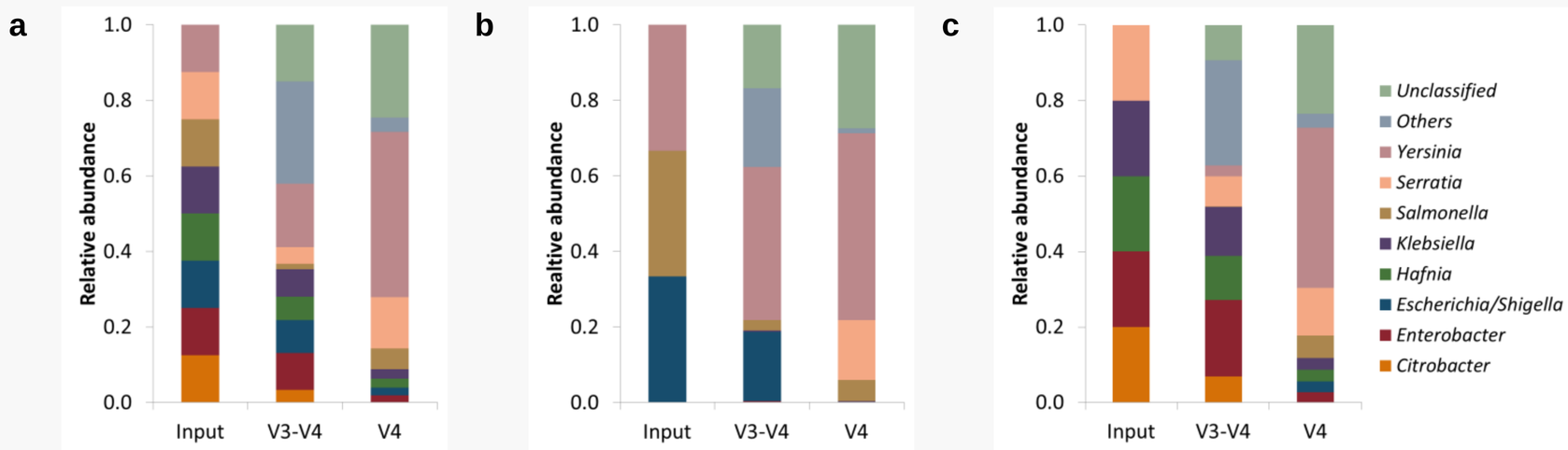

Fig. 1. Stacked column plots (100%) of PCR product mix A (a); B (b) and C (c) showing the distribution of Enterobacteriaceae genera in the sequencing input (PCR product mix of known concentrations) and the sequencing output based on the V3-V4 and V4 variable region of the *16S* gene. Sequencing reads that belonged to a genus not added to the input were aggregated in the artificial group *Others*.

Results and Discussion Community structures varied with sample processing such as DNA extraction methods (Fig. 2), polymerases used for initial amplification of community DNA (Fig.3) , and the 16S variable region used to characterize the community (Fig. 1)**.** Relative abundances of *Enterobacteriaceae* and genera thereof were affected by all variables tested (Fig.1, Fig. 2b, Fig. 3a). The observed variations in community compositions could be due to variable regions comprising different degrees of sequence dissimilarities, DNA extraction methods with distinct extraction efficiencies, and polymerases with biased amplification efficiencies.

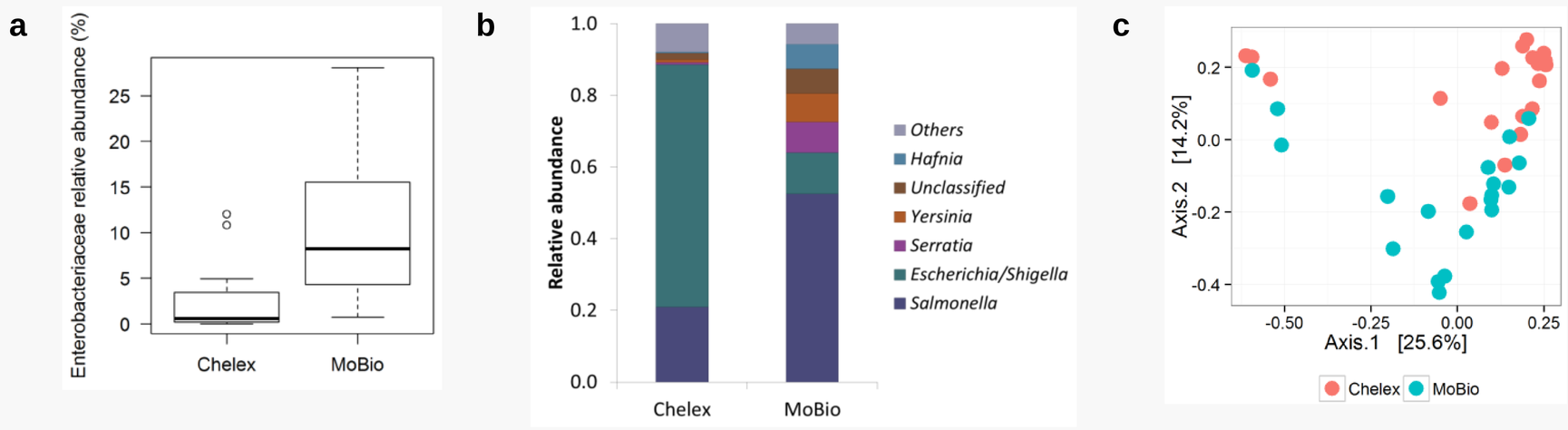

Fig. 2. (a) Boxplot showing the relative abundance of *Enterobacteriaceae* in Chelex samples compared to MoBio samples; (b) Stacked column plot (100%) showing the abundance of *Enterobacteriaceae* genera in Chelex and MoBio samples. Genera with an average of less than 100 reads per sample were aggregated in the artificial group *Others*; (c) Two-dimensional PCoA plot based on Bray-Curtis distance. Chelex samples (red) and MoBio samples (blue) are presented in single points and the explained diversity by each axis are shown.

Conclusion Our results indicate that conclusions from population studies based on 16S gene sequencing should be made with caution, especially if various sample types are analyzed that require different processing. However, being aware of the limitations, we believe that 16S rRNA gene sequencing offers great potential for microbial community studies in foods and process environments and may provide novel data for e.g. predictive modelling of food borne pathogens and identification of novel indicator bacteria.

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