**Title** Composition of the bacterial population of refrigerated beef, identified with direct 16S rRNA gene analysis and pure culture technique

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**Abstract**

The composition of the dominating population of freshly cut beef, and beef stored at 4 °C for 8 d, was studied by direct analysis of the 16S rRNA gene (PCR amplification, cloning and sequencing) and compared with pure culture technique where the isolates picked from the viable plate count were identified by sequencing of the 16S rRNA gene. The composition of the bacterial population was recorded at two different time points, at the start when the viable plate count of the meat was $4 \times 10^2$ colony forming unit (cfu) per $cm^2$ and when it was $5 \times 10^7$ cfu per $cm^2$. Direct gene analysis by PCR amplification generated 30 clones, and 79 isolates were picked from the plate count, and identified by 16S rRNA gene sequencing.

At the low initial bacterial load of the beef, the two sampling strategies showed variations in the composition of species. Direct 16S rRNA gene analysis revealed a domination of *Bacillus*-like sequences while no such sequences were found in isolates from the viable plate count. Instead the population of the plate count was dominated by *Chryseobacterium* spp. In contrast, the two sampling strategies matched on the multiplying beef population, where both methods indicated *Pseudomonas* spp. as the dominating group (99% of the population-sequences), irrespectively of sampling strategy. *Pseudomonas panacis*/*Pseudomons brennerii* was the dominating taxon (99% similarity to type strain), but sequences with highest similarity to *Pseudomonas lundensis* (99%), *Pseudomonas beteli* (99%) and *Pseudomonas koreensis* (100%) were also found.