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Yersinia enterocolitica grows and remains active in pork packaged under high-O_2 modified atmosphere (#597)

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Introduction

Yersinia enterocolitica 4/O:3is a frequent cause of sporadic human yersiniosis in Finland, and contaminated pork is considered its main vector. It is widely agreed that the contamination of meat with *Y. enterocolitica* takes mainly place during slaughtering and processing of carcasses. However, less is known what happens to *Y. enterocolitica* in the distribution chain and how the storage conditions and interaction with other bacteria growing in meat affect *Y. enterocolitica*. Thus, the purpose of this study was to asses if *Y. enterocolitica* is able to survive, remain active and grow as part of developing spoilage microbiome in high-O_2 modified atmosphere packaged pork steaks.

Methods

Fresh pork neck steaks (ca. 150-180 g) were obtained from a Finnish meat cutting plant. Steaks were cut to half, and the other half was spiked with *Yersinia enterocolitica* strain 4/O:3 (spiked samples) at levels of *ca* 6000 CFU/g, and the other half was left untreated (control). Steaks were packaged under modified atmosphere containing 70% O_2 and 30% CO_2, and stored at 5 °C. At days 2 to 9 and at the day 12, 3 spiked and 3 control packages were opened and sampled (25 g) for the following microbiological determinations: *Y. enterocolitica*, *Enterobacteriaceae*, aerobic microbes and lactic acid bacteria on CIN, VRBG, PCA and MRS medium, respectively. Metatranscriptomic analyses were conducted at day 3, 5, 7, 9 and 12: cells were collected from 10 g samples for RNA extraction followed by cDNA amplification, library construction, sequencing, quality filtering and adapter trimming of the reads before mapping of reads other than rRNA.

Results

Microbial numbers and pH: During the 12-day trial, *Y. enterocolitica* numbers increased from 2.6×10^4 to 3.3×10^6 CFU/g in spiked samples, whereas in control steaks, the numbers remained below the detection limit (100 CFU/g) throughout the trial. In most sample, also the numbers of *Enterobacteriaceae*, aerobic microbes and lactic acid bacteria increased during the trial, and at the day 12, colony counts of 2.0×10^7, 8.4×10^8, and 6.3×10^8 , respectively, were recorded. The pH values ranged from 5.74 to 6.59 being lowest at the days 5 and 6 (mean pH 5.91), and highest at the day 12 (mean pH 6.46)

Metatranscriptomic analysis: For the spikde samples, the proportion of RNA reads mapped to *Y. enterocolitica* ranged from 0% to 1.86%, whereas most of the reads obtained were mapped to lactic acid bacteria, mainly to species *Leuconostoc gelidum, Lactococcus piscium, Carnobacterium divergens* and *Carnobacterium maltaromaticum,* and to *Brochothrix thermosphacta.* For the control samples, 12 to 67% of the reads obtained were mapped to the above mentioned species of lactic acid bacteria, and 24 to 48% to *B. thermosphacta.* In additon, a varying proportion (0 to 42%) of RNA reads mapped to *Acinetobacter* spp., with highest (24-42%) proportion obtained in control steaks analyzed at the end of the trial.

Conclusion

Our work suggests that *Y. enterocolitica* 4/O:3 is well-adapted to grow in chilled pork packaged under a modified atmosphere with 70% O_2 and 30% CO_2: *Y. enterocolitica* 4/O:3 was able to grow and reach high numbers (>10^6 CFU/g) in pork and remained metabolically within the developing spoilage bacterial community until the end of the 12 -day trial. Furthermore, we showed that *B. thermosphacta, L. gelidum, L. piscium. C. maltaromaticum, C. divergens* and *Acinetobacter* spp. were dominating the bacterial communities and played a key role in the microbiomes of spoiled samples. Whereas *L. gelidum, L. piscium, Carnobacterium spp.* and *B. thermosphacta* are all known meat spoilers, *Acinetobacter* spp. has been less frequently reported in spoiling meat, and thus, its role in the spoilage process deserves further studies.

Notes