Evaluation of two sampling procedures to unveil the beef microbiota during storage through high-throughput sequencing

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High-throughput sequencing (HTS) is being a useful tool to unveil the microbiota of different food matrices. Sampling and DNA extraction are crucial steps of HTS, once only proper procedures will guarantee robust material for sequencing, assuring a reliable bioinformatics analysis. We aimed to evaluate two sampling protocols to characterize a beef microbiota through HTS. A vacuum-packed beef cut (shank) was cut in pieces of 2 x 2 cm in sterile conditions, distributed in sterile bags (50 g per bag), vacuum-packed and stored at 4 and 15 °C. One bag from each temperature was collected after 0, 5, 10, 15, 20 and 25 days, and subjected to two sampling procedures: 1) Exudate: the exudate was aseptically collected, and 2) Dilution: 25 g of the beef was homogenized with 25 mL of buffered peptone water, and the obtained dilution (1:1) was collected. DNA of the samples were extracted using magnetic beads and subjected to sequencing of the V3-V4 region of 16S rRNA using MiSeq Sequencing. Raw data was processed, resulting in reads that were grouped by operational taxonomy units (OTU), identified thought BLAST. The obtained frequencies were analyzed using XLSTAT and MicrobiomeAnalyst. OTU mean numbers obtained by exudate (7.8 ± 0.8) and dilution (6.8 ± 1.0) were not significantly different by ANOVA ($p = 0.445$), but exudate resulted a higher number of reads (11,614.1 ± 4,147.6) when compared to dilution (2,201.2 ± 912.4) (ANOVA, $p = 0.038$). Alpha-diversity indices did not present significant differences when exudate and dilution were compared based on genera and species by ANOVA: Chao1 ($p = 0.530$ and $p = 0.557$, respectively), Shannon ($p = 0.721$ and $p = 0.790$, respectively). Based on beta-diversity analysis (Jaccard) and PCoA, no significant trend of genera and species was observed for exudate and dilution (PERMANOVA, $p = 0.505$). Exudate revealed a core microbiota mainly composed by *Lactococcus*, *Dellaglioa* and *Leuconostoc* genera, and *Lactococcus piscium* and *Dellaglioa algida* species, and unidentified Enterobacteriaceae, while dilution presented a core microbiota mainly composed by *Lactococcus*, *Carnobacterium* and *Leuconostoc* genera, and *Lactococcus piscium*, *Carnobacterium maltaromaticum* and *Dellaglioa algida* species. Despite the absence of significant differences on alpha and beta diversity parameters, beef exudate allowed a higher number of reads when compared to dilution, leading to a more robust bioinformatics analysis for beef microbiota characterization.

Keywords: beef, microbiota, sampling, exudate, dilution

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