Bovine milk microbiota: Evaluation of different DNA extraction protocols for challenging samples

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Abstract

The use of an adequate protocol that accurately extracts microbial DNA from bovine milk samples is of importance for downstream analysis such as 16S rRNA gene sequencing. Although sequencing platforms such as Illumina are very common, there are reservations concerning reproducibility in challenging samples that combine low bacterial loads with high amounts of host DNA. The objective of this study was to evaluate six different DNA extraction protocols applied to four different prototype milk samples (low/high level of colony-forming units (cfu) and somatic cells). DNA extracts were sequenced on Illumina MiSeq with primers for the hypervariable regions V1V2 and V3V4. The different protocols were evaluated by analyzing the yield and purity of DNA extracts and the number of clean reads after sequencing. Three protocols with the highest median number of clean reads were selected. To assess reproducibility, these extraction replicates were re-sequenced in triplicates (n=120). The most reproducible results for alpha- and beta-diversity were obtained with the modified DNeasy Blood & Tissue kit after a chemical pre-treatment plus resuspension of the cream fraction. The unmodified QIAamp DNA Mini kit performed particularly weak in the sample representing unspecific mastitis. These results suggest that pre-treatment in combination with the modified DNeasy Blood & Tissue kit is useful in extracting microbial DNA from challenging milk samples. To increase reproducibility, we recommend that duplicates, if not triplicates, should be sequenced. We showed that high counts of somatic cells challenged DNA extraction, which shapes the need to apply modified extraction protocols.

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