

ABSTRACT

Recent work has demonstrated the existence of large inter-individual and inter-population variability in the microbiota of human milk from healthy women living across variable geographical and socio-cultural settings. However, no studies have evaluated the impact that variable sequencing approaches targeting different 16S rRNA variable regions may have on the human milk microbiota profiling results. This hampers our ability to make meaningful comparisons across studies. In this context, the main purpose of the present study was to re-process and re-sequence the microbiome in a large set of human milk samples ($n = 412$) collected from healthy women living at diverse international sites (Spain, Sweden, Peru, United States, Ethiopia, Gambia, Ghana and Kenya), by targeting a different 16S rRNA variable region and reaching a larger sequencing depth. Despite some differences between the results obtained from both sequencing approaches were notable (especially regarding alpha and beta diversities and Proteobacteria representation), results indicate that both sequencing approaches revealed a relatively consistent microbiota configurations in the studied cohorts. Our data expand upon the milk microbiota results we previously reported from the INSPIRE cohort and provide, for the first time across globally diverse populations, evidence of the impact that different DNA processing and sequencing approaches have on the microbiota profiles obtained for human milk samples. Overall, our results corroborate some similarities regarding the microbial communities previously reported for the INSPIRE cohort, but some differences were also detected. Understanding the impact of different sequencing approaches on human milk microbiota profiles is essential to enable meaningful comparisons across studies