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Supportingbiotechnologies Cryopreservationandcryobiology,diagnosticimaging,molecularbiologyand“omics”

COMPARISON BETWEEN GENETIC SEQUENCING METHODS (ILLUMINA X PACBIO) IN THE INVESTIGATION OF THE VAGINAL MICROBIOTA OF DAIRY COWS AND BACTERIAL IDENTIFICATION AT THE SPECIES LEVEL

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Resumo

The aim of this work is to characterize the vaginal microbiota of dairy cows by sequencing long DNA reads (PacBio sequencing) and comparing the data with the short reads technique (Illumina sequencing). To that end, vaginal swabs were collected from Holstein Black and White cows (HPB, n = 13), multiparous females (5 ± 1.3 years). Next-generation sequencing (SNG) of the V4 region of the 16S rRNA gene by the Illumina and PacBio platform. The sequence data was processed in the Mothur software (Illumina) and the DADA2 and SBanalyzer 2.4 software (PacBio). The statistical analysis were performed comparing the average of the phyla found in the different platforms (PacBio and Illumina) and the correlation between the indices (Chao, Simpson, Shannon) using the Minitab 18 software ($p \leq 0.05$). We obtained 366,509 readings of the vaginal microbiota from the PacBio platform, consisting of 27 phyla, 677 genera and 677 species identified. Five species represented about 51% of the vaginal microbiota, namely UCG-005_unclassified, UCG-010_ge_unclassified, Ureaplasma unclassified, Rikenellaceae_RC9_gut_group_unclassified and Bacteroides_unclassified. On the other hand, the Illumina platform identified 28 phyla and 662 genera, with 631,586 readings. The heterogeneity of community composition among individuals was confirmed. When comparing the averages of the two technologies for the main phyla, only one showed a great variation ($p<0.0001$), a set of bacteria without identification called ‘unclassified bacteria’, which was observed in greater quantity in the Illumina. It was not possible to directly compare the genera sequenced by PacBio with Illumina since the most abundant bacteria did not show similarities. The alpha diversity showed a strong and significant correlation ($r=0.758$, $p=0.003$) between the number of species and genera among the different techniques. Furthermore, when comparing PacBio and Illumina, sequencing long reads of the 16S rRNA gene provided Results similar and even more accurate than standard short-read sequencing platforms.