

**analysis of raw cattle milk microbiota in Sri Lanka employing Illumina MiSeq platform**

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Bacterial contamination of raw milk is a major concern worldwide in public health and economic viewpoint. Raw milk is highly susceptible to changes in temperature and humidity of the environment. The impact of tropical climatic conditions on the bacterial communities in raw milk has not yet been broadly examined. Therefore, this study was conducted to evaluate the bacterial composition of raw cattle milk produced in Sri Lanka, an island country located near the equator with year-round warm weather and considerable moisture. Ninety raw milk samples were collected from 18 dairy farms representing all three climatic zones of the country (wet, dry and intermediate zones) divided based on the rainfall distribution. Bacterial DNA extraction and purification was performed using Milk Bacterial DNA Isolation Kit as per the manufacturer's instructions. PCR amplification was carried out targeting the V3 and V4 regions of the 16S rRNA gene. Metagenomic sequences were obtained using IlluminaMiSeq platform at the Massey Genome Services (New Zealand). The operational taxonomic units (OTUs) clustering and classification at several taxonomic levels were performed using QIIME2 (version 2019.1) and Phyloseq (an R package). The OTUs belonged to 23 bacterial phyla, 110 orders, 381 genera, and 348 known species. Milk microbiota in terms of relative abundance (RA) was reported at the phylum, genus and species levels. The core microbiome was predominated by Firmicutes (48.33%) followed by Proteobacteria (22.66%), Actinobacteria (15.33%), Bacteroidetes (11.22%) and TM7 (0.91%) phyla. At the genus level, *Macrococcus* (10.30%) was the most abundant followed by *Streptococcus* (10.24%), *Elizabethkingia* (6.46%), *Staphylococcus* (3.48%), *Enhydrobacter* (3.14%), *Atopococcus* (3.04%), *Corynebacterium* (2.46%), *Arthrobacter* (2.13%), *Kocuria* (2.08%), *Acinetobacter* (1.66%), *Rothia* (1.32%), *Micrococcus* (1.14%) and *Bifidobacterium* (1.01%). The six most abundant (RA>1%) bacterial species identified were *Streptococcus agalactiae*, *Staphylococcus saprophyticus*, *Enhydrobacter aerosaccus*, *Atopococcus tabaci*, *Kocuria kristinae* and *Rothia nasimurium*. The members of *S. agalactiae* (5.61%), *S. saprophyticus* (3.48%), *E. aerosaccus* (3.14%) and *R. nasimurium* (1.06%) are known etiological agents in both human and animal diseases. The bacterial taxa diversity identified could be utilized to develop hygiene measures targeting the most problematic species.

**Keywords:** Metagenomic, Illumina MiSeq platform, Bacterial communities

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