

**TITLE:** MICROBIAL COMMUNITY STABILITY OF THE KEFIR GRAIN AND ITS FERMENTED BEVERAGE WITH RAW MILK

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**ABSTRACT:**

Consumption of the kefir has spread all over the world due its probiotic potential and beneficial health effects. Kefir beverage is usually produced by cow's milk fermentation using a kefir grain as the starter culture. These kefir grains are composed by an inert polysaccharide/protein matrix in which a complex microbial community coexists in a symbiotic relationship. The maintenance of the typical characteristics of kefir beverage and grain stability over the years may be associated with this complex symbiotic relationship established between the microbial members of these communities. The aim of this work was to monitor the ecological adaptation of bacterial communities of kefir grains and its fermented beverage (kefir) using raw goat's milk by next generation sequencing (NGS). The kefir grain was added to goat's milk (3%) and incubated by 25°C/96h. The kefir grain and kefir samples were collected at 0h, 24h, 48h and 96h of fermentation. For NGS, the universal primers 577f and 926r with sample-specific barcode were used for amplification of 16S rRNA gene and sequencing. Raw sequences were processed through the Ribosomal Database Project (RDP) pyrosequencing pipeline. A total of 24517 partial 16S rRNA sequences (>250 pb) were obtained varying from 3111 to 4909 sequences for sample. The goat's milk presented the highest rates of estimated richness and diversity calculated (Chao, Shannon and Ace) and after fermentation was observed a reduction of this diversity in the fermented beverage. The *Proteobacteria* were the most abundant phylum for goat's milk. However, the *Firmicutes* were the most abundant one for kefir grains and kefir beverage samples (G0h, G24h, G96h, L24h, L96h), with prevalence of the *Lactobacillus* genus in the kefir grains and *Lactococcus* in the beverage after 24h of fermentation. After 96h, it was observed a declined of *Lactococcus* and *Leuconostoc* in the kefir (L96h) and predominance of *Lactobacillus*, probably due to acidic pH of the milk fermented for 96 hours. According to NMS analyses kefir beverage and grains are more related among each other and goat's milk community were significantly different from them. Our results showed that the predominant bacteria in the raw goat's milk were not incorporated into the kefir grains or the beverage kefir and these data were useful to give an information about bacterial communities dynamics during fermentation process of kefir production using a raw milk.

**Key-words:** raw goat's milk; goat's kefir microbiome; kefir beverage stability; kefir grain stability.

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