Background: Aquakefir is a fermented beverage containing a simple microbial community of bacteria and yeasts. Since a fast method to characterize mixed communities is still unavailable, microbiological monitoring of mixed fermentations remains laborious.

Objective: To identify the species present in aquakefir through traditional culture-dependent and -independent approaches.

Methods: The media and incubation temperatures to plate microbial cells from aquakefir are: MRS, MRS at pH 5 (MRS-5), and KAA at 20, 28 and 37°C for lactic acid bacteria (LAB); DMS medium at 37 and 28°C for acetic acid bacteria (AAB); and MEA and AYM at 20, 28 and 37°C for yeasts. Following rep-PCR fingerprinting for dereplication, unique strains were identified using sequence analysis of ribosomal RNA and housekeeping genes. Denaturing gradient gel electrophoresis of variable rRNA regions was used to identify the dominant members and to evaluate the efficacy of the isolation methods.

Results: Aquakefir two months after bottling contains $10^8$ LAB CFU/mL on MRS and MRS-5 and $10^6$ LAB CFU/mL on KAA. In general LAB counts did not change significantly eight months after bottling. Among 122 isolates, 12 strains representing 9 LAB species were detected. $10^3$ and $10^0$ AAB CFU/mL were found in aquakefir two months and seven months after bottling, respectively; nine months after bottling no viable AAB were detected. All 20 AAB isolates belong or are related to Acetobacter lovaniensis. Finally, yeasts counts are $5\times10^4$ CFU/mL two months after bottling and approx. five-fold less eight months after bottling. Among 33 isolates, two strains of different species were identified.

Conclusions: The aquakefir population consists of an unexpectedly high number of different strains including 12 LAB, 1 AAB and 2 yeast strains. Accurate identification will be presented. LAB counts appeared unaffected eight months after bottling, while yeast counts decreased five-fold and AAB were no longer detected.