P19 & FP – **Evaluation of bioinformatic methods to reveal drinking water microbial community**

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The drinking water standards are set at levels necessary to protect the public from acute and chronic health risks associated with consuming contaminants in drinking water supplies. Although there are several indicators to evaluate treatment efficiency and product water quality, they do not allow to explore in depth the complex biological communities, an important field of research and public health, and the microbial population analysis is useful to optimize treatment systems and to verify the effectiveness of control and prevention actions as envisaged by the Water Safety Plan. Entire bacterial communities can be studied quickly and inexpensively using the environmental DNA metabarcoding approach, that it has increased dramatically in recent years for the detection of rare, elusive, invasive, or threatened wildlife species, helping us to routinely survey bacterial and fungal taxonomic communities, overcoming limits due to conventional culture-based methods. In this study, we compare two DNA metabarcoding bioinformatic pipelines to analyze HTS (High-Throughput DNA Sequencing) data for the monitoring of drinking water microbial community. In particular, we sequenced 84 samples in different steps of the potabilization belonging to process: raw water (groundwater), water passed through carbon filters and post-chlorination water. The sampling campaign lasted one year, with water samples collected monthly from a DWTP (Drinking Water Treatment Plant) located in Milan. Moreover, we had the opportunity to sample in parallel a second DWTP located in Milan for two months. We used the 16S ssuRNA molecular marker (V3-V4 hypervariable regions) for the diversity estimation of Bacteria. Sequences obtained with HTS technology were processed with two different pipelines designed with VSEARCH algorithm, calculating OTUs at a 97% of identity, a standard approach for Bacteria reads clustering, and the QIIME2 package, calculating ASV (amplicon sequence variants) with DADA2 algorithm. Reads were assigned with two different taxonomy classifier and two version of SILVA 16S database, a pre-clustered and curate database of 16S region with a formatted taxonomy. Our results showed the strength of samples' molecular information describing the differences in community composition in the three main sites, they allowed us to describe the taxonomy information in microbial community composition of drinking water and, moreover, highlighted the main differences between pipelines and database versions. From this study, we conclude that metagenomic amplicon sequencing is an informative method to support current compliance-based methods and can be used to reveal bacterial community information for the evaluation of drinking water quality, in order to protect the public health.

References

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