## One Health Approach: The Importance of Improving the Identification of Waterborne Bacteria in Austrian Water

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Abstract : The presence of various microorganisms (bacteria, fungi) in surface water and groundwater represents an important issue for human health worldwide. The matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF-MS) has emerged as a promising and reliable tool for bacteria identification in clinical diagnostic microbiology and environmental strains thanks to an ionization technique that uses a laser energy absorbing matrix to create ions from large molecules with minimal fragmentation. The study aims first to conceptualise and set up library information and create a comprehensive database of MALDI-TOF-MS spectra from environmental water samples. The samples were analysed over a year (2021-2022) using membrane filtration methodology (0.45 µm and 0.22 µm) and then isolated on R2A agar for a period of 5 days and Yeast extract agar growing at 22 °C up to 4 days and 37 °C for 48 hours. The undetected organisms by MALDI-TOF-MS were analysed by PCR and then sequenced. The information obtained by the sequencing was further implemented in the MALDI-TOF-MS library. Among the culturable bacteria, the results show how the incubator temperature affects the growth of some genera instead of others, as demonstrated by Pseudomonas sp., which grows at 22 °C, compared to Bacillus sp., which is abundant at 37 °C. The bacteria community shows a variation in composition also between the media used, as demonstrated with R2A agar which has been defined by a higher presence of organisms not detected compared to YEA. Interesting is the variability of the Genus over one year of sampling and how the seasonality impacts the bacteria community; in fact, in some sampling locations, we observed how the composition changed, moving from winter to spring and summer. In conclusion, the bacteria community in groundwater and river bank filtration represents important information that needs to be added to the library to simplify future water quality analysis but mainly to prevent potential risks to human health.

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