Heterotrophic plate count vs. *in situ* bacterial 16S rRNA gene amplicon profiles from drinking water reveal completely different communities with distinct spatial and temporal allocations in a distribution net

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Abstract

Heterotrophic plate count using ISO 6222 agar (HPC) vs. *in situ* bacterial (DF) community structure from corresponding samples of a drinking water distribution system were investigated by 16S rRNA gene-based polymerase chain reaction denaturing gradient gel electrophoresis (PCR DGGE) profiling. The investigation regime covered 10 different sampling locations and 2 points in time (t1, t2). In order to ensure accurate and reproducible 16S rRNA gene profile analysis, rigorous methodical evaluation and standardisation procedures were undertaken (DGGE optimisation, replication of PCR, multiple-lane standardisation, representative sampling volume determination, application of multiple similarity coefficients). The reproducibility level of the profile analysis was determined to be ≥ 90% similarity. Two completely different communities were revealed from HPC vs. DF as indicated by DGGE analysis and sequencing. HPC populations could be identified as ubiquitously occurring cultivable copiotrophic microbes, whilst most DF sequences could be allocated to sequences from microorganisms found in oligotrophic aquatic environments. Spatial- and temporal-based 16S rRNA gene amplicon profile analysis from recovered communities further revealed contrasting results. As proven by Jackknife simulations, DF profiles remarkably corresponded to sampling time, whereas HPC profiles revealed spatial associations within the distribution system. Recovered data demonstrate that cultivation based HPC vs. direct cell-based investigations can result in completely different results if used for monitoring purposes in distribution systems.

Keywords: heterotrophic plate count (HPC), *in situ* bacterial 16S rRNA gene population screening, bacterial cultivation vs. direct detection, PCR DGGE, drinking water, distribution system