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Questionable Specificity of Genetic Total Faecal Pollution Markers for Integrated Water Quality Monitoring and Source Tracking

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Characterisation of microbial faecal hazards in water is a fundamental aspect for target-orientated water resources management to achieve appropriate water quality for various purposes like water supply or agriculture and thus to minimize related health risks. Nowadays the management of water resources increasingly demands detailed knowledge on the extent and the origin of microbial pollution. Cultivation of standard faecal indicator bacteria, which has been used for over a century to test the microbiological water quality, cannot sufficiently meet these challenges. The abundant intestinal bacterial populations are very promising alternative targets for modern faecal indication systems. Numerous assays for the detection of genetic markers targeting source-specific populations of the phylum Bacteroidetes have been developed in recent years. In some cases markers for total faecal pollution were also proposed in order to relate source-specific marker concentrations to general faecal pollution levels. However, microbial populations in intestinal and non-intestinal systems exhibit a dazzling array of diversity and molecular analysis of microbial faecal pollution has been based on a fragmentary puzzle of very limited sequence information. The aim of this study was to test the available qPCR-based methods detecting genetic Bacteroidetes markers for total faecal pollution in terms of their value and specificity as indicators of faecal pollution.

We applied the AllBac (Layton et al., 2006) the BacUni (Kildare et al., 2007) and the Bacteroidetes (Dick and Field, 2004) assays on soil DNA samples. Samples were collected in well characterised karst spring catchments in Austria's Eastern Calcareous Alps. They were at various levels of altitude between 800 and 1800 meters above sea level and from several different habitats (woodland, alpine pastures, krummholz). In addition we tried to choose sampling sites representing a presumptive gradient of faecal pollution levels. For example sites with obvious faecal influence (e.g. right next to a cowpat) were included as well as more pristine sites without faecal influence from large animals (e.g. fenced areas).

Surprisingly, results from investigations with the AllBac assay showed concentrations of the total faecal marker in soil in the range of 10^6 to 10^9 Marker Equivalents per g of soil, which is equal or only slightly lower than the concentrations of this particular marker in faeces or raw sewage. Preliminary results from the other tested assays seem to confirm that the targeted markers are also highly abundant in soils. In addition, the markers were present in comparable concentrations in soils from pristine locations as well as in soils under the potential influence of faeces giving a strong indication that these methods also target non-intestinal, autochthonous soil populations. In contrast, source-specific markers (ruminant-specific BacR and human-specific BacH, Reischer et al., 2007, 2006) could only be detected in 30 to 50% of the soil samples at concentrations close to the detection limit, which is at least four orders of magnitude lower than in faecal samples of the respective target sources, ruminant animals and humans.

The achieved results call the applicability of the proposed qPCR-based assays for total faecal pollution into question. In fact the assays do not seem to be specific for intestinal Bacteroidetes populations at all and the respective marker concentration levels in pristine soils negate their applicability in the investigated areas. This study also emphasizes the need to test the specificity and sensitivity of qPCR-based assays for total faecal pollution on the local level and especially against non-intestinal environmental samples, which might contribute to marker levels in the aquatic compartment. In conclusion there is a strong demand for marker-based detection techniques for total faecal pollution in water quality monitoring and risk assessment but currently none of the tested assays seems to meet the methodical requirements.