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Soil metaproteomics – a comparison of extraction protocols

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Environmental metaproteomics has been proven to be a powerful tool to study the diversity of microbial communities in detail. This approach provides the possibility to link microbial community structure and function. In contrast to other environments soil is a complex matrix with high microbial diversity and low protein concentration. In this study we demonstrate that by a critical evaluation of the extraction protocols the quality of data can be enhanced, which is crucial when dealing with highly diverse natural samples such as natural soils.
The four different extraction procedures tested are based on protein extraction with (1) NaOH and following Phenol extraction modified from (Benndorf et al., 2007), (2) SDS extraction without Phenol, (3) SDS-Phenol extraction and (4) SDS – Phenol extraction with prior washing steps modified from (Wang et al., 2006).
These methods were applied to soil material from the A-layers of two contrasting soils: (i) a soil rich in organic matter from the botanical garden (BG) in Zurich, Switzerland and a Chromic Cambisol from the beech forest Schottenwald (SW) in Vienna, Austria. Proteins of the two different soil types were analyzed by two-dimensional liquid chromatography/tandem mass spectrometry (2D-LC-MS/MS). This approach enabled us to compare the number of identified proteins for each of the respective protocols. The number of unique proteins identified with the four protocols ranged from 116 to 182 and from 232 to 474 for BG and SW. Extraction with SDS-Phenol (3) resulted in the highest amount of unique proteins in both soil types. A spiking experiment confirmed the reliability of the method, where sterilized soil was amended with $\sim$10e8 Pectobacterium carotovorum cells per gram soil and analyzed with the two most efficient extraction protocols (1) and (3). The SDS-Phenol extraction resulted in a higher number of identified proteins in the spiked soil. Data evaluation was performed using a newly established semi-automatic bioinformatics pipeline. The microbial community in both soil types was dominated by bacteria, whereby Proteobacteria prevailed. The distribution of the community structure varied depending on the extraction protocol used. Based on our investigation we can recommend the SDS-Phenol extraction without washing for maximum extraction efficiency. This procedure proved suitable for the functional analysis of the metaproteome of garden as well as forest soil A-layers, where bacteria are dominating. The combination of this new technique of soil metaproteomics with classical soil enzyme analysis directly links enzyme origin, structure and function and provides a powerful tool for future assessment of soil quality and degradation.

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Wang W, Vignani R, Scali M, Cresti M (2006) A universal and rapid protocol for protein extraction from recalcitrant plant tissue for proteomic analysis. Electrophoresis 27:2782-2786