Gene expression and proteomics in soil

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Abstract
Molecular techniques have mainly been applied to soil to characterise DNA so as to assess microbial diversity whereas few studies have been carried out to monitor gene expression by extraction and characterization of mRNA and proteins. Novel techniques are available to extract mRNA from soil with successive characterisation. Functional metagenomics based on cloning of large genomic inserts containing operons and promoters can allow screening for specific functions and novel bioproducts. Soil proteomics can give useful information on cell-environment processes, by analysing proteins acting as biosensors, extracellular enzyme activities, stress proteins and metabolic proteins, and on homologous and heterologous cell-to cell interactions with analysis of proteins or peptides involved in quorum sensing and genetic exchange activities, or defining competition, predation, commensalims and symbiosis.

Key Words
Microbial diversity, genomics, proteomics, soil functionality.

Molecular techniques for detecting DNA sequences have been extensively used in soil and with metagenomic technologies it is possible to improve the determination of composition of complex microbial communities such as those inhabiting soil (Urich *et al*. 2008). However these DNA-based studies do not allow determining gene expression in soil and thus are not indicative of soil processes. Monitoring the expression of gene sequences at both transcription and translational levels is needed in soil science in analogy to post genomic studies of cultivated organisms. The analysis of the target mRNA is supposed to be an indicator of the transcription activity in soil. Despite the fact that extraction procedures of soil RNA and DNA are similar in principle, successful extraction and characterization of mRNA from soil has lagged behind those of DNA due to problems such as activity of nucleases and fast turnover rate of prokaryotic mRNA (Costa *et al*. 2004; Bakken and Frostegard 2006). However, several methods are now available to characterise mRNA and thus to measure gene expression in soil (Metcalfe *et al*. 2002; Krsek *et al.* 2006). An RNA meta-trascriptome approach involving the extraction of both mRNA and rRNA from a sandy soil, with reverse transcription to cDNA and with direct pyrosequencing, produced both cDNArRNA-tags and m-RNA-tags and this allowed the quantification of abundant microorganisms and information of the activity of enzymes involved in ammonia oxidation and CO_2 fixation (Urich *et al*. 2008).

Multiple protein isoforms can be synthesised by a single gene because mRNA molecules can be subjected to post-transcriptional control such as alternative splicing, polyadenylation and mRNA editing (Graves and Haystead 2002). The analysis of expressed proteins in pure culture is rapid and sensitive and involves extraction of proteins, their separation by 2-dimensional gel, the solubilisation of excised band and successive trypsin digestion followed by analysis of tryptic peptides by ionization mass spectrometers (Pandy and Mann 2000). The use of bioinformatics for processing a huge mass of data is also required.

The application of proteomic studies to soil should consider that on average microbial N accounts for 4% of organic N in soil whereas most of the total organic N is present as extracellular protein N or peptides N stabilised by soil colloids (Nannipieri 2006). In addition, information on microbial gene expression related to microbial activity should be based on the characterization of intracellular microbial proteins unless information on microbial processes involving extracellular enzymes is required. The characterization of extracellular proteins, protected against proteolysis by their association with soil colloids, should give insights on mechanisms responsible of such stabilization. The two approaches have been termed soil functional proteomics and soil structural proteomics, respectively (Nannipieri 2006). The study of functional proteomics can improve our understanding of degradation of organic pollutants and organic debris, nutrient cycling, blockage of inorganic pollutants, molecular colloquia between microorganisms, between plant roots and microorganisms and between plant roots (Nannipieri 2006). A successful extraction of intracellular proteins from soil should lyse microbial cells, inhibit proteases, avoid the adsorption of proteins with soil colloids once released after cell lysis and be representative of the status of microorganisms inhabiting soil. The manipulation of the sample prior to extraction should not alter microbial physiology.

Most of the reports on soil proteomics are based on the direct extraction method (Nannipieri 2006). Caution is required in using both colorimetric (Bradford method) or immunological (ELISA) methods to determine extracted proteins since these techniques can also determine phenolic compounds and litter and humic components (Rosier *et al*. 2006; Whiffen *et al*. 2007; Roberts and Jones 2008).

Proteins have been extracted from dissolved organic matter of forest soil, purified by gel filtration, with removal of humic acids, phenolic compounds and small molecules, and concentrated by ethanol before SDS-PAGE (Schulze 2004). After silver staining, each protein band was cut, digested by trypsin and mixtures of tryptic peptides separated by nanoflow liquid chromatography prior to analysis by mass spectrometry. The phylogenetic origin of proteins was evaluated and bacterial proteins were classified in ribosomal, transcription, membrane and enzyme proteins according to their function. The power of analytical MS tool was also shown by detecting chlorocatechol dioxygenases, enzymes involved in the degradation of 2,4-D (2,4-dichlorophenoxy acetic acid), after mass spectrometry analysis of tryptic peptides obtained from excised bands of SDS-PAGE gels, obtained by electrophoresis of protein preparations extracted by 0.1 M NaOH from 2,4-D treated soils and successively purified (Benndorf *et al*. 2007).

As it is done for the extraction of nucleic acids from soil, separation of microbial cells from soil particles and successive cell lysis with release of proteins is an alternative approach to extract microbial proteins from soil. The microbial extraction prior to cell lysis should give purer samples but with lower yields than the direct extraction method. However, artefacts due to possible changes in the physiology of microorganisms can occur during the extraction of microbial cells from soil (Nannipieri 2006) making it difficult to relate the microbial proteins to the effects of the studied factors (i.e. stresses, agriculture management, etc). Maron *et al*. (2008) used the indirect extraction method to show that copper or mercury pollution of soil stimulated the synthesis of protein with molecular weight ranging from 20 to 50 kDa; some of these proteins, such as those of heavy metal efflux pumps, were involved in heavy metal resistance mechanisms.

Phosphate is more efficient than other buffers in extracting proteins from soil. However each extraction procedure should be tested with a microbial strain with a known proteome so as to evaluate any effect of the used procedure on the microbial proteome. We have tested our extraction procedure on *Cupriavidus metallidurans* CH34.

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