ANNEX 4: FINAL REPORT (Final Publishable Summary Report)

INDIVIDUAL FELLOWSHIPS

Project n°: PIOF-GA-2009-235470
Project Acronym : ENVIPRO
Project Full Name: Environmental Proteomics: Methods development and characterization of proteinaceous compounds in environmental samples

Marie Curie Actions

IOF Final Report

Period covered: from September 2010 **to** November 2012
Period number: 1&2
Start date of project: September 2010
Project beneficiary name: Dr. Emmanouil Mandalakis
Project beneficiary organisation name: University of Crete
Date of preparation: January 21, 2013
Date of submission (SESAM): January, 28, 2013
Duration: 26 months
Version: 1

ENVIronmental PROteomics: Methods development and characterization of proteinaceous compounds in environmental samples (ENVIPRO)

Project summary

To date, substantial effort has been devoted to characterize the microbial communities in environmental samples, to understand the role of individual microbes and to unravel the key processes controlling the functioning of various ecosystems. Several culture-based methods and culture-independent DNA-based techniques have been developed for the assessment of microbial diversity, but these have some serious disadvantages (inefficient, laborious, slow, not informative about microorganisms function). Although these approaches enable the phylogenetic analysis of microbial communities, they do not necessary tell us what individual microorganisms can do and if they play any role in ecosystem functioning. The analysis of messenger-RNA has been suggested as an alternative approach for the retrieval of useful information about the cell's major activities, but mRNA molecules degrade very rapidly and their analysis is usually problematic.

In the last few years, there has been increasing interest in the potential applications of proteomics in the fields of environmental chemistry and ecology. Recently, proteomics opened a new avenue for the study of microorganisms in the different environmental compartments and the elucidation of their role in biogeochemical processes. Considering that proteins are the end products of gene expression, the identification characterization of proteinaceous molecules in environmental matrices can provide information not only for the processes taking place, but also for the identity of the active microbial species. The key goal of ENVIPRO project was to establish an appropriate proteomic workflow that will be properly tailored toward mass-spectrometry based characterization of proteins/peptides in environmental matrices and to enable the investigation of bacteria functioning under challenging environmental conditions.

Objectives

The main objectives of the ENVIPRO project can be summarized as follows:

- To offer extensive hands-on training in modern proteomic techniques to the Marie-Curie fellow
- To select specific "model" environmental samples and evaluate the efficiency of the different sample treatment steps involved in the extraction, purification and analysis of proteins
- To integrate the most efficient analytical steps and establish a proteomic workflow that will be best-suited for the application of proteomics in environmental samples
- To evaluate the applicability of the developed method in a number of easily accessible environmental samples, such as soils.
- To evaluate the applicability of proteomics in aerosol samples

Description of the work carried out in this project

The outgoing phase of the ENVIPRO project was held at the Barnett Institute (Northeastern University, Boston, MA). During this phase, the fellow [Emmanouil (Manolis) Mandalakis] received extensive hands-on training in modern proteomic techniques, which covered all sample processing steps, the analysis of protein samples using state-of-the-art nanoLC-MS/MS instruments and the analysis of proteomic data using sophisticated bioinformatic software. The fellow received additional training in several bacteria cultivation techniques and numerous monitoring methods for assessing the physiological state of microbial cells.

The establishment of mass-spectrometry based method for the analysis of bacterial proteins in environmental samples was a significant task of the outgoing phase. During hands-on training, a shotgun proteomic workflow that had been already optimized for biological samples (human and mouse cells) at the Barnett Institute, was tailored and applied to *Pseudomonas putida* F1, which is a ubiquitous bacterium in soil and water. This workflow included only a few analytical steps that were slightly modified to maintain simplicity and to avoid protein losses during sample treatment. The application of the developed proteomic workflow for a "model" environmental sample was another important task of the project. At this initial stage, the "model" environmental sample was

decided to be as simple as possible. As the presence of numerous different bacteria in soil samples would dramatically increase the complexity of proteomic data, a "model" sample consisting of a single bacterial species, *Pseudomonas putida F1*, was selected. This strain was employed because it is ubiquitous in natural systems, it has high biodegration capacity for numerous organic pollutants and its genome is fully sequenced (protein database was available).

In the context of this application, a comparative proteome analysis of *Pseudomonas putida* F1 under carbon-limited and carbon-excess conditions was carried out. The purpose was not only to prove the applicability of the established proteomic method but also to investigate the effect of starvation on bacteria. This is of high environmental importance because starvation is a rule rather than an exception in natural environments. In nature, many bacteria have developed mechanisms that allow them to survive starvation for essential nutrients and to reinitiate growth when nutrients again become available. Although this is of high biotechnological interest, the development of protective mechanisms at protein level has been poorly studied. The elucidation of proteins that bacteria express in response to starvation could be utilized to genetically engineer microorganisms with superior properties (greater tolerance to starvation conditions, higher survivability) for bioremediation purposes under real environmental conditions.

In a second phase, the fellow performed laboratory experiments to compare the efficiency of various methods reported in the literature for the extraction and purification of proteins from soil samples. A number of selected protocols were applied in soil samples and the yield/purity of extracted proteins was evaluated. In addition, the protocol showing the best performance was applied in samples of atmospheric aerosols and the possibility of using proteomics for the characterization of airborne microorganisms (bioaerosols) was examined.

Main results achieved during the project

The main outcome of the project was the establishment of the proteomic workflow, which enabled a comprehensive proteome profiling of soil bacteria. The adopted approach was based on shotgun proteomics which involves the gel-free separation of proteins/peptides by multidimensional liquid chromatography and their detection by mass spectrometry. This approach has been proven to be more powerful than the traditional 1D or 2D gel-electrophoresis methods. By using this workflow, the fellow investigated the changes taking place in the proteome of *Pseudomonas putida* F1 under carbon-limited (starvation) and carbon-excess (exponential growth) conditions. Moreover, this study was carried out for two different organic compounds (benzoate and citrate) to elucidate if the changes in protein expression under starvation stress depended on the quality of carbon source. Approximately 2100 proteins were identified in each experiments corresponding to a qualitative proteome coverage of 42%. More importantly, the proteomic results provided valuable insights about the adaptation strategies followed by bacteria under different growth conditions.

By carrying out a quantitative comparison of previously published methods for the extraction/purification of proteins from soils, significant information was obtained regarding their performance characteristics. The different protocols were applied in real soil samples and the quality of extracted proteins was evaluated. The method reported by Chourey et al. (J. Proteome Res., 2010, 9, 6615-6622) was found to be the most efficient in terms of protein yield and purity.

The application of proteomics for the investigation of airborne microsorganisms was the most ambitious goal of the project. Though, the protein content of aerosols was proved to be insufficient for the implementation of proteomic analysis. In contrast, DNA-based methods (i.e. 16s rRNA gene sequence analysis) enabled the characterization of airborne microorganisms. The collection of very large amounts of atmospheric particles will be necessary, if the analysis of individual proteinaceous molecules in aerosols will be attempted in the future.

Contact information

Scientist in charge: *Spiros A. Pergantis, e-mail: spergantis@chemistry.uoc.gr*
Marie Curie Fellow: *Emmanouil (Manolis) Mandalakis, e-mail: mandalakis@chemistry.uoc.gr*
Dept. of Chemistry, University of Crete, GR-71409, Heraklion, Greece, Tel.: +30 2810 545084