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Metaproteomics of soils from semiarid environment: Functional and phylogenetic information obtained with different protein extraction methods

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ABSTRACT

Microbial populations fulfil a critical role in the soil sustainability and their functionality can be ascertained by proteomics based on high-performance mass spectrometry (MS) measurements. However, soil proteomics is compromised by methodological issues, among which extraction is a limiting factor, and still has not been adequately applied in semiarid soils, which usually are nutrient limited. We aim to evaluate the functional and phylogenetic information retrieved from three semiarid soils with distinct edaphic properties and degradation levels. Three extraction methods with different physico-chemical bases were tested [1–3]. The HPLC-amino acid quantification of the extracted protein pellets revealed a tremendous inefficiency of the extraction methods, with a maximally 6.8% of the proteinaceous material being extracted in comparison with the protein content in the bulk soil. The composition of the proteomes extracted was analysed after SDS-PAGE and liquid chromatography coupled with electrospray-MS/MS. Chourey's method, based on boiling and DTT, yielded a high diversity of bacterial proteins and revealed differences in the community composition at the phylum level among the three soils. The overall metabolic information obtained by both extraction methods was similar, but Chourey's method provided additionally valuable bio-geochemical insights which suggest an ecological adaptation of microbial communities from semiarid soils for carbon and nitrogen fixation.

Biological significance
Microbial communities inhabiting the soil perform critical reactions for the sustainability of the planet. At biochemical level, soil proteomics is starting to provide incipient insights into the microbial functionality of soils. However, methodological comparisons are needed to assess which methods are more suitable. Precisely, such information under arid and semiarid environments is missing. By using amino acid quantification of extracted proteomes and LC–MS/MS based proteomics, we provide a novel methodological evaluation of the functional, phylogenetic and bio-geochemical information obtained by three extraction methods in semiarid soils with distinct edaphic properties.

1. Introduction

The study of the proteins collectively expressed by all the microorganisms within an ecosystem, the so-called

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metaproteomics, has received increasing attention in recent years [4,5]. The identification of functional proteins involved in the metabolic processes of ecosystems is an open area in microbial ecology and would provide information regarding the functional capabilities of the microorganisms inhabiting a wide variety of habitats [6–9]. In this respect, obtaining further insights into the microbial functioning of soil ecosystems is of paramount importance for the sustainability of the planet. However, soils harbour an extraordinary microbial diversity and, hence, diverse biochemical pathways are expected, which makes soil proteomics a highly-challenging topic in microbial ecology [10].

The development of soil proteomics is technically difficult. Soil heterogeneity and the need for powerful chromatography and high-performance mass spectrometry (MS) equipment are currently key factors required for the successful identification of proteins from the soil [5,11]. Additionally, the absence of sample metagenome is an important factor limiting soil proteomics [9,10]. Besides these issues, the primary limitations to protein identification in the soil are probably the scarce knowledge on protein extraction methods and, particularly, the application of inadequate protein quantification methods — even when it is known that co-extraction of humic compounds interferes with standard colorimetric assays for protein quantification [5,12]. Despite this drawback, some soil proteome studies still rely on colorimetric methods [13]. Instead, a more-suitable method for the quantification of the extracted proteins is required. For this purpose, the evaluation of extraction methodologies by the quantification of amino acids may be a successful strategy.

Despite these limitations, a few soil proteomic studies have provided valuable information regarding intracellular metabolic processes, by using cellular-based separation methods [14,15] or direct protein extraction from bulk soil [2,3,8]. Indeed, recently soil proteomics has been applied towards understanding key processes in the soil [3,8]. However, the identification of proteins with potential ecological roles is very limited and hardly discussed in these published studies. Among the proteins with ecosystemic value, it is worth mentioning those involved in the bio-geochemical cycling of carbon, nitrogen or phosphorus, proteins related to carbon and nitrogen fixation and enzymes able to oxidise organic matter.

Definitively, these proteins and enzymes are responsible for key processes related to soil quality and sustainability. Despite the fact that the activity of these enzymes was first assayed more than 30 years ago [16,17], their direct identification by MS remains obscure, contrasting with the high extracellular stability which has been proposed for some of them (phosphatases, glucosidases, ureases, *etc.*) due to linkage with humic compounds and mineral surfaces [18,19].

It is now critical to move soil proteomics forward in order to achieve its full potential for explaining the functionality of a given ecosystem. Recently, Keiblinger et al. [8] compared different protein extraction protocols in a forest soil. However, as concluded in this study, the validity of extraction protocols must be tested in different soils. For instance, the validity of proteomic methods in soils developed under semiarid climates is not known, even when such soils occupy a land extension of 2.37×10^9 ha on the Earth [20]. This information is crucial since many of these soils have a nutrient limitation based on a low organic matter content, which hampers microbial functionality [21,22]. In this work, we will focus on three representative semiarid soils with differing edaphic properties such as organic matter content and texture. The microbial biomass of these soils is usually low due to the insufficient organic matter and low moisture [21,22] and such low biomass would limit the amount of proteins that can be extracted.

Overall, we evaluate soil protein extraction methodologies in order to obtain functional and phylogenetic information in semiarid ecosystems. In particular, the objectives of this study are: i) to compare the yields of different protein extraction protocols for different semiarid soils; ii) to evaluate the metabolic and ecological information obtained with each method and soil, and iii) to elucidate if soil proteomics is valid for the identification of proteins with a potential role in bio-geochemical cycling.

2. Material and methods

2.1. Experimental area and soil characteristics

The studied soils are located in the province of Murcia (SE Spain), under a semiarid climate with an average rainfall of around 250 mm per year and an average temperature of 18 °C. The soils were selected on the basis of their differing organic carbon contents and textures. These are factors that may affect the protein extraction yields [13,23]. All these soils represent typical substrates in semiarid areas of the planet. Within each area of 300 m^2, each soil was sampled in duplicate from the top 15 cm of the soil, in September 2012. Each replicate was composed of eight homogeneously-mixed subsamples in an attempt to minimise the spatial variability of the soil. The plant remains were removed, to prevent them from influencing the analyses carried out, and the soil samples were sieved (2 mm) and kept at 3 °C. Protein extraction was performed within one week after sampling. Protein extractions and further determinations were performed on biological replicates (n = 2).

All the chosen sites suffered agricultural abandonment many years ago and differ in their vegetation cover, which is responsible for their distinct organic matter contents. A previous study established different levels of biological degradation in these soils on the basis of a lower amount of nutrients and microbial activity [22]. SF is a non-degraded forest soil with loam texture, high organic carbon content and a plant cover of 80%, and is dominated by *Pinus halepensis* Millar. JL is a sandy-loam soil with medium organic carbon content and is covered by xerophytic shrubs (30%). AB, a soil developed from marsh lithological substrates with a sandy-clay texture and a very-low organic carbon content of 2.9 g kg^{-1}, represents a very-degraded soil from the chemical, physical and microbiological points of view and is not covered by vegetation. More details are described by Bastida et al. [22].

2.2. Physico-chemical and chemical analysis of soils

The electrical conductivity and pH were measured in a 1/5 (w/v) aqueous solution, in a Crison conductivimeter and pH meter, respectively. Texture analysis was performed using the method of Guitian and Carballas [24]. The total N was determined using