Soil Biology & Biochemistry 42 (2010) 1148–1156

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Metaproteomic characterization of a soil microbial community following carbon amendment

Mark A. Williams*, Erin B. Taylor, Himaya P. Mula

Department of Plant and Soil Sciences, Mississippi State University, 470 Dorman Hall, Mississippi State, MS 39762, USA

ARTICLE INFO

Article history:
Received 18 December 2009
Received in revised form
22 March 2010
Accepted 23 March 2010
Available online 9 April 2010

Keywords:
Soil metaproteome
Protein
SDS-PAGE
Soil microbial community
16S rRNA genes
Density gradient centrifugation

ABSTRACT

Metaproteomics has the potential to transform the study of microbial community functioning in soil. To test this potential, experiments were conducted to characterize the microbial community proteome in (1.) toluene-amended soil and (2.) toluene-amended soil microbial inoculated cultures. A glucose-amended soil was also used as a contrast to the effects of toluene. A total of 47 proteins were identified at a 95% confidence interval. The toluene-amended communities shared many proteins and showed a high degree of similarity to previous studies of toluene impacted bacteria. For example, the microbial communities in the toluene-amended soil and cultures but not the glucose impacted microbial communities, showed the presence of glutamine synthetase (Gln), ABC transporters (e.g. Glt1), extra-cellular solute-binding proteins, and outer membrane proteins (Omp). These proteins may be upregulated for the purpose of removing toluene from bacterial cells. Toluene-amended cultures also showed the presence of arginine deiminase (ArcA), and cold-shock protein (Csp), while microbial protein from toluene-amended soil showed the presence of the stress proteins superoxide dismutase (SodB) and chaperonin (GroEL). Fatty acid methyl ester analysis indicated that toluene-exposed microorganisms increased the rigidity of their membranes through a 3 to 12$\times$ increase in the ratio of saturated to monounsaturated fatty acids. Moreover, the 16S rRNA gene analysis of bacterial communities in the toluene amended soil showed a high degree of dominance, whereby $\sim$80% of the OTUs were described by representatives most closely related to genus *Bacillus*, This research has shown that proteomic studies can be used to provide useful descriptions of microbial function in soil.

© 2010 Elsevier Ltd. All rights reserved.

1. Introduction

It is well known that microbial community composition and structure are diverse across a broad range of soil, habitat, vegetation, and management conditions, but extraordinarily little is known about the functional role of these organisms. In this regard, proteins are direct expressions of cellular function and play the primary role in carrying out cellular activities encoded by the genome (Stress and Tiedje, 2006). Thus, at the most fundamental level, the proteomes of microbial communities are responsible for the vast majority of functions and processes in soil. Yet, there is a dearth of information about the types of microbial proteins and how they are expressed in soil microbial communities.

Metagenomic studies have backed up the idea of extraordinary microbial functional richness (Handelsman et al., 1998; Rondon et al., 2000; Daniel, 2004). But, to understand microbial functions in soil and how these functions change in response to various environmental drivers, proteomics may be more useful. Studies of functional protein activities, for example, such as nitrogenase, dehydrogenase, protease, and urease (Braker et al., 2001; Jimenez et al., 2002; Schloter et al., 2003) to name a few, have deepened our understanding of microbial functioning. Yet, a single prokaryote may contain 5000 different functional proteins. Meta-functional diversity in soil is far greater. To investigate the dynamics and confirm an array of microbial community functions, a proteomic approach is the gold standard (Stress and Tiedje, 2006).

Advances in the study of the soil microbial proteome have occured over the last few years. *In situ* study of the upregulation of a 1,2-dioxygenase protein involved in dichlorophenoxy acetic acid (2,4-D) metabolism was observed in soil percolated with 2,4-D (Benndorf et al., 2007). However, only 4 or 5 bands on the gels were resolute enough for picking and protein identification using LC-MS-MS-ESI. Treatment of soil microparticles with strong acid (10% HF) resulted in the release and identification of cellulases, laccases, proteases, and phenoloxidases (Schulze et al., 2005). More recently, deep aquifer gravel and sand sediments were

* Corresponding author. Tel.: +1 662 325 2762; fax +1 662 325 8742.
E-mail address: mwilliams@pss.msstate.edu (M.A. Williams).

0038-0717/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2010.03.021

probed to assess microbial community responses to benzene. Several hundred spots on 2-D gels were observed and several proteins were found with potential linkages to benzene metabolism (Benndorf et al., 2009). However, low confidence in MS peptide matching reduced the certainty of protein identification, perhaps a reflection of the effort to directly extract protein from soil and the inability to remove contaminates that interfere with MS/MS. Other recent work has shown that protein extracted directly from soil can be used to produce ∼10 resolute bands on SDS-PAGE gels that have different banding patterns based soil management practices (Chen et al., 2009). Identification of these proteins would likely reveal information about microbial functioning. Similarly, proteins extracted from soil microbial communities first isolated from soil using density gradient centrifugation have produced up to ∼40 resolute bands on SDS-PAGE gels containing protein amenable to MS/MS identification, but the approach is limited to soils receiving organic amendments (Taylor and Williams, 2010). These and others studies have set the stage to show that proteomics can be used to extract and measure a diversity of proteins and to answer novel questions about the ecology of microbial communities (Banfield et al., 2005; Ram et al., 2005; Wilmes and Bond, 2006; Maron et al., 2007).

In this study we assessed the proteins expressed in soil microbial communities following the addition of glucose and toluene. To further understand the nature of microbial community protein expression and for comparison to the proteins observed in soil communities, we also assessed the proteomic complement of microbial cultures growing in soil extract broth amended with toluene. We hypothesized that toluene-amended community proteins would show evidence for microbial toluene exposure and be very different from those amended with glucose.

2. Materials and methods

2.1. Soil descriptions

Soil (0–10-cm) was collected from plots managed for the production of herbaceous crops such as St John's Wort (*Hypericum perforatum*) and Hyssop (*Hyssopus officinalis L.*). The soil is described as a thermic Aquic Paleudults (Quitman fine sandy loam) with a pH of 5.9–6.2 and a carbon content of 0.6%. Soils were sieved to pass a 5-mm mesh and stored frozen (−10 °C). Before experimentation, soils were taken from the freezer and stored at 4 °C for up to one month.

2.2. Toluene and glucose addition and incubation

Fifty grams (dry weight) of moist (−33 MPa) soil was placed in four 1-L canning jars. Two jars remained unamended and two jars were amended with 1 mL of toluene spread evenly over the soil surface (15.8 mg toluene-C g^{-1} soil). The jars were sealed and incubated in the dark at 25 °C. The incubation proceeded for 3 weeks, with the amended soils receiving an additional 1 mL of toluene after 1 and 2 weeks.

A similar experiment was set up for the addition of glucose at a rate of 1.0 mg glucose-C g^{-1} soil. Soils were incubated for 24 h at 25 °C in the dark instead of 3 weeks; this period of time resulted in 20% of the glucose-C being converted to CO_2 (data not shown). If 200 μg C was converted to biomass, then a doubling of microbial C would have been expected. Following incubation, the soils were removed from the incubator, and transferred to zip-top bags and stored at 4 °C for 1–2 days.

2.3. Growth of enrichment cultures containing toluene

To culture microorganisms from soil receiving toluene for 3 weeks, 1 g of soil was combined with 99 mL of sterile liquid media containing 0.50 g K_2HPO_4, 0.25 g $MgSO_4$, 900 mL tap water, and 100 mL soil extract (James, 1958) per liter. Soil extract was prepared by autoclaving 100 g soil and 200 mL tap water for 1 h at 12 °C and 15 psi. After autoclaving, the solids were allowed to settle and the liquid poured into a 250 mL bottle and centrifuged at 3500×*g* for 15 min. The resulting supernatant was poured into a 250 mL flask and frozen at 20 °C until use. Toluene was then added to the media at a final concentration of 200 μg/mL. Each culture was covered in parafilm and incubated in a 2 L flask in the dark at 25 °C with shaking at 200 rpm. After 5 days of incubation, following the development of a turbid solution, the cultures were removed from the incubator and soil particles allowed to settle for ∼15 min. The solution was removed and the bacteria were harvested using density gradient centrifugation (Section 2.4).

2.4. Separation of soil microorganisms using density gradient centrifugation (DGC)

Microorganisms were isolated from soil or culture using density gradient centrifugation (Courtois et al., 2001) as modified by Taylor and Williams (2010). Briefly, 20 g soil or 50 mg of culture were combined with 50 mL sterile 0.9% NaCl. The sample was blended (Oster, Model 4119, 45,000 rpm) three separate times for 1 min with 1 min cooling on ice between each cycle and quantitatively transferred to 2 ultracentrifuge tubes. Then, 10 mL of Nycodenz (Sigma) ($\rho = 1.3$ g/mL) was pipetted under the sample in each tube. The samples were centrifuged at 10,000×*g* for 40 min at 4 °C in a Beckman SW-28 rotor and the layer of microbial cells was carefully removed from each tube and centrifuged again to pellet the cells. Cells were rinsed 2× with sterile ddH_2O to remove traces of Nycodenz and the cell pellet subjected to protein extraction.

2.5. Extraction and quantification of protein from microbial pellets

Microbial pellets were combined with 0.5 mL of extraction buffer containing 0.5 M Tris–HCl (pH 8.7), 0.9 M sucrose, 0.05 M EDTA, 0.1 M KCl, and 2% 2-mercaptoethanol and were homogenized using a mortar and pestle (Hurkman and Tanaka, 1986). After homogenization, the samples were transferred to 2 mL centrifuge tubes and 0.5 mL phenol was added to the tube. Each sample was vortexed for 2 min and centrifuged at 3000×*g* for 10 min at 4 °C. The top (phenol) layer was removed and placed in a new tube. An equal volume of extraction buffer was added to the phenol and once again vortexed for 2 min and centrifuged at 3000×*g* for 10 min at 4 °C. The phenol layer was removed and placed in a new tube. Five volumes of methanol solution containing 0.1 M ammonium acetate and 1% β-mercaptoethanol were added to the phenol and the samples were placed at −10 °C overnight to precipitate proteins. After overnight precipitation, the samples were centrifuged at 3000×*g* for 20 min at 4 °C to pellet protein. The supernatant was decanted and the pellet was washed two times with 80% acetone. The protein pellets were resuspended in SDS-PAGE sample buffer (50 mM Tris–HCl (pH 6.8), 100 mM DTT, 10% glycerol, 2% SDS) and quantified using the BioRad RCDC protein assay (Lowry et al., 1951).

2.6. FAME analysis

Immediately following cessation of the soil glucose and toluene incubation experiments, 5-g of the collected soil was frozen (−10 °C) and analyzed for fatty acid methyl esters (FAME) using the

Download English Version:

https://daneshyari.com/en/article/2026275

Download Persian Version:

https://daneshyari.com/article/2026275

Daneshyari.com