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Note

A sequential co-extraction method for DNA, RNA and protein recovery from soil for future system-based approaches

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ABSTRACT

A co-extraction protocol that sequentially isolates core biopolymer fractions (DNA, RNA, protein) from edaphic microbial communities is presented. In order to confirm compatibility with downstream analyses, bacterial T-RFLP profiles were generated from the DNA- and RNA-derived fractions of an arid-based soil, with metaproteomics undertaken on the corresponding protein fraction.

Soil-based microorganisms drive key subsurface bioprocesses, including chemical and nutrient cycles (Bell et al., 2014), decomposition and mineralisation pathways (Moore et al., 2010) and the removal of pollutants (Bissett et al., 2013). For studying edaphic microorganisms an array of methods is available to recover nucleic acids from the soil matrix (Paulin et al., 2013; Petric et al., 2011), with recent developments in protein isolation (Keiblinger et al., 2012). However, there is still a fundamental lack of methods available for isolating each fraction concurrently for parallel downstream molecular analyses. This method gap is not comparable with the rapid advances made in high-resolution omic-based technologies (Jansson et al., 2012). Indeed, an integrative systems-based strategy, whereby multiple omic-datasets (metagenomic, metatranscriptomic and metaproteomic) are employed holds significant promise for elucidating discrete microbial community dynamics (Muller et al., 2013), such as the linkage of metabolic processes with functional phylotypes (Yu and Zhang, 2012). Here we present a co-extraction protocol for the simultaneous recovery of each biopolymer fraction from individual soil samples. This method should facilitate the effective and reproducible application of system-based approaches for studying diverse soil habitats.

Two types of soil samples were used, reflecting distinct environmental regimes. Soil type-A; oligotrophic dry-land soil from the Namib Desert (23°33′ S, 15°02′ E) and Soil type-B; organic rich soil from a local site in Gauteng, South Africa (25°45′ S, 28°13′ E). Specific characteristics for each soil-type are presented (Table 1). For molecular analysis, each soil sample (~10 g) was first supplemented with 50% (v/v) RNAlater at the point of sampling (Sigma-Aldrich, Copenhagen, Denmark), in order to preserve RNA and protein for further analysis (Rodrigo et al., 2002; Saito et al., 2011).

A method adapted from Griffiths et al. (2000) was used to recover the nucleic acid fraction from each soil sample. One-gramme aliquots of powdered soil (−20 °C; IKA® A11 homogeniser) were transferred to sterile 2 ml screw cap tubes (WhiteSci, Gauteng, SA). Ten grammes was required from Soil type-A, while only 1 g of soil was required to recover sufficient DNA, RNA and protein from Soil type-B. Thereafter, 0.5 ml of a 10% (w/v) hexadecyltrimethylammonium bromide (CTAB; Merck, Gauteng, SA) extraction buffer (10 mM Tris, 0.1 mM EDTA, 0.7 M NaCl, 5 mM $MgCl_2$; pH 8) and 0.5 ml phenol:chloroform: isoamyl alcohol (25:24:1; pH 8; Sigma) were added to each 2 ml sample tube. Each tube was also supplemented with 0.25 g of zirconia beads (0.1 and 0.5 mm; BioSpec, Bartlesville, OK, USA). Cell lysis was achieved by bead beating, with the aqueous phase pooled and phenol removed by a chloroform–isoamyl alcohol (24:1) phase separation (Fig. 1A) Total nucleic acids were precipitated by 30% polyethylene glycol 8000 (Sigma) and −1.6 M NaCl at 10 °C for 2 h with 70% (v/v) ice-cold ethanol wash steps (twice).

The organic phase containing the protein was also pooled and retained in a separate tube at 4 °C. Residual liquid was removed from the original sample tubes containing the soil and 0.5 ml of fresh 1% sodium dodecyl sulphate (SDS; Merck) extraction buffer (10 mM Tris, 5 mM $MgCl_2$; pH 8), supplemented with protease inhibitor cocktail (10 μl/ml; Sigma) (Fig. 1A). A second bead-beating extraction step was applied with subsequent centrifugation and benzonase

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Table 1
Soil characteristics and extract data.

	pH	Sand/silt/clay (%)	CEC[a]	Org C (%)	DNA yield (ng/μl)	DNA quality (A260/280)	RNA yield (ng/μl)	RNA quality (A260/280)	Protein yield (ng/μl)
Soil type-A	6.7	85/11/4	5.2	0.07	123.3 (±12.2)	1.62 (±0.05)	86.9 (±9.3)	1.42 (±0.05)	504.4 (±46.5)
Soil type-B	8.1	57/17/26	22.1	1.34	96.8 (±17.8)	1.52 (±0.02)	39.6 (±7.4)	1.51 (±0.02)	690.2 (±90.1)

Standard deviation is in parenthesis reflecting 3 replicates each.
[a] Cation exchange capacity cmol(+) kg^{-1}.

treatment (250 U/μl; Sigma) undertaken on the pooled supernatants to remove any remaining nucleic acids. Thereafter, an additional phenol:chloroform:isoamyl alcohol (25:24:1; pH 8) step was applied to partition the organic phase, which was then added to the organic fraction from the first extraction step (Fig. 1A). Five volumes of 0.1 M ammonium acetate in methanol was used to precipitate the protein (−20 °C, overnight) with additional washing steps using ice-cold methanol (twice) and acetone (80% v/v; twice). The resulting pellets were resolubilised in 6 M guanidine buffer supplemented with 10 mM DTT, 10 mM Tris, and 5 mM $CaCL_2$ (pH 8) with 1 min of sonication (sonicator bath VWR USC2600).

Successful co-extraction of DNA and RNA was confirmed and quantified using a Nanodrop 1000 spectrophotometer (NanoDrop Products, Wilmington, DE, USA) and gel electrophoresis (Table 1; Fig. 1B). Average yields for oligotrophic Soil type-A were between 5 and 6 μg of DNA and 3 and 4 μg of RNA per gramme of (dry weight) soil, comparable to previous studies where nutrient-rich soil was used (Mettel et al., 2010; Wang et al., 2009). In order to determine the effectiveness of this co-extraction protocol, subsequent molecular analysis of Soil-type A was performed.

Reverse transcription (RT) of total RNA was undertaken using a method adapted from Corgié et al. (2006). Prior to cDNA generation, the RNA was further purified (RNeasy; Qiagen, GmbH, Hilden, Germany). The incorporation of this step was crucial for successful cDNA generation, as without it no cDNA could be detected by subsequent PCR analysis. The 16S rRNA gene was amplified using the bacterial primers 341F-FAM and 908R (Angel et al., 2010) for both DNA and cDNA. The PCR reactions and conditions were as described by Makhalanyane et al. (2013). Overall, 57 DNA-based operational taxonomic units (OTUs) were identified, with 48 RNA-based OTUs recorded (Fig. 1C). These results were consistent with recent studies where commercial DNA extraction kits were employed (Makhalanyane et al., 2013; Stomeo et al., 2013), suggesting that representative phylogenetic information was recovered from oligotrophic soil using this approach. We noted that 21 OTUs were unique for the RNA-derived cDNA samples, confirming the importance of incorporating metatranscriptomics for comprehensive community analyses in arid systems.

Protein samples from Soil-type A were reduced, digested and processed for Q-Exactive LC–MS/MS analysis (Fig. 1D). Through this novel gel-free metaproteomic workflow, a total of 110 proteins were identified with significant protein scores ($P < 0.05$) from Mascot searches of peptide mass fingerprints against the NCBInr database. This level of resolution is comparable with a recent soil metaproteomic study (Lin et al., 2013). Proteins assigned to both archaeal and bacterial groups were identified, representing various functional categories including biosynthesis, DNA repair and membrane transport (Fig. 1D; Table 2). The identification of numerous proteins assigned to methanogens (e.g. HMD, Q02394, ACDA1, Q49161) was surprising and merits further detailed analysis to determine the presence and functional capacity of methanogens in near-surface arid-based soil communities.

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