

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

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Running title: Soil co-extraction method for meta-omic approaches

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Abstract

A co-extraction protocol is presented that sequentially isolates core biopolymer fractions (DNA, RNA, protein) from edaphic microbial communities. 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.

Keywords: Co-extraction; Nucleic acids; Protein; T-RFLP; Metaproteomics

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 are 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., 2012**), such as the linkage of metabolic processes with functional phylotypes (**Yu & 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), 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-gram aliquots of powdered soil (-20°C; IKA® A11 homogeniser) were transferred to sterile 2 ml screw cap tubes (WhiteSci, Gauteng, SA). Ten grams was required for 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

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)

^a Cation exchange capacity
Standard deviation is in parenthesis reflecting 3 replicates each.

bromide (CTAB; Merck, Gauteng, SA) extraction buffer (10 mM Tris, 0.1 mM EDTA, 0.7M NaCl, 5 mM MgCl₂; pH 8) and 0.5 ml phenol:chloroform:isoamyl alcohol (25:24:1; pH 8; Sigma) was 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) -1.6M 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 MgCl₂; pH 8), supplemented with protease inhibitor cocktail (10 µl/ml; Sigma) was added (**Fig. 1A**). A second bead-beating extraction step was applied with subsequent centrifugation and benzonase treatment (250U/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 0.1M 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 6M guanidine buffer supplemented with 10mM DTT 10mM Tris 5mM CaCl₂ (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 to 4 µg of RNA per g (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.

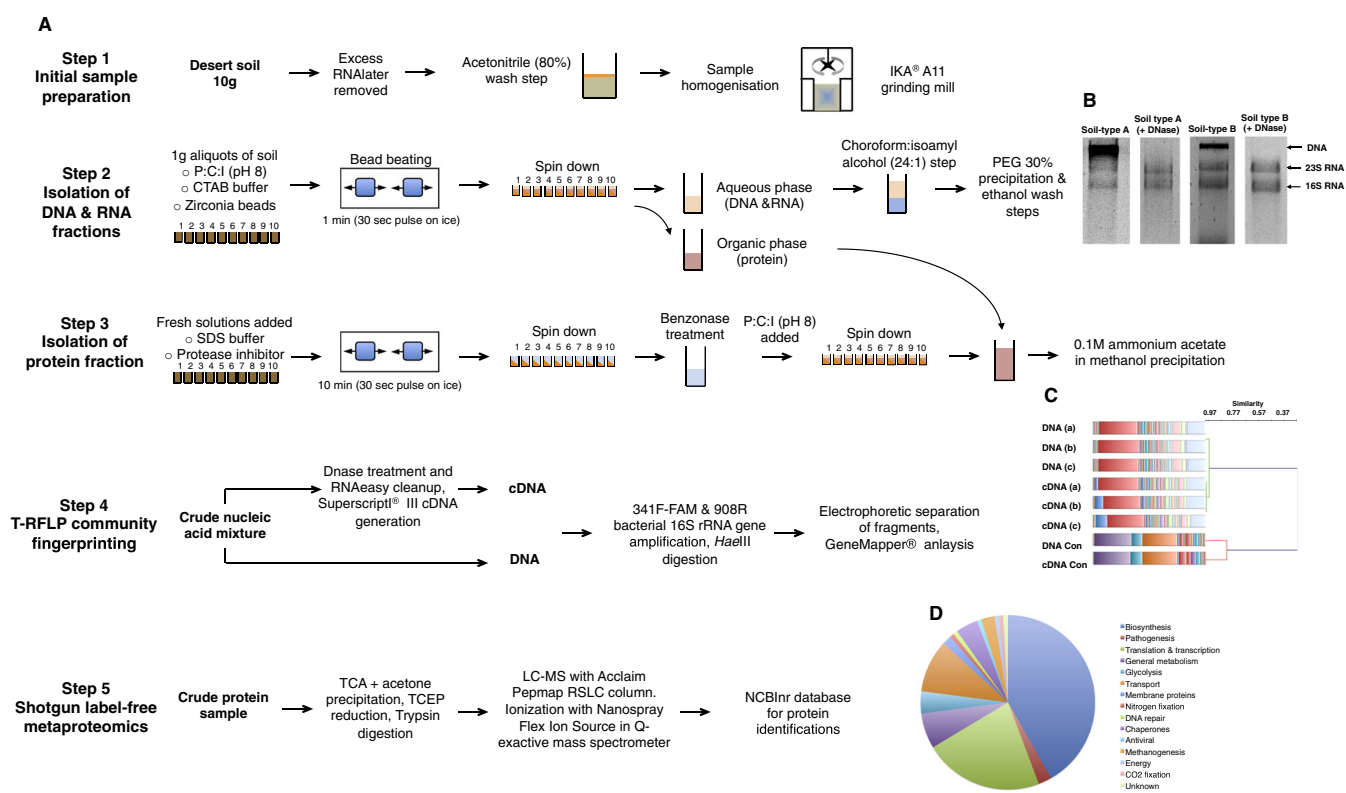


Figure 1 (A) Workflow of the sequential co-extraction protocol for the isolation of (B) nucleic acids with (C) T-RFLP profiles generated and (D) protein with metaproteomic functional categories for Soil-type B shown.

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 NCBI nr 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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Table 2 Proteins identified in Soil type-A organic fraction

Accession number	Protein name	Score ^a	Num. of matches ^b	Putative Biological function	Phylogenetic classification (Family/Phylum)	
Archaea						
Antiviral						
CAS2B_SULSO	CRISPR-associated endoribonuclease	32	2	Antiviral defence	<i>Sulfolobaceae</i>	Crenarchaeota
Biosynthesis						
G1PDH_IGNH4	Glycerol-1-phosphate dehydrogenase	41	38	Lipid biosynthesis	<i>Desulfurococcaceae</i>	Crenarchaeota
COFD_HALMA	2-phospho-L-lactate transferase	34	33	Cofactor biosynthesis	<i>Halobacteriaceae</i>	Euryarchaeota
F16PA_NATPD	Fructose-1,6-bisphosphatase	33	4	Carbohydrate biosynthesis	<i>Halobacteriaceae</i>	Euryarchaeota
HEM3_PYRAE	Probable porphobilinogen deaminase	31	18	Porphyrin-containing compound metabolism	<i>Thermoproteaceae</i>	Crenarchaeota
IDI2_PYRHO	Isopentenyl-diphosphate delta-isomerase	30	79	Isoprene biosynthesis	<i>Thermococcaceae</i>	Euryarchaeota
SYI_SULAC	Isoleucine--tRNA ligase	27	34	Protein biosynthesis	<i>Sulfolobaceae</i>	Crenarchaeota
TYSY_METJA	Putative thymidylate synthase	25	9	Nucleotide biosynthesis	<i>Methanocaldococcaceae</i>	Euryarchaeota
THIM_PYRAB	Hydroxyethylthiazole kinase	24	36	Thiamine biosynthesis	<i>Thermococcaceae</i>	Euryarchaeota
GCST_PYRKO	Probable aminomethyltransferase	23	11	Glycine catabolic process	<i>Thermococcaceae</i>	Euryarchaeota
GATD_METST	Glutamyl-tRNA(Gln) amidotransferase subunit D	23	23	Protein biosynthesis	<i>Methanobacteriaceae</i>	Euryarchaeota
ASPD_UNCMA	Probable L-aspartate dehydrogenase	23	83	Pyridine nucleotide biosynthesis	<i>Uncultured methanogenic archaeon</i>	Euryarchaeota
PYRC_SULTO	Dihydroorotase	22	40	Pyrimidine biosynthesis	<i>Sulfolobaceae</i>	Crenarchaeota
PDXS_META3	Pyridoxal biosynthesis lyase	21	8	Pyridoxal phosphate biosynthetic process	<i>Methanococcaceae</i>	Euryarchaeota
COBY_METJA	Adenosylcobinamide-phosphate guanylyltransferase	21	28	Cobalamin biosynthesis	<i>Methanocaldococcaceae</i>	Euryarchaeota
VATA_META3	V-type ATP synthase alpha chain	20	31	ATP synthesis	<i>Methanococcaceae</i>	Euryarchaeota
GATE_NATPD	Glutamyl-tRNA(Gln) amidotransferase subunit E	20	11	Protein biosynthesis	<i>Halobacteriaceae</i>	Euryarchaeota
G1PDH_PYRIL	Glycerol-1-phosphate dehydrogenase	20	6	Lipid biosynthesis	<i>Thermoproteaceae</i>	
IF1A_METJA	Translation initiation factor 1A	19	40	Protein biosynthesis	<i>Methanocaldococcaceae</i>	Euryarchaeota
GFPS_METMA	Geranylarnesyl diphosphate synthase	18	21	Isoprenoid biosynthetic process	<i>Methanosarcinaceae</i>	Euryarchaeota
ADEC_METKA	Adenine deaminase	18	10	Adenine catabolic process	<i>Methanopyraceae</i>	Euryarchaeota

ARLY_METST	Argininosuccinate lyase	17	32	Amino-acid biosynthesis	<i>Methanobacteriaceae</i>	Euryarchaeota
EF1A_METMP	Elongation factor 1-alpha	16	14	Protein biosynthesis	<i>Methanocaldococcaceae</i>	Euryarchaeota
PYRH_PYRIL	Uridylate kinase	15	39	Pyrimidine biosynthesis	<i>Thermoproteaceae</i>	Crenarchaeota
COFH_METM5	FO synthase subunit 2	15	6	Coenzyme F0 biosynthesis	<i>Methanocaldococcaceae</i>	Euryarchaeota
GGR1_METTH	Digeranylgeranylglycerophospholipid reductase	14	10	Lipid biosynthesis	<i>Methanobacteriaceae</i>	Euryarchaeota
SYFB_PYRAB	Phenylalanine--tRNA ligase	13	7	Protein biosynthesis	<i>Thermococcaceae</i>	Euryarchaeota
Chaperones						
DNAK_METTP	Chaperone protein DnaK	40	40	Heat shock	<i>Methanosaetaceae</i>	Euryarchaeota
RAD50_THEAC	DNA double-strand break repair Rad50 ATPase	27	72	DNA repair	<i>Thermofilaceae</i>	Crenarchaeota
PFDA_HYPBU	Prefoldin subunit alpha	16	26	Protein folding	<i>Pyrodictiaceae</i>	Crenarchaeota
SMC_PYRFU	Chromosome partition protein Smc	16	25	DNA repair	<i>Thermococcaceae</i>	Euryarchaeota
CBPX2_SULSO	Thermostable carboxypeptidase	15	21	Proteolysis	<i>Sulfolobaceae</i>	Crenarchaeota
CO₂ Fixation						
CAPPA_SULAC	Phosphoenolpyruvate carboxylase	28	103	CO ₂ fixation	<i>Sulfolobaceae</i>	Crenarchaeota
Energy						
TIAS_METV3	tRNA(Ile2) 2-agsmatinylcytidine synthetase	16	10	ATP binding	<i>Methanococcaceae</i>	Euryarchaeota
Methanogenesis						
ACDA1_METM A	Acetyl-CoA decarboxylase/synthase complex subunit alpha	27	21	Methanogenesis from acetate	<i>Methanosarcinaceae</i>	Euryarchaeota
HMD_METTH	5,10-methenyltetrahydromethanopterin hydrogenase	26	5	Methanogenesis from CO ₂	<i>Methanobacteriaceae</i>	Euryarchaeota
HMD_METKA	5,10-methenyltetrahydromethanopterin hydrogenase	14	3	Methanogenesis from CO ₂	<i>Methanopyraceae</i>	Euryarchaeota
Transcription & Translation						
ECX2_ARCFU	Probable exosome complex exonuclease	29	40	RNA catabolic process	<i>Archaeoglobaceae</i>	Euryarchaeota
DPOL_THELI	DNA polymerase	26	33	DNA replication	<i>Thermococcaceae</i>	Euryarchaeota
PCNA_THESM	DNA polymerase sliding clamp	15	9	DNA replication	<i>Thermococcaceae</i>	Euryarchaeota
PRIL_METKA	Probable DNA primase large subunit	21	88	DNA replication	<i>Methanopyraceae</i>	Euryarchaeota
RS8_THEPD	30S ribosomal protein S8	19	26	Translation	<i>Thermofilaceae</i>	Crenarchaeota
PCNA_METBF	DNA polymerase sliding clamp	19	12	DNA replication	<i>Methanosarcinaceae</i>	Euryarchaeota
RL31_AERPE	50S ribosomal protein L31e	18	13	Translation	<i>Desulfurococcaceae</i>	Crenarchaeota
RL37_METST	50S ribosomal protein L37e	17	9	Translation	<i>Methanobacteriaceae</i>	Euryarchaeota
FTSZ1_PYRW	Cell division protein FtsZ	15	19	Cell division	<i>Thermococcaceae</i>	Euryarchaeota
O						

TF2B2_PYRKO	Transcription initiation factor IIB 2	14	8	Transcription	<i>Thermococcaceae</i>	Euryarchaeota
DP2L_METHJ	DNA polymerase II large subunit	13	63	DNA replication	<i>Methanospirillaceae</i>	Euryarchaeota
Transport						
SRP54_HALLT	Signal recognition particle 54 kDa protein	52	47	Insertion of nascent membrane proteins into the cytoplasmic membrane	<i>Halobacteriaceae</i>	Euryarchaeota
MTAD_THEON	5-methylthioadenosine/S-adenosylhomocysteine deaminase	24	4	Metal ion binding	<i>Thermococcaceae</i>	Euryarchaeota
Y4342_METAC	Putative ABC transporter ATP-binding protein	23	18	Transport - cell membrane	<i>Methanosarcinaceae</i>	Euryarchaeota
Y427_METS3	UPF0095 protein Msm_0427	15	6	Magnesium ion binding	<i>Methanobacteriaceae</i>	Euryarchaeota
Bacteria						
Biosynthesis						
PDXA_GEOMG	4-hydroxythreonine-4-phosphate dehydrogenase	55	38	Pyridoxine biosynthesis	<i>Geobacteraceae</i>	Proteobacteria
PURA_CAMLR	Adenylosuccinate synthetase	7	3	Purine biosynthesis	<i>Campylobacteraceae</i>	Proteobacteria
LEU1_SYNR3	2-isopropylmalate synthase	42	22	Amino-acid biosynthesis	<i>Synechococcaceae</i>	Cyanobacteria
TRPE_HELPJ	Anthranilate synthase component 1	41	32	Amino-acid biosynthesis	<i>Helicobacteraceae</i>	Proteobacteria
EX7L_SHESA	Exodeoxyribonuclease 7 large subunit	39	11	DNA catabolic process	<i>Shewanellaceae</i>	Proteobacteria
LEU3_THIDA	3-isopropylmalate dehydrogenase	39	32	Amino-acid biosynthesis	<i>Hydrogenophilaceae</i>	Proteobacteria
SYD_HELMI	Aspartate--tRNA ligase	37	12	Protein biosynthesis	<i>Heliobacteriaceae</i>	Firmicutes
CBID_PROMA	Putative cobalt-precorrin-6A synthase	36	10	Cobalamin biosynthesis	<i>Synechococcaceae</i>	Cyanobacteria
GUAA_ANAVT	GMP synthase [glutamine-hydrolyzing]	34	20	Purine biosynthesis	<i>Nostocaceae</i>	Cyanobacteria
ASSY_BORPE	Argininosuccinate synthase	34	17	Amino-acid biosynthesis	<i>Alcaligenaceae</i>	Proteobacteria
UBIA_ACIET	4-hydroxybenzoate octaprenyltransferase	33	12	Ubiquinone biosynthesis	<i>Comamonadaceae</i>	Proteobacteria
COAE_BURMA	Dephospho-CoA kinase	32	7	Coenzyme A biosynthesis	<i>Burkholderiaceae</i>	Proteobacteria
KAD_DESPS	Adenylate kinase	31	14	Nucleotide biosynthesis	<i>Desulfobulbaceae</i>	Proteobacteria
YEGS_XANCP	Probable lipid kinase YegS	31	19	Lipid biosynthesis	<i>Xanthomonadaceae</i>	Proteobacteria
RF3_ECO24	Peptide chain release factor 3	31	12	Protein biosynthesis	<i>Enterobacteriaceae</i>	Proteobacteria
DAPB_LACPL	4-hydroxy-tetrahydrodipicolinate reductase	30	14	Amino-acid biosynthesis	<i>Lactobacillaceae</i>	Firmicutes
GUAA_MOOTA	GMP synthase [glutamine-hydrolyzing]	27	42	Purine biosynthesis	<i>Thermoanaerobacteriaceae</i>	Firmicutes
GCH4_AZOVD	GTP cyclohydrolase FolE2	27	13	Cofactor biosynthesis	<i>Pseudomonadaceae</i>	Proteobacteria
PLSX_AZOC5	Phosphate acyltransferase	26	12	Lipid biosynthesis	<i>Xanthobacteraceae</i>	Proteobacteria
SYL_BACWK	Leucine--tRNA ligase	22	7	Protein biosynthesis	<i>Bacillaceae</i>	Firmicutes

METX_PSEE4	Homoserine O-acetyltransferase	21	5	Amino-acid biosynthesis	<i>Pseudomonadaceae</i>	Proteobacteria
LPXK_BARHE	Tetraacyldisaccharide 4'-kinase	19	12	Lipid biosynthesis	<i>Bartonellaceae</i>	Proteobacteria
ACCA_HELP2	Acetyl-coenzyme A carboxylase carboxyl transferase subunit alpha	18	4	Fatty acid biosynthesis	<i>Helicobacteraceae</i>	Proteobacteria
GATA_SYNY3	Glutamyl-tRNA(Gln) amidotransferase subunit A	16	13	Protein biosynthesis	<i>Oscillatoriohycideae</i>	Cyanobacteria
Chaperones						
ADDA_MOOTA	ATP-dependent helicase/nuclease subunit A	26	4	DNA repair	<i>Thermoanaerobacteriaceae</i>	Firmicutes
General metabolism						
PTH_RICB8	Peptidyl-tRNA hydrolase	36	89	Aminoacyl-tRNA hydrolase activity	<i>Rickettsiaceae</i>	Proteobacteria
ERA_ALKMQ	GTPase Era	34	35	Ribosome biogenesis	<i>Clostridiaceae</i>	Firmicutes
RLMG_SHEPA	Ribosomal RNA large subunit methyltransferase	33	5	rRNA processing	<i>Shewanellaceae</i>	Proteobacteria
OCD_AGRT4	Ornithine cyclodeaminase	33	47	Lyase	<i>Rhizobiaceae</i>	Proteobacteria
XYND_BACSU	Arabinoxylan arabinofuranohydrolase	29	3	Carbohydrate metabolic process	<i>Bacillaceae</i>	Firmicutes
NAGB_CORU7	Glucosamine-6-phosphate deaminase	28	11	Carbohydrate metabolism	<i>Corynebacteriaceae</i>	Actinobacteria
CITD1_SALPA	Citrate lyase acyl carrier protein 1	27	5	Lyase	<i>Enterobacteriaceae</i>	Proteobacteria
MI43_MYCIT	27 kDa lipoprotein antigen	23	5	Lipoprotein	<i>Mycobacteriaceae</i>	Actinobacteria
PGK_LISIN	Phosphoglycerate kinase	29	11	Glycolysis	<i>Listeriaceae</i>	Firmicutes
PGK_ONYPE	Phosphoglycerate kinase	26	10	Glycolysis	<i>Acholeplasmataceae</i>	Tenericutes
GLPK_MYCCT	Glycerol kinase	25	35	Glycolysis	<i>Mycoplasmataceae</i>	Firmicutes
PGK_MESSB	Phosphoglycerate kinase	25	11	Glycolysis	<i>Phyllobacteriaceae</i>	Proteobacteria
ENO_PROM4	Enolase	25	4	Glycolysis	<i>Synechococcaceae</i>	Cyanobacteria
Membrane proteins						
HFLD_AZOVD	High frequency lysogenization protein HflD homolog	31	5	Negative regulator of phage lysogenization	<i>Pseudomonadaceae</i>	Proteobacteria
YABM_BACSU	Uncharacterized membrane protein YabM	21	7	Transmembrane transport	<i>Bacillaceae</i>	Firmicutes
Nitrogen fixation						
NIFM_KLEOX	Putative peptidyl-prolyl cis-trans isomerase NifM	28	14	Nitrogen fixation	<i>Enterobacteriaceae</i>	Proteobacteria

Pathogenesis						
LOVHK_PSES M	Blue-light-activated protein	29	7	Virulence	<i>Pseudomonadaceae</i>	Proteobacteria
HLYE_ECOLI	Hemolysin E, chromosomal	23	11	Pathogenesis	<i>Enterobacteriaceae</i>	Proteobacteria
Transcription & translation						
RL3_MYCCT	50S ribosomal protein L3	37	22	Translation	<i>Mycoplasmataceae</i>	Firmicutes
RS2_WOLPM	30S ribosomal protein S2	31	44	Translation	<i>Rickettsiaceae</i>	Proteobacteria
RTCA_ECOHS	RNA 3'-terminal phosphate cyclase	30	4	RNA processing	<i>Enterobacteriaceae</i>	Proteobacteria
RL28_HYDS0	50S ribosomal protein L28	26	4	Translation	<i>Aquificaceae</i>	Aquificae
NREC_STAEQ	Oxygen regulatory protein NreC	24	3	Transcription	<i>Staphylococcaceae</i>	Firmicutes
Transport						
LIPA_PSEFS	Lipoyl synthase	33	24	Transferase	<i>Pseudomonadaceae</i>	Proteobacteria
PSTS_XYLFA	Phosphate-binding protein PstS	33	10	Phosphate transport	<i>Xanthomonadaceae</i>	Proteobacteria
SECA_BLOFL	Protein translocase subunit SecA	31	23	Protein transport	<i>Burkholderiaceae</i>	Proteobacteria
SECB_SHEON	Protein-export protein SecB	29	6	Protein transport	<i>Shewanellaceae</i>	Proteobacteria
RNFA_SODGM	Electron transport complex protein RnfA	24	7	Electron transport	<i>Enterobacteriaceae</i>	Proteobacteria
PRMA_PROM2	Ribosomal protein L11 methyltransferase	24	10	Transferase	<i>Synechococcaceae</i>	Cyanobacteria
PNP_NEIM0	Polyribonucleotide nucleotidyltransferase	23	2	Transferase	<i>Neisseriaceae</i>	Proteobacteria
NUOB1_ROSC S	NADH-quinone oxidoreductase subunit B	19	11	Electron transport	<i>Chloroflexaceae</i>	Chloroflexi
UREF_ALHEH	Urease accessory protein UreF	16	2	Nickel cation binding	<i>Ectothiorhodospiraceae</i>	Proteobacteria
Unknown						
Y159_HAEIN	Uncharacterized protein HI_0159	25	6	Unknown	<i>Pasteurellaceae</i>	Proteobacteria

^a MS/MS MOWSE score (Mascot, Matrix Science)

^b Number of identified peptides

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