PROTEOMIC PROTOCOL

Protein extraction

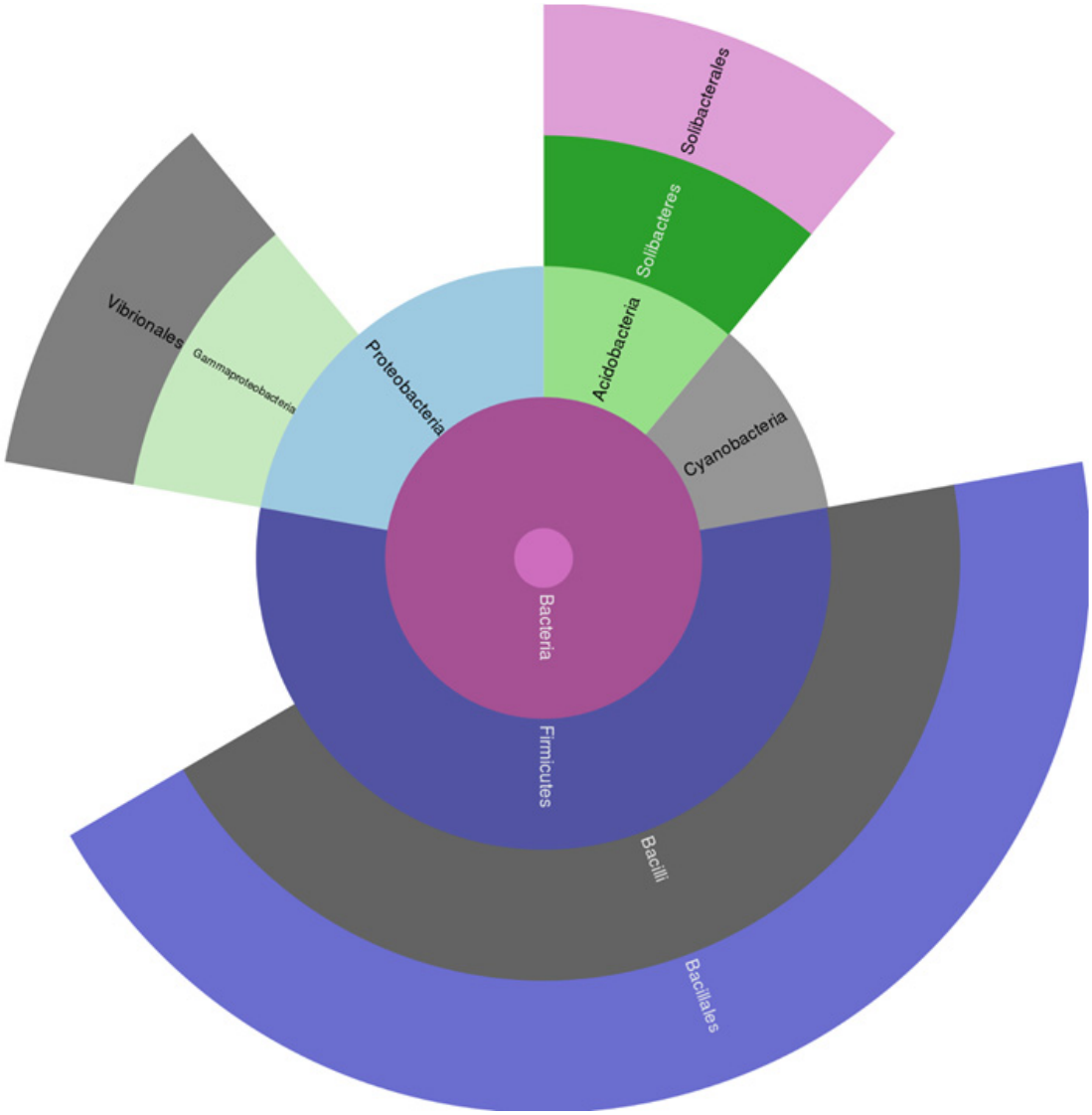
Protein isolation was performed according to Wang et al. [20], with some modifications. From each composite sample, 3 g of soil were added to 9.0 mL of extraction buffer [0.85 M sucrose, 0.1 M Tris-HCl (pH 8.0), 2% (w/v) sodium dodecyl sulfate (SDS), 1 mM phenylmethylsulfonyl fluoride and 2% (w/v) polyvinylpolypyrrolidone]. After that, 3 mM Protease Inhibitor Cocktail Powder (Sigma-Aldrich) and 70 mM dithiothreitol (DTT) were added. Samples were incubated at room temperature per 10 min before performing five events of sonication (30 s duration per event and 30 s intervals between them). Each sample was fractioned in eight microtubes and homogenized individually with 700 μL saturated phenol (pH 8.0). After centrifugation at 14,000 rpm per 7 min at 4 °C, the phenolic phase of each aliquot was collected and combined into new microtubes, following by other centrifugation at 14,000 rpm per 7 min at 4 °C to remove any residue of SDS or the aqueous phase. The phenolic phase was collected and proteins were precipitated by incubating the samples with 800 μL 0.1 M ammonium acetate (prepared in absolute methanol at -20 °C) at -80 °C overnight. Samples were centrifuged at 14,000 rpm per 4 min at 4 ºC and the supernatant was carefully discarded. The remaining protein pellet was washed two times with 1.5 mL 80% (v/v) ice-cold acetone and ice-cold ethanol. Finally, the resulting pellet was dried in a vacuum concentrator for 7 min and proteins were solubilized by adding 50 μL of 0.2 % RapiGest™ Surfactant (Waters) and stored at -80 °C until protein digestion. We used Qubit 2.0 Fluorometer (Invitrogen) to estimate protein concentration.

Protein digestion and sample desalting

Thirty micrograms of protein were quantified per sample, ammonium bicarbonate (50 mM) was added to obtain a final volume of 100 μL and to dilute RapiGest™ Surfactant to 0.1%. The preparation for digestion involved protein reduction with dithiothreitol (DTT, 5 mM) and incubation for 25 minutes at 56 °C, followed by alkylation with iodoacetamide (IAA, 14 mM) for 30 minutes at room temperature. Residual IAA was removed by adding DTT (5 mM) and incubating for 15 minutes at room temperature, followed by the addition of $CaCl_2$ (1 mM) and treatment with trypsin (20 ng/μL) for 20 hours at 37 °C. Subsequently, trifluoroacetic acid (TFA) was added to a final concentration of 0.4% to terminate the enzymatic reaction. Samples were incubated for 90 minutes at 37 °C, then centrifuged at 14,000 rpm at 6 °C for 30 minutes. Finally, the supernatant was transferred to appropriated vials and the pH solution was adjusted to 10 with 1 N ammonium hydroxide for effective trapping on the first-dimension column of the Ultra-Performance Liquid Chromatography (UPLC).

BACTERIA POPULATIONS

A)



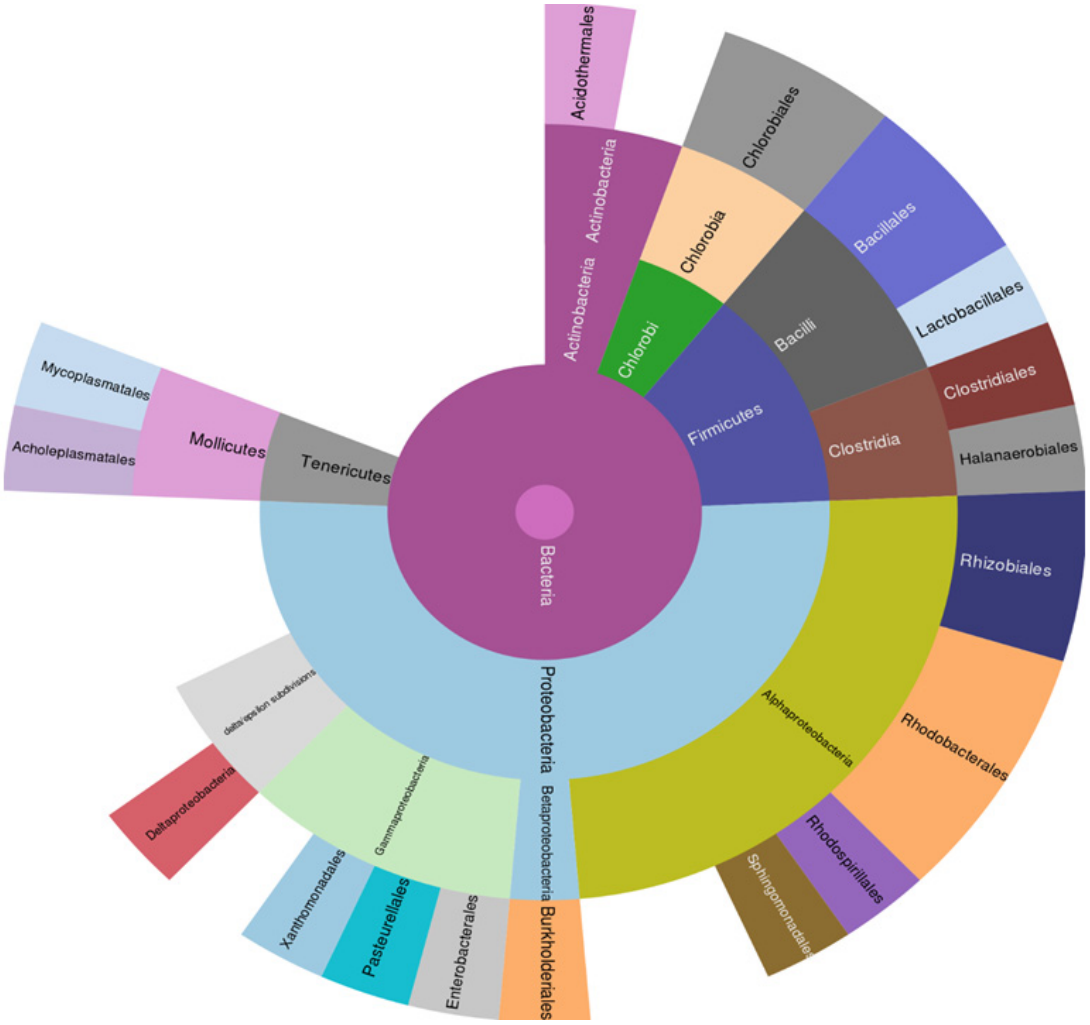
B)


Bacteria
Proteobacteria
Firmicutes
Bacteroidetes
Cyanobacteria
Chloroflexi
Chlorobi
Acidobacteria
Actinobacteria
Aquificae
Spirochaetes
Verrucomicrobia
Deltaproteobacteria
delta/epsilon subdivisions
Gammaproteobacteria
Betaproteobacteria
Alphaproteobacteria
Bacilli
Clostridia
Bacteroidia
Chlorobia
Solibacteres
Spirochaetia
Methylacidiphilae
Solibacterales
Frankiales
Aquificales
Bacteroidales
Chlorobiales
Herpetosiphonales
Synechococcales
Prochloraceae
Bacillales
Lactobacillales
Clostridiales
Rhizobiales
Rhodospirillales
Rickettsiales
Sphingomonadales
Burkholderiales
Nitrosomonadales
Alteromonadales
Chromatiales
Enterobacterales
Pasteurellales
Pseudomonadales
Spirochaetales
Methylacidiphilales

C)


Bacteria
Actinobacteria
Aquificae
Cyanobacteria
Firmicutes
Proteobacteria
Tenericutes
Mollicutes
Mycoplasmatales
Betaproteobacteria
Gammaproteobacteria
Vibrionales
Pseudomonadales
Enterobacterales
Nitrosomonadales
Burkholderiales
Alphaproteobacteria
Rhizobiales
Rhodobacterales
Rhodospirillales
Sphingomonadales
Acidithiobacillia
Acidithiobacillales
Clostridia
Clostridiales
Bacilli
Lactobacillales
Synechococcales
Synechococcaceae
Prochloraceae
Aquificales
Micrococcales

D)


Bacteria
Actinobacteria
Chlorobi
Firmicutes
Tenericutes
Proteobacteria
Acidothermales
Actinobacteria
Chlorobia
Chlorobiales
Bacillales
Bacilli
Lactobacillales
Clostridiales
Clostridia
Halanaerobiales
Mycoplasmatales
Acholeplasmatales
Mollicutes
delta epsilon subdivisions
Deltaproteobacteria
Gammaproteobacteria
Betaproteobacteria
Alphaproteobacteria
Rhizobiales
Rhodobacterales
Rhodospirillales
Sphingomonadales
Xanthomonadales
Pasteurellales
Enterobacterales
Burkholderiales

E)

Figure S5. Peptide abundance in the phyla, classes, and order of bacteria (respectively, from the central circle of "bacteria"), built by Unipept 4.0 software. The size of the slices corresponds to the number of peptides identified in the respective taxonomic level. **A)** Nonrehabilitated stage, **B)** Early rehabilitation (4 years), **C)** Advanced rehabilitation (12 years), **D)** Advanced rehabilitation (14 years), **E)** Native soil (reference).