A long-read 16S rRNA sequencing workflow enhances bacterial diversity recovery from rhizospheric soils of a salt flat in the Atacama desert.

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BACKGROUND

Salt flats are extreme ecosystems characterized by high mineral content and harsh environmental fluctuations. Despite these conditions, *Puccinellia frígida* thrives in these habitats, interacting with soil microorganisms that enhance its stress tolerance. Studying these rhizospheric communities helps reveal microbial adaptability and their role in plant survival under extreme conditions.

We analyzed the rhizospheric bacterial community of *P. frígida* in the Salar de la Isla (Atacama, Chile) using long-read 16S rRNA sequencing with DeCodi-Fi High-Fidelity Polymerase and Oxford Nanopore Technology.

This workflow improved taxonomic resolution and enabled the detection of low-abundance bacterial taxa. Between 31–51 genera were unique to specific microhabitats, while 121 genera were shared among all samples.

Soil analyses revealed spatial variation in Na, Mg, Cl, K, Ca, Mn, and Fe, correlating with local hydrological patterns and associated differences in bacterial community composition.

This approach enhances bacterial diversity detection in low-biomass, heterogeneous soils, supporting its application in environmental microbiome studies using long-read sequencing.

METHODS

The study was conducted at Salar de la Isla (25°39.2012'S, 68°38.1044'W), Atacama Region, Chile. Rhizospheric sediment samples were collected in triplicate from a vegetated patch dominated by *Puccinellia frígida* (Figure 1). Total DNA was extracted using the E.Z.N.A.® Soil DNA Kit (OMEGA).

PCR amplification was performed using two DNA polymerases to evaluate yield and taxonomic richness: LongAmp® Taq DNA Polymerase, commonly recommended in 16S ONT protocols, and DeCodi-Fi™ High-Fidelity Polymerase, tested as an alternative to improve species resolution. The V1–V9 region of the 16S rRNA gene was sequenced using Oxford Nanopore Technology (ONT) to characterize bacterial community composition.

Additionally, the soluble fraction of chemical elements in each sample was analyzed by Total Reflection X-Ray Fluorescence (TXRF) (Bruker).

Figure 1. Sampling site at Salar de la Isla.
(A) Location and general view of the study area.
(B) Aerial view of a *Puccinellia frígida* vegetation patch and its surrounding rhizospheric sampling zones (RZ1, RZ2, RZ3, RZ4).

RESULTS

To determine the most suitable enzyme for the workflow, amplification performance was compared between two DNA polymerases: LongAmp® Taq DNA polymerase, commonly recommended in Oxford Nanopore protocols, and DeCodi-Fi™ high-fidelity polymerase optimized for low-input and challenging environmental samples.

DeCodi-Fi™ produced higher and more consistent amplification yields across four rhizospheric zones, while LongAmp® showed lower and more variable efficiency. Moreover, **DeCodi-Fi™ resulted in greater bacterial species richness and improved taxonomic resolution.** Therefore, data generated using the DeCodi-Fi™ high-fidelity polymerase were used to assess bacterial abundance profiles.(Figure 2)

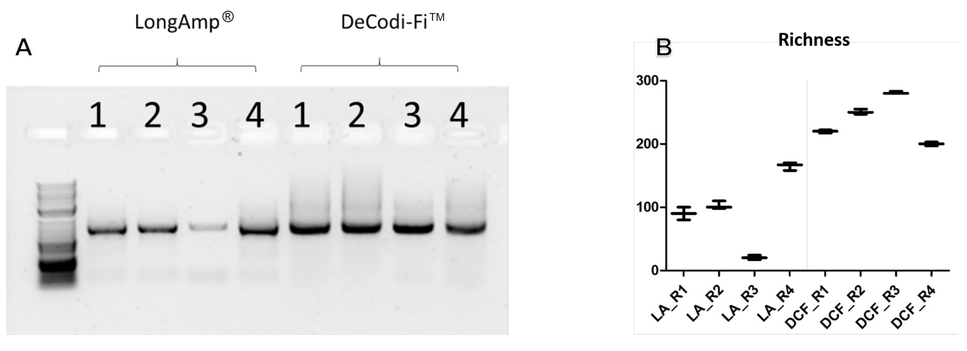

Figure 2. 16S rRNA amplification performance and bacterial richness.
(A) Representative agarose gel showing amplification differences between the LongAmp® and DeCodi-Fi™ DNA polymerases across four rhizospheric sampling zones.
(B) Bacterial richness for each rhizospheric sampling zone based on 16S rRNA gene amplification using LongAmp® (LA) and DeCodi-Fi™ (DCF) enzymes.

Bacterial communities in *P. frígida* rhizospheres showed spatial variation among rhizospheric zones. The dominant phyla were Pseudomonadota, Bacteroidota, and Cyanobacteriota. A total of 121 genera were shared, while each zone hosted unique genera (13–51), highlighting microhabitat-driven diversity (Figure 3).

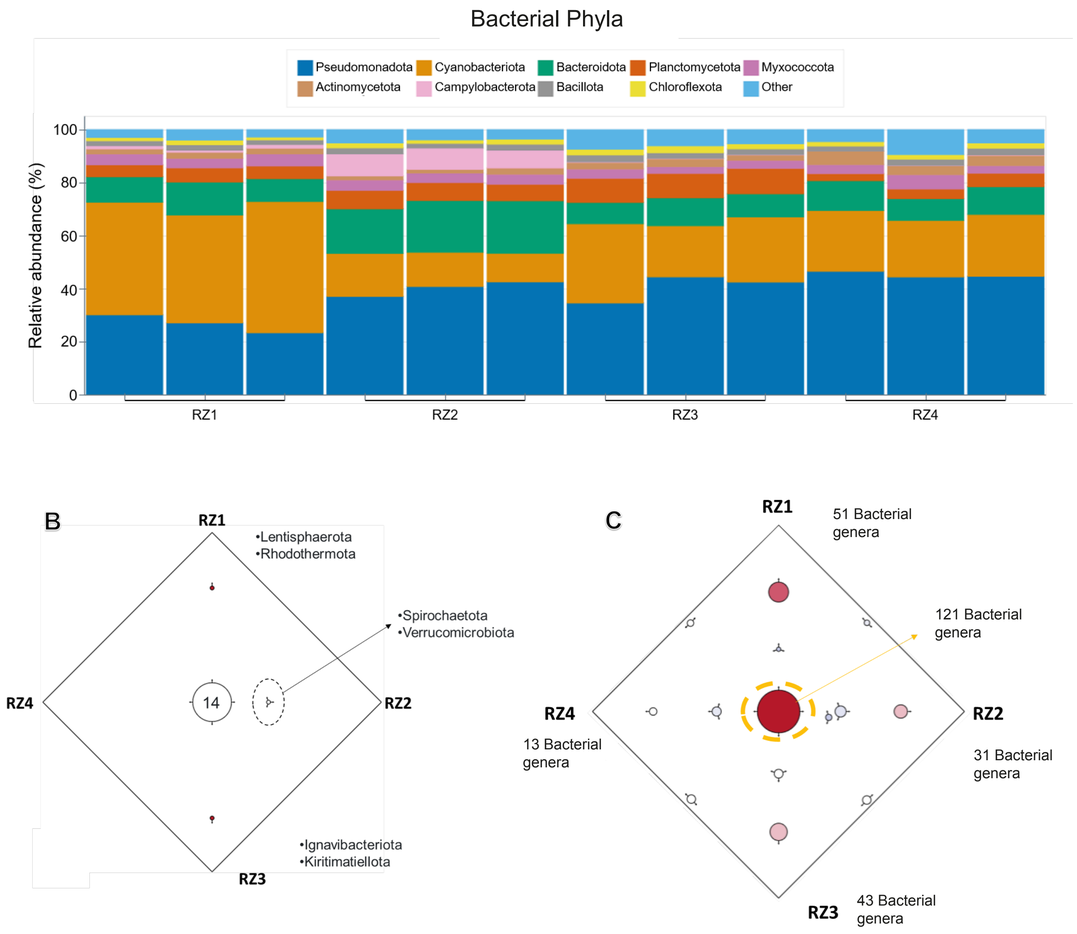

Figure 3. Taxonomic and diversity analysis of *P. frígida* rhizospheric samples.
(A) Relative abundance of bacterial phyla.
(B) Fourteen phyla were shared among all zones, while others were unique to specific sites (noted on the sides of the diagram)
(C) Shared(121 genera) and unique bacterial genera identified in *P. frígida* rhizosphere samples.

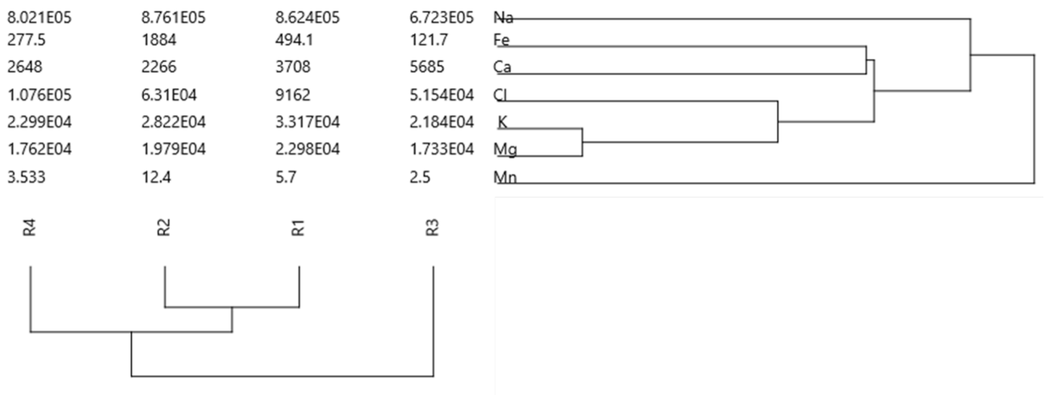

Figure 4. Clustering of soluble chemical elements in the rhizosphere of *P. frígida*, as determined by Total Reflection X-Ray Fluorescence (TXRF), showing compositional relationships among elements and sampling zones.

CONCLUSIONS

Rhizospheric bacterial communities associated with *Puccinellia frígida* in the Salar de la Isla show notable variability in composition, influenced by the edaphic heterogeneity and water flow of the study site.

These results reveal microbial adaptability and their potential role in plant tolerance under extreme salinity and nutrient availability through their participation in soil biogeochemical cycles. **The use of DeCodi-Fi™ High-Fidelity Polymerase with Oxford Nanopore sequencing improved the detection of low-abundance bacterial taxa and enhanced taxonomic resolution in low-biomass, heterogeneous soils.**