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COMMUNITY SUCCESSION DURING TOP-DOWN ENRICHMENT AT INCREASING SALINITIES ALLOWS SCREENING OF KEY MICROBIAL PLAYERS OF MICROBIAL PAH-DEGRADING CONSORTIA

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Soils impacted by hydrocarbon exploitation in Argentina are characterized by high salinity levels, exposing microorganisms to multiple stressors such as carbon starvation, osmotic stress, and oxidative stress. Given the resilience of halophilic microorganisms in degrading hydrocarbons and other contaminants, it is crucial to explore their full potential for remediating contaminated hypersaline ecosystems. However, assembling simplified microbial consortia from complex environmental communities remains a significant challenge due to the vast diversity and intricate biotic interactions involved. This study employs a top-down strategy to identify and obtain key microorganisms in the degradation of polycyclic aromatic hydrocarbons (PAH) in saline environments. An initial enrichment culture (CE) was obtained from saline hydrocarbon contaminated soil. Through three sequential transfers, performed every 30 days with phenanthrene as the sole carbon source in a saline medium (2% NaCl), the SAL49 consortium was obtained. Further sequential transfers, also every 30 days but in a higher saline medium (3% NaCl), yielded the SAL59 consortium. We assessed the diversity and key microbial players within CE and both consortia using 16S rRNA gene metabarcoding (Novaseq-QIIME2) and co-occurrence analysis with the SparCC tool (bootstrap 1000, $p < 0.05$), retaining associations with correlations $> |0.5|$. The degradation potential of both consortia was evaluated using HPLC-UV. Additionally, two strains were isolated from SAL59 and were physiologically characterized (Substrate degradation spectra, Biolog Ecoplate analysis, PAH degradation capabilities and Minimum Inhibitory Concentration of NaCl (CIMS). The PAH degradation rate and bacterial diversity decreased as culture pressure increased. Specifically, SAL49 and SAL59 showed 99.1% and 60% PAH degradation, respectively, after 21 days. Throughout the enrichment process, *Pseudomonadota* remained dominant across various saline pressure stages. Co-occurrence network analysis predicted/identified the keystone genera in CE as *Sphingobium*, *Bordetella*, *Brevundimonas*, *Achromobacter*, and *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*. In SAL49 the keystone genera were predicted to be

Ochrobactrum and *Sphingobium*. From SAL59, two isolates, *Pseudomonas* oryzihabitans V1 and *Ochrobactrum* intermedium V3, were obtained, both of which demonstrated PAH degradation capacity and belonged to genera known for salinity resistance. The phenotypic fingerprint provided by Biolog offered insights into the analysis of the individual isolates, revealing robust kinetic growth and metabolism, predominantly by the V3 strain. These findings highlight the potential effectiveness of a "top-down" microbial screening approach in selecting preadapted key PAH-degrading microorganisms for designing microbial assemblies useful for pollutant degradation.

Palabras clave: Biorremediación - Saline soil – Microbial interactions- Molecular Ecological Networks