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Cover image:

Mineral–microorganisms interactions Mlewski EC¹, Gérard E²

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A Confocal Laser Scanning Microscopy image of a resin-embedded microbialite from Laguna Negra (Puna-Catamarca), stained with calcein (a fluorescent dye that produces a stable complex in the presence of calcium and fluoresces in the green region of visible light). Mineral aggregates are observed in blue. Their surfaces are partially stained with calcein, indicate the presence of free Ca²⁺ ions. Diatoms and *Rivularia halophila* filaments are visible in red thanks to their photosynthetic pigments.

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COMPARATIVE GENOMIC ANALYSIS OF THE *Fructobacillus* GENUS REVEALS IMPORTANT DIFFERENCES IN AMINO ACID METABOLISM

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The Fructobacillus genus is a group of obligatory fructophilic lactic acid bacteria, recently reclassified from Leuconostoc due to phylogenetic and biochemical differences. These bacteria require the use of fructose or another electron acceptor for its growth because of the lack of an alcohol-acetaldehyde dehydrogenase gene (adhE). Previously, some genomic differences were reported in Fructobacillus with respect to Leuconostoc, suggesting a reductive evolution in carbohydrate metabolism caused by an adaptation to fructose-rich niches. In this work, we performed a comparative genomic analysis in the genus Fructobacillus to evaluate possible genomic and metabolic differences among species. For this, nine Fructobacillus genomes were used. As expected, the GC content was highly similar among this genus (43.9-44.8 % mol); however, some differences were found in genome size and number of CDS being the genomes of F. durionis DSM 19113 and F. sp. CRL 2054 smaller (1,32 Mb) than F. tropaeoli genomes (1.66–1.68 Mb). Six intact prophage regions (20.6–30.5 kb) were identified in four strains, whereas Type II CRISPR-Cas systems were found in five genomes. A bacteriocin-coding gene was only found in F. durionis DSM 19113. Plasmids and antibiotic resistance genes were not detected in the studied genomes. Phylogenetic analyses were done based on 16S rRNA sequences and Fructobacillus core-genome. Both phylogenetic trees allowed us to distinguish two different phylogroups in this genus. Phylogroup 1 was composed of F. sp. CRL 2054, F. durionis and F. fructosus strains, whereas F. ficulneus, F. pseudoficulneus, F. sp. EFB-N1, and F. tropaeoli strains formed part of phylogroup 2. A pangenome analysis showed important differences in the presence/absence of genes between both groups. Consequently, annotation of genomes in COG and KEGG databases was performed to classify genes according to their metabolic function. The number of genes involved in COG categories E, F, H, and P related to metabolism was significantly lower (p < 0.05) in phylogroup 1. This group of strains also presented an important decrease in the number of genes involved in the synthesis of five amino acids when comparing to phylogroup 2 and L. mesenteroides ATCC 8293. This reduction in the amino acid metabolism was also observed experimentally in F. sp. CRL 2054 and F. tropaeoli CRL 2034. Finally, the presence of genes involved in central carbohydrate metabolism was evaluated among the strains. Differences were detected in genes related to fructose metabolism (*fk*, *gpi*) and genes related to the use of electron acceptors (D- and L- *ldh*, *adh*, *budC*, *yjlD*); however, those variations were not always related to the phylogeny between organisms. In conclusion, two groups with important genomic differences were identified in Fructobacillus; moreover, the presence of genes involved in NADH reoxidation and fructose intake was variable throughout the genus.

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COPING WITH OXIDATIVE STRESS IN EXTREME ENVIRONMENTS: DISTINCTIVE ROLES OF *Acinetobacter* sp. VER 3 SUPEROXIDE DISMUTASES

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High-altitude Andean lakes (HAAL) are distributed along the central Andes area in South America, located at an average altitude of 4,000 m. HAAL are characterized by extreme environmental conditions such as high UV radiation, low nutrient concentration, presence of heavy metals, high salinity levels, and large daily temperature fluctuations. Acinetobacter sp. Ver 3 is a polyextremophilic strain isolated from HAAL shown to display high tolerance to prooxidants as UV radiation, H₂O₂, and methyl viologen (MV) when compared with collection strains used as controls. Catalases and superoxide dismutases (SODs) are the most important enzymes involved in the protection against oxidative stress. The SODs are metalloenzymes widely distributed in nature classified into four types based on the metal cofactors at their active sites: manganese SOD (MnSOD), iron SOD (FeSOD), copper/zinc SOD (CuZnSOD), and nickel SOD (NiSOD). Based on these data, we studied the role of SODs enzymes from the Ver 3 strain in the defense against oxidative stress. The complete genome of the Ver 3 strain was sequenced, showing the presence of two putative genes coding for (SODs) enzymes: the sodB gene coding for an iron SOD (FeSOD) and the sodC gene, which codes for a copper/zinc SOD (CuZnSOD). We analyzed both sod genes transcriptional levels by means of a qPCR assay and established that while the sodB levels remained unalterable under all the tested conditions, the sodC gene was upregulated in the presence of H₂O₂ and MV. Bioinformatic analysis indicated that FeSOD might be a cytosolic protein while the CuZnSOD has a predicted signal peptide, suggesting it is secreted into the periplasmic space. This bioinformatic prediction was confirmed by subcellular fractionation assays, revealing that the FeSOD is exclusive to the cytosolic fraction while the CuZnSOD can be found in the periplasmic soluble and insoluble fractions. In order to determine the prevalence of sod genes in the Acinetobacter genus, we performed a comparative genomic analysis in 31 Acinetobacter strains available at the NCBI GenBank database. The study reveals that all the species encoded at least one sodB gene (FeSOD), except for A. apis, which only encodes a MnSOD. Furthermore, 90% of the strains contained a sodC gene (CuZnSOD) with its predicted signal peptide in the corresponding amino acid sequence. Interestingly, most of the Acinetobacter strains lacking a sodC gene alternatively encoded a sodA gene (MnSOD) with a putative signal peptide, indicating that this enzyme could replace the oxidative stress-protective function exerted by the CuZnSOD protein in the periplasmic space. We concluded that the CuZnSOD from Acinetobacter sp. Ver is a periplasmic protein, and its ubiquity in the Acinetobacter genus supports the notion that this enzyme fulfills an important role in the defense against oxidative stress in the periplasmic space.