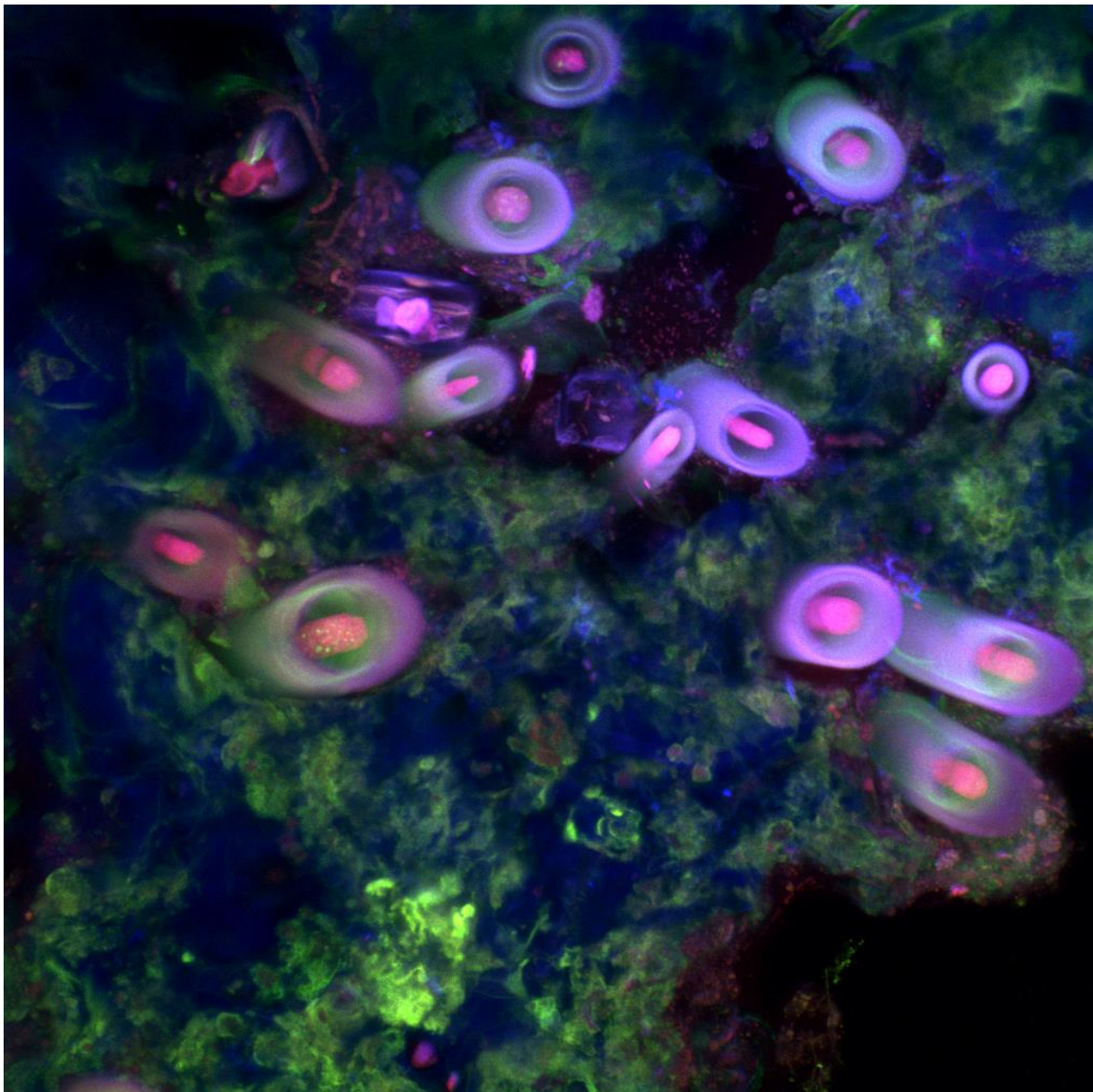




LVI SAIB Meeting – XV SAMIGE Meeting



SAIB-SAMIGE Joint Meeting 2020 – *Online*

Cover image:

Mineral–microorganisms interactions

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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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MI-C31-204

MECHANISMS ASSOCIATED WITH PROLINE METABOLISM AND REDOX BALANCE IN PEANUT MICROSymbionTS EXPOSED TO WATER STRESS

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The exposure of microorganisms to adverse environmental conditions can affect the possibility of establishing interaction with plants. Proline –an osmoprotective amino acid and determinant of cellular redox balance– could increase tolerance to drought stress. Thus, proline addition to peanut inoculants would mitigate drought stress in crops. The objective was to elucidate the fundamental mechanisms of protection against water stress mediated by proline in peanut microsymbionts, exploring the participation of its metabolism in the cellular redox balance. To evaluate whether the addition of proline activates the catabolism of the enzyme and the antioxidant system, we used the microsymbionts recommended as peanut inoculants, *Bradyrhizobium* sp. SEMIA6144 and *Bradyrhizobium* sp. C-145. Cultures in exponential phase were treated with different proline doses (0–50 mM) for 0–60 min to determine viability (CFU mL⁻¹), the transcription of genes from proline catabolism (*putA*) and antioxidants, catalase (*cat*) and thioredoxins (*trx*). Next, we analyzed the effect of proline on growth and redox metabolism of microsymbionts exposed to water stress. Proline concentration was selected by studying microorganisms' viability and priming effect on peanut seeds. The drought stress condition was imposed by polyethylene glycol (PEG) addition, whose concentration was selected in viability tests. The treatments were: (I) control; (II) 50 mM proline; (III) 30 mM PEG 6000; and (IV) 30 mM PEG 6000 + 50 mM proline. Bacterial response was determined through viability, proline content, production of a reactive oxygen species (hydrogen peroxide, H₂O₂), oxidative damage to lipids by thiobarbituric acid reactive substances (TBARs) and specific activity of the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT). The results showed that activation of proline degradation, revealed by elevated *putA* transcript levels, was related to up-regulation of transcripts coding for antioxidants (*cat* and *trx*). The addition of 50 mM proline increased the viability of PEG-treated *Bradyrhizobium* sp. C-145, reaching control values. In the presence of water stress, bacterial cells revealed an increase in proline content and SOD and CAT activities, while upon exogenous proline addition, they showed basal levels. In PEG-treated *Bradyrhizobium* sp. SEMIA 6144, the amino acid addition did not modify the decreased viability and elevated H₂O₂ and specific activities of SOD and CAT. In conclusion, the transcription of genes coding for the bifunctional enzyme of proline catabolism (*putA*) could be associated with the generation of excess electrons that react with oxygen, activating a redox-dependent transcription factor and enhancing the antioxidant response of bacterial cells (*cat* and *trx*). Besides, the addition of proline to the culture medium had a protective effect on *Bradyrhizobium* sp. C-145 growth in the presence of stress, which can be associated with the maintenance of redox balance.

MI-C32-217

IN-DEPTH BIOINFORMATIC CRISPR RECONSTRUCTION FROM METAGENOMIC DATA DISCLOSE PHAGE-HOST EVOLUTION IN COMPLEX ENVIRONMENTS

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Bacteriophages are highly abundant and ubiquitous in almost any habitat, where they play a critical role in shaping the microbiomes by infecting bacteria and archaea which carry out important processes to the environment. Lab-scale studies revealed that bacterial hosts respond to phage attacks by using a number of mechanisms that allow them to evade phage predation. In turn, genome rearrangements, mutations, and antibacterial defense systems allow phages to overcome these barriers, leading to an evolutionary arms race. However, laboratory settings do not necessarily reflect the more complex interactions that bacteria and phages experience in natural ecosystems. Metagenomics may complement this gap in information, but unfortunately, universal phylogenetic markers, such as the 16S rRNA gene of prokaryotes, are not present in phages. Therefore, investigating the diversity of phage communities and prediction of phage-host relationships is not straightforward. Taking advantage of the CRISPR (clustered regularly interspaced short palindromic repeats) system, which is present in most archaea and nearly 40% of bacteria, we developed a bioinformatic pipeline to provide a comprehensive picture of phage-host coevolution in naturally evolving populations within a complex environment from metagenomic data. The CRISPR-Cas systems are composed by Cas enzymes and an array of short DNA sequences, called spacers, separated by a repetitive sequence. Spacers are incorporated into CRISPR during unsuccessful phage attacks and it acts as an immune system, protecting the cell against future infections of the same phage. At the same time, it keeps a chronological register of previous attacks. In this approach, reads containing repetitive CRISPR sequences from multiple samples were used to reconstruct all the detectable variants of each particular CRISPR array. This resulted in a network of all possible spacers (nodes) connected by repeats (edges), which represent the spatio-temporal universe of CRISPR diversity. This network thus could be used to reconstruct the events of phage infections and identified the rise of new host populations. Phages were matched to their specific bacterial host by searching the corresponding protospacers within the metagenome and their genomes were reconstructed. This methodology was applied to predict phage-*Gordonia* associations and to assemble bacterial and phage variants in an environmental biotechnology system. By looking closely at single nucleotide variants and resolving CRISPR spacers that were present even at low abundance across a temporal series, we gained insight into the complexity of virus-host