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whose concentration was selected in viability tests. The treatments were: I) control; II) 50 mM proline; III) 30 mM PEG 6000; IV) 30 mM PEG 6000 + 50 mM proline. Bacterial response was determined through viability, proline content, production of a reactive oxygen species (hydrogen peroxide, H_2O_2), 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´s 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 H_2O_2 and specific activities of SOD and CAT. In conclusion, the transcription of genes coding for the bifunctional enzyme of proline catabolism (p*utA*) 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 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, call 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 by 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 arise 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 interaction at the population level in a real-world setting.

MI-C33-229

HIGH POTENTIAL FOR THE BIOSYNTHESIS OF NEUTRAL LIPID STORAGE COMPOUNDS IN CHRONICALLY-POLLUTED SUBANTARCTIC SEDIMENTS

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Microorganisms in intertidal sediments of Ushuaia Bay (Tierra del Fuego, Argentina) are adapted to extreme conditions, including low temperatures, high UV-B radiation levels and the presence of various environmental pollutants. Due to tidal cycles, these organisms are also exposed to periods of drought, as well as rapid changes in temperature, salinity and nutrient availability. Members of a limited number of phyla are known to accumulate wax esters (WE) and triacylglycerol (TAG) as an adaptation response to stressful environmental conditions similar to those present in intertidal sediments of Ushuaia Bay. The goal of this work was to study the abundance and diversity of bacteria with the potential to biosynthesize these neutral

lipid storage compounds in intertidal sediments polluted with aliphatic and polycyclic aromatic hydrocarbons, retrieved near a pier of a fuel storage facility. Homolog sequences of the key enzyme for WE and TAG biosynthesis, the wax ester synthase/acyl-CoA diacylglycerol acyltransferase (WS/DGAT), were identified in a metagenomic dataset from sediments of this site. Out of the 682,972 protein coding sequences of the dataset, 166 contained the wax ester synthase-like Acyl-CoA acyltransferase pfam domain commonly used to identify this enzyme (PF03007, E-value $\leq 10^{-5}$), the 74% of them full-length. A WS/DGAT C-terminal domain (PF06974) was also detected in the majority of the sequences. The relative abundance of WS/DGAT homolog sequences in the dataset was 1.42 ± 0.18 times the number of sequences of single-copy genes coding for ribosomal proteins (average ± standard deviation of 12 genes), suggesting a high prevalence of WE/TAG biosynthesis potential in the microbial community. Sequences were highly diverse, as 108 and 44 clusters were recovered using distance thresholds of 80% and 40% identity at the amino acid level, respectively. Furthermore, 64% of the putative enzymes shared low to moderate identity values with WS/DGAT homologs identified in bacterial genomes, indicating the presence of novel organisms with WE/TAG biosynthesis potential in the sediments. The taxonomic assignment of scaffolds containing WS/DGAT homologs (1 to 43.4 Kb, N50 = 35 Kb) indicated that members of the Actinobacteria (46 %), Proteobacteria (33 %), Bacteroidetes (3 %) and Acidobacteria (1 %) phyla could be the origin of the majority of the scaffolds, while 17% of them could only be assigned to Bacteria. These results suggest the presence of phylogenetically diverse and abundant microbial populations with the potential to biosynthesize neutral lipid storage compounds in intertidal sediments of this polluted site. This study is the starting point for more in-depth analyses of these metagenomic fragments, in order to increase our understanding of the mechanisms used by these diverse bacterial populations to adapt to environmental stressors in this extreme environment.

MI-C34-230

RECONSTRUCTING NEUTRAL-LIPIDS METABOLIC PATHWAYS OF A METAGENOMIC DATASET FROM USHUAIA BAY SEDIMENTS

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Bacterial production of neutral lipids such as triacylglycerides, wax-esters and polyhydroxyalcanoates (TAG, WE and PHA-B, respectively) has been reported in *Gammaproteobacteria* and *Actinobacteria*. Within them, there is a short list of microorganisms with an in-depth study of the metabolic route involved in the synthesis of these compounds. To increase our knowledge of the potential of sediment bacteria in relation to this process, we analyzed homolog sequences of the key enzyme involved in TAG biosynthesis, the wax synthase/diacylglycerol acyltransferase (WS/DGAT), from a metagenomic dataset of a chronically-polluted Subantartic coastal environment, and their genomic context. Almost half of putative WS/DGAT sequences were related to those identified in genomes from members of the *Actinobacteria* phylum, mainly from the *Acidimicrobiia, Actinobacteria* and *Nitriliruptoria* classes, while 34% of the sequences shared higher identity values with WS/DGAT homologs from Proteobacteria (*Gammaproteobacteria*, followed by *Alpha-*, *Beta-* and *Deltaproteobacteria*). Phylogenetic analyses showed that most metagenomic sequences were more closely related to sequences from genomes assembled from metagenomes, generated from environmental samples collected worldwide, including seawater, marine sediments, groundwater, seashore sand and freshwater, as well as biological wastewater treatment plants. Gene clusters potentially related to neutral lipid biosynthesis pathways were identified in scaffolds of the metagenomic dataset containing putative WS/DGAT sequences. A number of scaffolds shared highly similar genetic arrangements with genome fragments from a variety of organisms. Among them, some loci included genes that potentially encode other steps in neutral lipid biosynthesis, such as putative Type-2 PAPs and HAD-type hydrolases, glycerol- and acylglycerol- phosphate *O*-acyltransferases. In Proteobacteria, the gene clusters presented novel distributions of genes involved in TAG, WE and/or PHA, suggesting that they are intertwined. Most scaffolds contained genes from related metabolic pathways, such as fatty-acids metabolism and its regulation, implying that recycling of carbon might drive the flux to one or another neutral lipid synthesis. In addition, genes encoding osmoregulated periplasmic transporters for uptake of organic acids were present, revealing how the environment could also be influencing the studied process. This work is a pioneer study on the diversity of neutral lipid metabolic routes present in sediment bacteria based on metagenomic data. It enriches our knowledge of the metabolic potential of these microbial communities in relation to a process with an inherent biotechnological interest.

MI-C35-241

IMPACT OF ALTERNATIVE GRAPE MUSTS ON THE GROWTH OF INDIGENOUS NON-*SACCHAROMYCES* YEASTS

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Spontaneous fermentations of must from Isabella (*Vitis labrusca* L.) grapes, harvested from Colonia Caroya vineyards, show ethanol contents ~1% (v/v) lower than expected from their initial concentration of total reducing sugars. This phenomenon,