M-16 - NMR-Based Metabolite Profiling of Two A. thaliana Lines Overexpressing Ricinus communis Malate Synthase Gene

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INTRODUCTION: Malate synthase (MLS) is an enzyme that catalyzes the conversion of malate to acetyl-CoA and glyoxalate, connecting important energy-generating pathways in plants. Nuclear magnetic resonance (NMR) has been widely used in the mapping of metabolic changes in different biological systems, since it is a robust and highly efficient technique for metabolomics studies. OBJECTIVES: To assess the metabolite profile of two *Arabidopsis thaliana* lines overexpressing *R. communis* malate synthase gene (MLSA and MLSB). MATERIALS AND METHODS: Overexpressing lines were obtained by stable transformation of *A. thaliana* flowers. Extracts were obtained by grinding 20 mg of dry seeds in 500 µL of 0.1 M phosphate buffer, pH 7.4. ^{1}H-NMR spectra were obtained on a Varian 500 MHz Spectrometer and processed using two different programs: MestReNova and NMRProcFlow. Uni and multivariate statistical analysis were performed. DISCUSSION AND RESULTS: Eighteen metabolites were identified: three carbohydrates (glucose, sucrose and G6P), ten amino acids, and three organic acids. MLSA and MLSB lines showed significant differences in metabolite composition as compared to the wild type (Col-0). For example, sucrose and glucose content was up to four times higher in MLSA and MLSB than in Col-0. Similarly, the content of some amino acids such as asparagine were up to four times higher in MLSA and MLSB than in Col-0. Principal component analysis demonstrated that transgenic lines have metabolic signatures close to each other but different from Col-0. CONCLUSION: Overexpression of *R. communis* malate synthase gene affected the metabolome as it was observed an increase in several carbohydrates and amino acids. These changes demonstrate that overexpression of MLS altered metabolic homeostasis leading to greater accumulation important energy-generating molecules, which are crucial for proper seedling germination and development. The observed biochemical modifications may justify the better germination of the transgenic lines at high temperatures as compared to Col-0. **Keywords:** abiotic stress, metabolomics, transgenic / **Supported by:** UFBA, PGQA-UNEB, FAPESB, CNPq and CAPES

M-17 - Development of Target Proteomics Methods for Casbene Synthase Analysis in Jatropha curcas tissues

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INTRODUCTION: *Jatropha curcas* is a Euphorbiaceous plant that has biotechnological value since the seed oil is an alternative source of biodiesel. The seed also accumulates phorbol ester, a diterpenoid compound that confer toxicity to the oil and seedcake. Although it is known that phorbol esters accumulates in seeds, there is scarce information on its synthesis. Literature says that casbene synthase (CS) is considered a key enzyme for the synthesis of phorbol ester. OBJECTIVES: The project consists in developing target proteomics methods to investigate the presence and abundance of specific enzymes present in the synthesis pathway of phorbol esters of *J. curcas*. MATERIALS AND METHODS**:** Heavy peptides from CS were optimized for the development of the SRM method. For roots and endosperm tissue, protein extracts were reduced with DTT, alkylated with iodoacetamide and digested with trypsin. The optimization and SRM tests were carried out on an EASYII-nano LC system coupled to an nESI-TSQ Quantiva. Data were analyzed with the Xcalibur v. 2.2 and Skyline v.3.7 softwares. DISCUSSION AND RESULTS: We optimized the detection of 35 heavy peptides from CS and established the SRM method. These peptides were used as internal standards for the identification and absolute quantification of the target endogenous peptides. Target proteomics showed that there is no evidence of CS in endosperm and that it was possible to identify 6 endogenous peptides in roots that comprehend 6 gene models of CS isoforms. CONCLUSION: The SRM method for the analysis of CS were successfully developed. Our preliminary results indicate no evidence of CS in endosperm, but its presence in roots. The next step is to perform a broad analysis of root, endosperm and leaf samples from two different variants of *J. curcas*, one with high and the other with low level of phorbol ester, using the established SRM method. **Keywords:** Casbene synthase, Jatropha curcas, Target proteomics / **Supported by:** CAPES, FAPERJ, CNPq

M-18 - Analysis of microbiota associated to biodigesters fed with bovine and swine slurry

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INTRODUCTION: The biogas production in biodigester is a multi-step process, driven by microbial communities. Know these communities is essential for understanding system dynamics and the role of the main organisms, an important step to improve the gas production. OBJECTIVES: The microbial diversity in anaerobic swine and bovine biodigesters was examined using metagenomic 16S rRNA gene next-gene sequencing approach. MATERIALS AND METHODS: amples from two biodigesters swine-CSRT (Continuous Stirred-Tank Reactor) and bovine-CVB (Continuous Vertical Biodigestor) were taken. DNA extraction was performed using FastDNA™ SPIN Kit**.** 16S rRNA genes (V4 region) was amplified, sequenced on the Illumina MiSeq platform, and subjected to taxonomic identification and phylogenetic and statistical analyses using Qiime 1.9.1 and Greengenes reference database with 97% cut off identity. DISCUSSION AND RESULTS: esults pointed out to different microbial communities between swine and bovine substrates. The data were normalized at 11.456 sequences per sample. representing 60 bacterial phyla, with Bacteroidetes (38.2%), Firmicutes (23.1%) and Proteobacteria (6.0%) being predominant. Bovine and swine microbiota were distinct according to pCoA and nearly 250 genus were statistically different between both environments. CONCLUSION: his analysis of the global microbiota diversity in AD systems can guide future studies to further examine the microbial diversity involved in AD and development of comprehensive analytical tools. **Keywords:** biodigesters, 16S RNA, metagenomic. **Supported by:** Capes, CNPQ