Metabolomic Analyses of Associated Endophytic Fungi from *Senna Spectabilis* Using NMR and Computational Dereplication Models

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Introduction

With advent of the modern analytical tool the seek for bioactive substances in complex matrices has allowed application of a systemic vision which one can compare the chemical information of metabolic profile or fingerprint. Thus, one can correlate biosynthetic pathway and its interactions in another cellular level such proteins, genes and so on. This emerging field is called metabolomics and it consists on the qualitative and quantitative analysis of the molecular composition of an organism in given time aiming comprehension of environmental, stress or gene modification. This project aims to develop NMR analytical methods associated to dereplication computational models, in order to evaluate the metabolic profile produced by endophytic fungi associated with *Senna spectabilis*, a plant known to produce chemotypes with acetylcholinesterase inhibitory properties.

Experimental

Crude extracts from endophytes isolated from the Brazilian plant species, *Senna spectabilis*, were analyzed by following the protocol established in Verpoorte [1], using a 10-100 mg/mL solution of extract and MeOD. The samples were vortexed at room temperature for 1 minute. The samples were sonicated for 10-20 minutes and then centrifuged for 5 minutes in a microtube centrifuge. Six hundred µL of the supernatant was transferred to a 5mm NMR tube (5mm x 15 mm, Norell Inc.) with 100 µL of TMS 0.03% as an internal standard. Sixty µL of this solution was transferred to a 1.5mm NMR tube (1.5mm x 15 mm, Norell Inc.).

The data were acquired using 600 MHz NMR spectrometer with conventional 5mm probe and NHMFL specialized 1.5mm HTS probe [2] Standard 1H, 13C, TOCSY, HSQC, HMBC, experiments were performed and were processed using NMRPipe [3] and exported to MATLAB for the construction of the metabolic profile.

Results and Discussion

Initially it was performed 1D proton and the 1D ^{13}C experiments (Fig 1) for 45 samples of endophyte fungus from *Senna spectabilis*. Data were analyzed by application of multivariate analyses (PLS and PCA) and it was observed the separation of different groups based on the metabolic profile of each sample. Since that the metabolite profile presented differences in its constitution it will be use for chemotaxonomy studies and will allow classify the endophytes species through to their metabolite production in the given substratum [4].

The next step was to choose a representative sample for each cluster group and it were performed all the 2D analysis on each one. The available metabolomics databases on the Internet will be used to do the dereplication studies in order to identify and classify the chemical compounds.

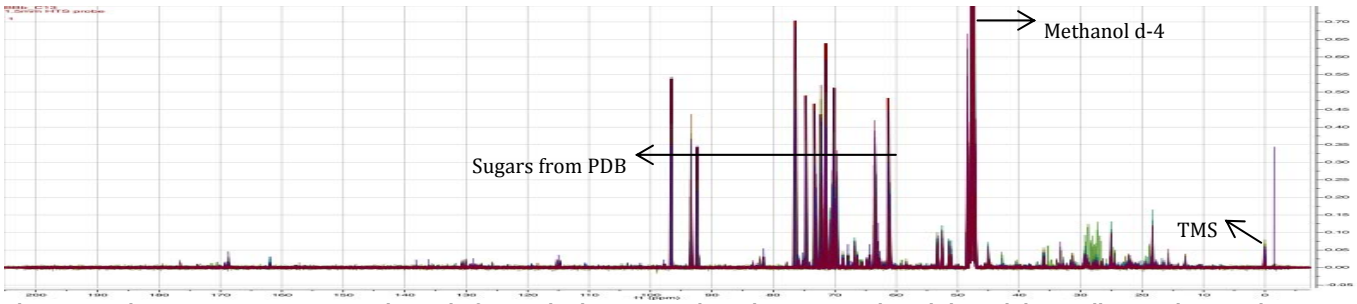

Figure 1 Carbon-13 NMR spectra overlapped of 45 endophytes samples. The TMS, methanol-d4 and the media PDB (potato dextrose broth) peaks are shown on the figure. All other peaks are from unknown compounds produced by the metabolism of the endophytes.

Conclusions

By the use of multivariate analyses based on Proton and C13 1D NMR, were possible to classify the endophytes according to their metabolite production and the rate of their metabolism. The next step is to identify what are the compounds that generate this separation and we hope to find new natural products substances.

Acknowledgements

This work was supported by the FAPESP (São Paulo Research Foundation) and NHMFL.

References

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