

THE APPLICATION OF ION TRAP MS IN METABOLOMICS; DETERMINING THE METABOLIC OUTCOMES OF GENE KNOCK-OUTS

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Targeted disruption of genes with an unknown function is a commonly applied strategy in functional genomics. However, the method is highly dependent on the ability to detect differences in the resulting phenotype. When the disrupted genes are involved in metabolism, the resulting phenotype is often highly similar to the wild type. Depending on the bioinformatics data, either an unbiased metabolic profiling or targeted methodology is then necessary to determine the role of the gene. We have applied both strategies to metabolically profile *Epichloë festucae* endophytes containing a targeted gene replacement of specific genes of interest of unknown function.

When no hypothetical function was known we applied a direct infusion ion trap mass spectrometry (DIMS) method for unbiased metabolic profiling. The principle of the method is based on the idea that the fragmentation of each ion in the mass spectrum delivers highly discriminative information about the chemistry of the metabolites present. To obtain this information crude solvent extracts were infused into the mass spectrometer. Each experiment was run for 6 minutes during which time the mass spectrometer collected a full mass spectrum of the extract together with spectra from a series of collision-induced dissociation reactions. Within a 6-minute run this process yielded around 250 MS² spectra, of which around 60 % yielded a MS³ spectrum, depending on the concentration of the metabolites. The sequence of one specific gene of interest showed homology to siderophore producing non-ribosomal peptide synthetase genes of other fungi. We therefore adapted a LCMS method for siderophores¹ for a linear ion trap MS.

Both unbiased and targeted methods have proved to be successful. The DIMS method was extremely sensitive in determining metabolomic differences between samples in a number of experiments. The collected fragmentation patterns facilitate rapid identification of the ions of interest to at least the chemical class level. On the basis of the multivariate statistics the differences could be pinpointed to the levels of specific ions, which could be identified by their MS² and MS³ spectra. The high density of chemical information obtained with this method makes it extremely useful in metabolomics studies. The targeted LCMS method was developed using the known siderophore ferrochrome. We were able to detect the presence and absence of two metabolites in respectively the wild type strain and gene knock out *E. festucae* strains. Based on the fragmentation data we could classify these compounds as hydroxamate siderophores, either bound or free of iron. Currently we are working on the complete structure elucidation of this siderophore.