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P1–MAKING SENSE OF THE METABOLOME

Organised by M.M. Burrell and N. Smirnov

P1.1 Chemical derivatization and mass spectral libraries in metabolic profiling by GC/MS and LC/MS/MS

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Gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/mass spectrometry (LC/MS) are two major hyphenated techniques employed in metabolomics and complement direct methods such as atmospheric pressure ionization (API) quadrupole time-of-flight MS, API Fourier transform MS and NMR. In GC/MS, the analytes are normally derivatized prior to analysis in order to reduce their polarity and facilitate chromatographic separation. The electron ionization mass spectra obtained are reproducible and suitable for library matching, mass spectral collections being readily available. In LC/MS, derivatization and library matching are at an early stage of development. Chemical derivatization can dramatically increase the sensitivity and specificity of LC/MS methods for less polar compounds and provided additional structural information. The potential of derivatization for metabolic profiling in LC/MS is demonstrated by the enhanced analysis of plant extracts. The important role of mass spectral library creation and usage in these techniques is discussed and illustrated by examples.

P1.2 Rapid analysis of complex biological systems using metabolic fingerprinting and machine learning of simple, robust rules

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In the early stages of functional genomics programmes we have a scenario where our knowledge is minute, that is to say we have no ideas about the role of an orphan ORF and there are few if any hypotheses to test. However, we can design experiments based, for example, on

gene knockouts and controlled over-expression and observe the effect on the phenotype of the organism. Alternatively we might expose an isogenic organism to different abiotic and biotic stresses to assess how it adapts to these new environments.

Post-genomic science is therefore producing bounteous data floods, and the extraction of the most meaningful parts of these data is key to the generation of useful new knowledge. A typical metabolic fingerprint or metabolomics experiment is expected to generate thousands of data points (samples *times* variables) of which only a handful might be needed to describe the problem adequately. Evolutionary algorithms (EAs) such as genetic algorithms (GAs) and genetic programming (GP) are ideal strategies for mining such data to generate useful relationships, rules and predictions. We have pioneered the use of these algorithms within the metabolomics setting and have shown that these are very powerful methods that allow one to ascertain which inputs are important in the mapping from input to output. This presentation describes these techniques and highlights their usefulness within metabolomics. In addition, genetic search methods are highly effective for optimising experimental conditions and an example of this for the effective analysis of intact proteins by ESI-MS will be given.

P1.3 Applications of NMR and MS profiling in plant metabolomics

M.H. Beale, J.L. Ward, J. Lewis, J. Baker. National Centre for Plant and Microbial Metabolomics, Rothamsted Research, Harpenden, Herts AL5 2JQ, UK

Techniques to carry out large-scale metabolite analysis of plants have great potential in post-genomic plant science, and are also being applied to the study of substantial equivalence in genetically modified plants. A variety of well-known spectroscopic techniques are being applied to plant metabolite analysis. Traditionally, for accurate quantitative analysis, the analytical chemist would aim to purify particular compounds, or classes of metabolite, prior to analysis by techniques such as nuclear magnetic resonance (NMR) and mass spectroscopy

(MS). This 'classical' targeted analytical approach will continue to be important in the study of plant biochemical pathways, but demand for high-throughput global snapshots, consisting of data on levels of a large number of metabolites from many pathways, has resulted in the development of methods to generate fingerprints that can be compared computationally to screen for 'differences' in plant lines/treatments.

The talk will demonstrate how NMR fingerprints can be obtained and utilised in high-throughput screens to highlight differences in plants and to indicate areas of plant biochemistry for further investigation. Examples, drawn from the GARNet Arabidopsis project and a programme on GM wheat will be used to illustrate the NMR technique and how further MS analysis can be targeted to confirm biochemical consequences of mutation and/or genetic manipulation.

P1.4 Metabolite profiling in plants using NMR

P. Krishnan, N.J. Kruger and R.G. Ratcliffe, Plant Sciences, University of Oxford, UK

Although less sensitive than mass spectrometry, nuclear magnetic resonance (NMR) spectroscopy provides a powerful and complementary alternative technique for the identification and quantification of metabolites, and for the measurement of intracellular fluxes between different components in the metabolic network. The lower sensitivity of NMR is compensated by the speed and convenience of the analysis, and by the extra information that is available on compound identification. Moreover *in vivo*, NMR makes a unique contribution to the provision of metabolic data by revealing features of metabolism that would be lost during extraction and by facilitating the measurement of time courses and metabolic perturbations. While NMR and mass spectrometry can be considered to be equally useful for metabolic flux analysis, NMR is less versatile than mass spectrometry in the field of metabolomics, being more suitable for the important but less inclusive objectives of metabolite profiling and metabolite fingerprinting. The profiling and fingerprinting applications of NMR in plant metabolism will be reviewed and the usefulness of such investigations for the definition of metabolic phenotypes will be discussed. In principle such phenotypes might be useful for identifying gene function, for example through the metabolic analysis of mutant and transgenic plants, or for assessing the metabolic impact of physiological perturbations. As well as drawing on the literature, the talk will include a description of a recent investigation in which ^1H NMR spectroscopy was used as a fingerprinting technique to analyse the effect of heat stress on rice (*Oryza sativa*) seedlings.

P1.5 The role of metabolic profiling in the refinement of plant metabolic engineering

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Many attempts have been made to understand and manipulate plant metabolic pathways by the use of reverse genetic approaches. The generation of transgenic potato plants with modifications in carbon metabolism and partitioning have had mixed success when assessed from a biotechnological standpoint. Recently we have used a combination of GC-MC based metabolic profiling techniques and contemporary statistical tools in order to allow the biochemical phenotyping of these transgenics. It was our hope that greater knowledge of both direct and pleiotropic effects of the introduced transgenes would lead to greater understanding of the interactions involved in plant metabolic networks. Along the same lines we have established a *Solanaceous* microarray that allows the assessment of around one thousand genes, primarily concerned with central metabolism. These tools allow us not only to characterise genetic diversity but also to replicate or *phenocopy* it by the application of diverse environmental conditions. Results from these strategies allow several important conclusions to be made about heterotrophic carbon metabolism but they also suggest that yet further improvements are required with respect to the analytical tools used before our understanding allows routine metabolic engineering. In parallel we have begun to use metabolic profiling on breeding populations of tomato as a means to the same end. Preliminary results from these experiments will also be presented.

P1.6 Enzymes, metabolites and fluxes

D.A. Fell, Oxford Brookes University, UK

An implicit assumption behind metabolomics is that measuring changes in metabolite concentrations in different physiological states, or for different genotypes, will yield information about the causes of the differences. In order to argue from the changes back to the causes, however, it is useful to understand the types of metabolic effect produced by simple, known alterations in metabolism. In the case of changes in the activity of a single enzyme, Metabolic Control Analysis offers a framework for understanding why, usefully for metabolomics, changes in metabolite concentrations are often much larger than changes in metabolic rate. The explanation relates to the metabolite concentration control coefficients and the flux control coefficients and their different summation theorems.

A useful tool for further analysis is the co-response coefficient, essentially the ratio of the control coefficients of an enzyme on two variables – either a concentration and a metabolic rate, or two metabolite concentrations. Two enzymes in the same ‘metabolic module’ have similar co-response coefficients on pairs of metabolites in distant modules, but are likely to have different co-response coefficients on metabolites in their own module. This has potential applications in narrowing down the metabolic site of action of a gene of unknown function or a novel inhibitor.

P1.7 Metabolic network analysis in plant systems: moving from measurement of metabolites to determination of fluxes using steady-state stable-isotope labelling techniques

N.J. Kruger, V. Mittova and R.G. Ratcliffe, University of Oxford, UK; A. Roscher, Université de Picardie, France

For genes encoding enzymes, metabolic flux is the ultimate expression of genotype and, in such instances, comprehensive analysis of gene function relies on accurate determination of metabolic activity *in vivo*. In micro-organisms, metabolic network analysis, based on a determination of the distribution of label after the system has reached isotopic steady-state, is a powerful technique for determining fluxes through individual sections of a metabolic pathway. In plants this analysis can be complicated by the widespread and variable subcellular duplication of many enzymes, and by the existence of alternative pathways resulting in the formation of the same product. This talk will discuss developments in the application of the technique in higher plants by focusing on our recent attempts to determine the relative contributions of the cytosol and plastids to flux through the primary pathways of carbohydrate oxidation in maize root tips and a cell suspension culture of *Arabidopsis*. Our approach involves labelling cells to isotopic steady state with [¹³C]glucose and determining the fractional abundance of the ¹³C-cumomers of various metabolites using ¹H and ¹³C NMR following extraction of the cells using perchloric acid. From such analyses it is possible to resolve: (i) flux through the glycolysis and the oxidative pentose phosphate pathway (oxPPP) in both the cytosol and plastids; (ii) recycling of hexose phosphates through the oxPPP in each compartment; (iii) reversible exchange of intermediates in the non-oxidative section of the oxPPP; and (iv) exchange of intermediates between the cytosol and plastids.

P1.8 MALDI Imaging to Reveal Metabolite Profiles in Potato Tubers

J. Bunch, Sheffield Hallam University, M.M. Burrell, University of Sheffield, M.R. Clench, Sheffield Hallam University, UK

Recently imaging using Matrix Assisted Laser Desorption Ionisation Mass Spectrometry has become a subject of interest. MALDI has been used for imaging peptides and proteins and drugs in animal and human tissue. The initial step in imaging MALDI-MS is the application of a thin, homogeneous layer of matrix to the sample. The chemistry of the sample can then be imaged by rastering a laser across the surface, acquiring spectra from each location. Images are produced by plotting the spatial distribution, x and y, versus ion intensity. We present here a back ground to imaging MS and methods for profiling plant metabolites by MALDI-TOF-MS. A cross section of potato tuber was blotted onto a membrane that was pre-coated in matrix material. This ‘imprint’ of the tuber section was analysed by MALDI-TOF-MS on an Applied Biosystem ‘‘Qstar pulsar-I’’ hybrid quadrupole time of flight mass spectrometer. Mass spectra were acquired at 0.2mm increments over the entire membrane area. Ion imaging software (MDS Sciex) was used to produce images depicting the spatial distribution of selected analyte ions.

Images showing the anatomical detail of the cross section have been produced by mapping an ion of m/z 852, which is thought to be Aceto-acetyl CoA, an intermediate involved in lipid biosynthesis and terpenoid metabolism. To date method development has been focused on metabolites of primary metabolism. In particular we have examined the distribution of glucose-6 phosphate. These preliminary investigations indicate that this novel approach may be used to obtain information on the spatial distribution of plant metabolites.

P1.9 Metabolic Footprinting: A High Information Strategy for Functional Genomics

W. Dunn, D.B.Kell, Irena Spasic, UMIST, Manchester; J. Allen, H. Davey, D. Broadhurst and J. Rowland, Aberystwyth; S.G. Oliver, University of Manchester, UK

The metabolome describes the complete range of low molecular weight compounds within a defined biological system. Intra-cellular metabolites are generally analysed, though after the time consuming and technically difficult task of rapid metabolic quenching and appropriate extraction of metabolites from cells. However microbes secrete a large number of metabolites into their external environments as a consequence of overflow metabolism and these secreted extra-cellular metabolites are rich in

biochemical information and reflect intra-cellular metabolic activity.

The metabolic footprint is defined as the information provided by the group of extra-cellular metabolites and avoids the need for metabolite extraction and metabolism quenching. The large dilution of metabolites secreted into a growth medium effectively minimises further metabolic activity. The growth medium can be analysed directly to qualitatively or quantitatively determine a wide range of metabolites whose concentrations can vary widely. The metabolic footprint can be used, for example, within the field of functional genomics for the yeast *Saccharomyces cerevisiae* to elucidate gene function of novel genes by analysis of yeast single gene deletants generated by the EUROFAN project.

This presentation will describe, by employing the yeast *Saccharomyces cerevisiae*, the technique of metabolic footprinting employing a range of analytical technologies (GC-MS, direct injection electrospray TOF and FTICR mass spectrometry). Subsequently the robustness of the technique will be demonstrated as well as its ability to discriminate between samples of differing biological status.

P1.10 Combining 'fingerprinting' and profiling to understand plant metabolic phenotypes

J. Draper, University of Wales Aberystwyth, UK

The development of a metabolomics technology platform for the future requires maximal capture of reproducible information on the metabolite content of plants and it is essential that data sets can be compared meaningfully. Metabolomic approaches involving 'profiling' (e.g. GC-MS and LC-MS) require unambiguous peak identification (and also quantification) in the absence of chemical standards for most metabolites and both profiling and 'fingerprinting' approaches (e.g. FT-IR, NMR) demand also reproducible data production over long periods of time, during which instrument 'drift' is a significant source of variance.

In two collaborative projects led by teams in Aberystwyth we are exploring the utility of a range of metabolite profiling and metabolite fingerprinting technologies to develop standardised methods that will provide an integrated, global overview of the metabolomes of both potato and *Arabidopsis* (see Beckmann, P1.21 and Johnson, P1.29 for details). A database schema named ArMet (An Architecture for Metabolomics) has been developed to store the high dimensional analytical chemistry data and associated meta-data produced in metabolomics experiments (see Smith, P.128). Within these projects we have undertaken research to understand and compensate for instrument-derived variance in metabo-

lite data tables. Significant effort has centred on the validation of appropriate data analysis methodology to cope with analytical system variance in GC-tof-MS profiling. An overview of progress in handling large metabolomics data sets will be presented with examples drawn from genotyping experiments involving both field grown potatoes and *Arabidopsis* grown under controlled conditions.

P1.11 Metabolite profiling of fungi and yeast: From phenotype to metabolome though MS and informatics

Jørn Smedsgaard and Jens Nielsen, Center for Industrial Biotechnology, BioCentrum-DTU, Technical University of Denmark

Filamentous fungi and yeast (**Ascomycetes** belonging to the kingdom **Mycota**) from the genera: *Saccharomyces*, *Penicillium*, *Aspergillus*, *Fusarium* are well known for their impact on our life as pathogens, though food spoilage by degradation or toxin contamination and also for their wide use in biotechnology for fermentation and production of e.g. beverages, chemicals, pharmaceuticals and enzymes.

The genomes of these eukaryotic microorganisms range from yeasts (*S. cerevisiae*) about 6000 genes to more than 10000 genes in filamentous fungi (*Aspergillus sp.*). Yeast and filamentous fungi are expected to share much of their primary metabolism; therefore much understanding of the central metabolism and regulation can be learned from comparative **metabolite profiling** and **metabolomics**. Filamentous fungi have a very active diverse secondary metabolism in which many of the additional genes present in fungi, compared to yeast, are likely to be involved. Although the "blueprint" of a given organism is represented by the genome, its behaviour is expressed as the **phenotype** thus growth characteristics, cell differentiation, respond to environment, the production of secondary metabolites and enzymes. Therefore the profile of (secondary) metabolites – fungal **chemodiversity** – is important for functional genomics and in search of new lead compounds that may serve as biotech products but is an equally efficient tool for taxonomy.

Metabolite profiling for functional analysis or lead discovery requires integration of high performance analytical methodology and efficient data handling techniques. One very efficient approach is direct infusion **Mass Spectrometry** (diMS) integrated with **automated data handling**, but a full metabolic picture requires more analytical techniques combined.

P1.12 Abstract not supplied

P1.13 Effects of varying nutrient regime on plant metabolic profiles

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The role of inorganic nitrogen assimilation in the production of amino acids is one of the most important biochemical processes in plants. One of the significant sources of inorganic nitrogen for plants is nitrate, which also act as a signal metabolite invoking rapid change in metabolism. For this reason, we performed a detailed broad range characterisation of metabolic response to different levels of nitrate and light conditions in tomato leaves (*Lycopersicon esculentum* Mill.). Tomato plants were grown hydroponically on liquid culture on three different nitrate conditions, saturated (8mM NO₃⁻), replete (4mM NO₃⁻) and deficient (0.4mM NO₃⁻) in two environment-controlled growth cabinets, in which the applied light regime was identical with the exception of the light intensity (which was set at 200 and 900 μE). The leaves were collected after 7, 14 and 21 days of nitrate replacement. Additionally, an experiment for the short response (1, 24, 48 and 94 hours after replacement) was performed at the high light intensity. The levels of amino acids, Krebs cycle intermediates, sugars, sugar alcohols, and several representative compounds of secondary metabolism were analysed using a gas chromatography-mass spectrometry with substantial changes in primary metabolites observed particularly under the high light growth. The obtained results will be discussed and compared with previously reported transcriptomics studies of varying nutrient regimes

P1.14 Metabolic fingerprinting: Two case studies in tomato and silage

H.E. Johnson, D. Broadhurst, G.W. Griffith, A.R. Smith; IBS, University of Wales Aberystwyth; R.J. Merry, M.K. Theodorou, IGER, Aberystwyth; R. Goodacre, D.B. Kell; UMIST, UK

The study of the metabolome is the measurement of the total biochemical composition (typically low molecular weight molecules) of a cell or tissue sample at any given time [1], with the aim of unravelling systems biochemistry and/or elucidating gene function. Metabolic fingerprinting, an area of metabolome research, involves the analysis of crude metabolite mixtures for discrimination, classification and hypothesis development. Here we describe how the approach of metabolic fingerprinting through the use of FT-IR spectroscopy and chemometrics was adopted to study i) the effect of salinity on

tomato fruit [2] and ii) silage fermentation dynamics [3]. FT-IR spectroscopy predominantly measures the vibrations of functional groups and highly polar bonds producing a biochemical fingerprint of the sample. The resultant spectra are complex making visual interpretation difficult; hence chemometric and machine learning methods are used to investigate these multivariate data. Methods used in these studies include principal components analysis (PCA), discriminant function analysis (DFA) and Evolutionary algorithms. Both these studies demonstrate an inductive experimental approach where the derivation of hypotheses is the output rather than the input, providing a direction for future research [4].

[1] Oliver, S.G. et al. (1998) Trends in Biotechnology 16, 373–378.

[2] Johnson, H.E. et al. (2003) Phytochemistry 62, 919–928.

[3] Johnson, H.E. et al. (2004) Applied and Environmental Microbiology In Press.

[4] Kell, D.B. (2002) Trends in Genetics 18, 555–559.

P1.15 Metabolomics, Proteomics, and Integrated Functional Genomics of *Medicago truncatula*

L. Sumner, The Noble Foundation

The magnitude in which biology is queried is rapidly evolving toward the large-scale quantitative and qualitative profiling of gene expression products at the mRNA, protein, and metabolite levels. These large-scale approaches are generally referred to as functional genomics, systems biology, or large-scale biology. The large volume of data generated from these experiments represent global snapshots of the dynamic biochemistry of life. When these snapshots are compared before and after genetic perturbations, differences can be used to decipher gene function or to evaluate the holistic response of biological systems. The Noble Foundation has initiated an integrated functional genomics approach to study the model legume *Medicago truncatula* that includes transcriptomics, proteomics, and the emerging science of metabolomics. In this presentation, the technologies used in metabolomics and proteomics will be summarized and specific examples of their use with *Medicago truncatula* will be presented. Special attention will be focused on data acquisition, dataset composition, methods of data processing and visualization, current bioinformatic needs, and secondary metabolism. Efforts to incorporate these technologies into an integrated functional genomics program will also be described.

P1.16 Using metabolic profiling to exploit natural variation for the nutritional enhancement of folates

L.C Garratt, C. Ortori, G.A Tucker, D.A Barrett, and M.J Bennett, University of Nottingham, UK

Specific and sensitive quantification of folates, including their polyglutamated derivatives, has been developed using high-performance liquid chromatography-tandem electrospray mass spectrometry (LC-MS/MS). Folates play a pivotal role in the methylation cycle and DNA synthesis by acting as cofactors for carbon one transfer. Dietary deficiencies of which have been associated with the prevalence of several chronic diseases, including vascular diseases, certain cancers, neurological dysfunction and birth defects. The complex range of mono and polyglutamated tetrahydrofolates, including their biosynthetic precursors, has been quantified in a range of plant species, including *Arabidopsis*, *Brassica* and wheat. Quantitative variation in constitutive tetrahydrofolate pools shown to exist between ecotypes is being exploited to identify key quantitative trait loci that regulate the biosynthesis of tetrahydrofolates and flux between their derivative forms. In combination with less specific more high-throughput methodology, the LC-MS/MS developed here is also being applied to identify novel *Arabidopsis* mutants affecting folate biosynthesis. Together, these approaches should expand our understanding of the flux through, and regulation of folate biosynthesis, allowing the manipulation of these essential plant micronutrients for the improvement of human health.

P1.17 The relationship between metabolomes, transcriptomes and phenotypes of glyoxylate cycle mutants

J.E. Cornah, V. Germain, University of Edinburgh; J. Ward, M. Beale, Rothamsted Research; and S.M. Smith, University of Edinburgh, UK

The glyoxylate cycle is essential for growth of microorganisms on 2-carbon substrates or fatty acids, and is regarded as necessary for gluconeogenesis from fatty acids in plants. *Arabidopsis* mutant seedlings lacking the glyoxylate cycle enzyme isocitrate lyase (ICL), are unable to convert lipid into sugars and they grow poorly (Eastmond et al., PNAS 97, 5669–5674, 2000). We now find that lipid utilisation and seedling growth are significantly more effective in mutants lacking malate synthase (MLS) than in those lacking ICL. The transcriptome of *mls* mutant seedlings differs little from that of wild type, while *icl* mutant seedlings show many differences suggesting that they are carbohydrate limited, including

the induction of transcripts associated with amino acid catabolism. Analysis of the metabolomes of each mutant using ¹H-NMR confirm that *icl* mutant seedlings are lacking sugars, and reveals differences in amino acid content. The metabolome of *mls* seedlings is not deficient in sugars but is characterised by elevated glycine and serine. Direct measurements of sugar contents show that *mls* seedlings can generate higher levels of sugars than do *icl* seedlings during the transition from heterotrophic to phototrophic growth. When fed with ¹⁴C-acetate, appreciably more ¹⁴C-labelling of sugars was detected in *mls* seedlings than in *icl* seedlings. These results show that in the absence of MLS, an alternative pathway for the conversion of 2-carbon compounds to sugars can operate in seedlings. The elevated levels of glycine and serine in *mls* seedlings are consistent with the operation of the photorespiratory pathway.

P1.18 Metabolic profiling of low ascorbate *Arabidopsis thaliana*

Nicholas Smirnoff, University of Exeter, UK; Oliver Fiehn, and Stephan Gatzek, Max Planck Institute for Molecular Plant Physiology, Golm, Germany

Ascorbate (vitamin C) is a ubiquitous constituent of plants and has roles as an antioxidant and as a cofactor of dioxygenase enzymes. In some species it is a precursor of oxalic and tartaric acids. In an attempt to understand more about the function of ascorbate we have investigated the consequences of ascorbate deficiency by metabolic profiling. We have produced low ascorbate *Arabidopsis thaliana* plants containing 30–50% of wild type ascorbate levels by antisense suppression of L-galactose dehydrogenase (GalDH). This is the second last enzyme in the mannose/L-galactose ascorbate biosynthesis pathway and it is likely that the only direct effect of reducing GalDH activity will be on this pathway. Plants were grown at high light intensity or at low temperature: conditions that increase photo-oxidative stress and induce ascorbate accumulation in leaves. Ascorbate accumulated to a lesser extent in antisense GalDH plants than in wild type plants. Leaf extracts from these plants have been analysed by GC-MS and the results will be discussed in relation to the proposed functions of ascorbate.

P1.19 Genetic modification of SnRK1 and impact on metabolism in *Arabidopsis*

D. Jhurreea, Y. Zhang, J. Andralojc, Rothamsted Research; M. Bennett, University of Nottingham; N. Halford, M. Paul, Rothamsted Research, UK

SnRK1 (sucrose non-fermenting-1-related protein kinase-1) has a central role in the regulation of carbo-

hydrate metabolism in plants. A strategy has been planned to define further details of this role in metabolism and the genes affected downstream of SnRK1 in the model plant *Arabidopsis thaliana*. *Arabidopsis* has two active SnRK1 genes, AKIN10 and AKIN11. Conventional antisense has been used to down-regulate both genes together. Lines have been generated with reduced SnRK1 activity. As the two genes have different expression profiles in planta, a strategy has also been undertaken to down-regulate the two genes separately using RNAi and to use recently available SnRK1 mutants. Sugar feeding experiments are being performed on the plant material and latest data will be presented.

P1.20 Metabolic profiling of isoprenoids in GM and non-GM tomatoes

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Isoprenoids comprise one of the largest groups of secondary metabolites in plants and include the carotenoids, sterols, tocopherols and side chains of chlorophylls and quinones. We have generated a broad range of transgenic tomato lines that have been transformed with isoprenoid genes (especially those for carotenoid formation), under the control of constitutive and fruit-specific promoters. In order to analyse the changes in isoprenoids in the GM fruit, and to evaluate cross talk between isoprenoid pathways and unintended effects of the transformations, we have developed robust and reproducible extraction protocols and chromatographic techniques to identify and quantify these metabolites. Rapid reverse-phase hplc, using C₃₀ columns with a diode array detector has been used to separate and identify carotenoids, tocopherols and chlorophylls in a single chromatographic run. Sterols have been analysed separately by gas chromatography.

Results will be presented to demonstrate the utility of the profiling to a range of GM and non-GM tomato lines and to elucidate the effects of transgene expression on the various branches of the isoprenoid pathway. The effects on isoprenoid profiles of using different promoters with the same transgene will also be discussed.

P1.21 Metabolomics as a technology platform to contribute to the assessment of compositional differences between GM and conventionally bred crops

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Substantial Equivalence embodies the idea that a conventional food may be used as a basis of comparison for novel foods as part of a safety assessment. This concept has sparked much political and scientific controversy centring on lack of a definitive meaning. We aim to explore metabolomics as tool to describe compositional differences between field-grown transgenic potatoes modified in fructan metabolism and conventional potato cultivars.

Within the FSA G02 programme a comprehensive analysis of global metabolite profiles has been undertaken based on GC-MS and direct injection ESI-MS coupled with computational analysis. This should allow non-biased detection of both intended and unintended effects of a deliberate genetic change in an organism at the level of metabolism. An experimental strategy, including experiment design, data capture and statistical data analysis is described, which aims to provide a validated technology platform for determination of compositional differences between plants.

Statistical experiment design to capture systematic variability was important to enable metabolome variability to be measured and in these large experiments cumulative variance due to instrument drift has a major influence. Analyses based on either GC-MS profiling or ESI-MS fingerprinting was able to distinguish transgenic potatoes from conventional cultivars. Particularly using supervised data analysis techniques individual potato cultivars could be recognised by a range of clustering and classification methods. A small number of metabolites strongly associated with the GM lines were identified, all of which were predicted to be precursors or breakdown products of fructans thus representing expected changes in metabolome associated with transgene expression.

P1.22 Application of metabolite profiling to the identification of traits in a population of tomato introgression lines

S.A. Overy, H.J. Walker, W.B. Dunn and W.P. Quick, Animal and Plant Sciences, University of Sheffield; H. Major and S. Preece, Waters, UK

Metabolite profiling is a potentially informative technique for screening a large number of plants for differences in metabolism. When compared to analyses that generally focus on one chemical group, the relatively non-selective approach allows simultaneous analysis of chemically diverse metabolites and may reveal unexpected differences. We are using LC-MS-based techniques to profile metabolites in two tomato species – *Lycopersicon esculentum* and *L. pennellii*, and subsequently a population of *L. esculentum* plants containing defined chromosomal introgressions from *L. pennellii**. Two analytical techniques have been employed. One method involves infusion of the extracts into an ESI-TOF-MS, without chromatography. Each sample is run in triplicate, in both ES+ and ES–, and the resulting peak tables (mass vs. intensity) combined to select only those masses present in all three replicates, thereby removing background noise. These peaks then form the metabolite profile for that sample.

The second method employs chromatographic separation prior to detection using ESI-TOF-MS. Each sample is analysed in both ES+ and ES–. The resulting data is then processed using Waters MarkerLynx software, which incorporates a deconvolution package that allows detection and alignment of peaks across the samples. The data is then combined into a single matrix of mass and retention time pairs with associated normalised intensities for each component detected across all the samples. This table can either be analysed by PCA within MarkerLynx or exported to third party multivariate data analysis software packages.

Data resulting from the two methods will be discussed. *Eshed & Zamir (1995) Genetics 141, 1147-1162

P1.23 Using Metabolomics and Feeding Experiments with Putative Substrates to “Proofread” the Proposed Taxol Biosynthetic Pathway

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Trees in the genus *Taxus*, or yews, produce over 350 diterpenoids presumably derived from the olefin, taxadiene. The important chemotherapeutic drug, Taxol, and its precursors represent only 5% of these taxoids. The challenge of elucidating the Taxol biosynthetic path-

way, and isolation of the relevant genes in the pathway, requires a thorough understanding of all of the taxoids produced by the experimental system used for this elucidation – in this case cell suspension cultures of *Taxus*. After formation of the first committed step in Taxol biosynthesis, cyclization of geranylgeranyl diphosphate to taxadiene by taxadiene synthase, the next step in the pathway is believed to be oxidation of the olefin by a cytochrome P450 monooxygenase to form 5 α -hydroxy-taxadiene. Feeding studies with this radiolabeled taxoid to suspension-cultured cells have failed to show convincing incorporation of radioactivity into Taxol or related highly functionalized taxoids. Feeding with additional radiolabeled taxoids, in addition to unlabeled taxoids as “cold traps,” have implicated either a different order of oxygenation or an alternate pathway in Taxol biosynthesis. Discussion of the results of these cell-feeding experiments will include speculation on the order of oxygenation as well as the difficulty associated with dissecting a complex biosynthetic pathway from an even more complex network of related pathways.

P1.24 Metabolite analysis of Arabidopsis mutants altered in the tetrapyrrole biosynthesis pathway

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The tetrapyrrole biosynthetic pathway leads to the formation of 3 important prosthetic groups (chlorophyll, heme, siroheme) and of the chromophore of phytochrome. The regulation of this pathway has to be tightly controlled, each end-compound being required in different locations (chloroplast, mitochondria, peroxisome and nucleus) at different concentrations (the chlorophyll content might be 100 times more than the heme content). In addition, the phototoxic intermediates must not accumulate in the cell. Several mutants altered in tetrapyrrole biosynthesis have been characterized and some of them have *gun* (genome uncoupled) phenotype, in which plastid-nuclear signaling is disrupted. This indicates the important role of tetrapyrroles in the communication between the chloroplast and the nucleus. Recent work shows that the Mg-protoporphyrin may be involved (3). On another hand, the analysis of the mutants have revealed 2 kind of regulation, the first involve the heme branch and the second the chlorophyll branch synthesis (1, 2). Each of them inhibits the formation of the initial precursor, aminolevulinic acid. With the aim of determining the part of each branch in the regulation of tetrapyrrole biosynthesis, we are establishing a method for metabolite analysis of *Arabidopsis* mutants altered in the tetrapyrroles biosynthesis pathway

by LC/MS analysis. This will be coordinated with a comprehensive analysis of the transcriptome of these mutants by microarrays.

1. Cornah JE, Terry MJ, Smith AG. 2003. *Trends Plant Sci.* 8: 224–30
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P1.25 Metabolomics of Host-Pathogen Interactions in *Brachypodium distachyon*

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Rice blast caused by the filamentous fungus *Magnaporthe grisea* can inflict disease in many species of the grass (*poacea*) family. It is the most damaging disease of rice crops, annually causing the loss of between 11% and 30% of worldwide rice production. Rice blast is of enormous economic importance and biological interest as the pathogen is genetically tractable and grows *in vitro* on defined media. The plant model used in the project is *Brachypodium distachyon*. This plant has recently been described as a good developmental model for host-pathogen interactions in grasses and is a compatible host for rice blast.

There is now the opportunity to try and determine what changes in global metabolite profile are associated with the successful colonization of plant tissue by the rice blast fungus. Whether of fungal or plant origin the identification of such chemicals may provide novel leads for changes in metabolism required either in the fungus for compatible growth *in planta* or alternatively, may reveal changes directly induced by the fungus in the plant metabolome associated with/required for colonisation. Metabolomics thus may provide tools for looking for novel virulence attributes (potential fungicide targets). Similarly, it will be possible to treat infected plants with known fungicides to determine whether chemicals with similar expected modes of action (MOA) will trigger metabolomic changes that cluster significantly, and determine whether any changes in particular metabolites can be determined and explained in this complex biological system. This may provide an important new tool for MOA studies in the future.

P1.26 Application of metabolite profiling to studies of biodiversity in *Solanum* species

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Globally, potato is an important foodstuff and source of nutrition. Traditionally potatoes have been developed for agronomic traits such as yield and disease resistance. To meet changes in consumer demands, considerable effort is being put into improving additional characteristics such as nutritional value and organoleptic properties.

At the Scottish Crop Research Institute we have access to a wide range of potato germplasm which is being used to explore phenotypic and phytochemical diversity and to relate specific characteristics to metabolite distribution. Several genotypes of the potato species *Solanum phureja*, with favourable organoleptic properties, have been developed and are the subject of studies to relate tuber metabolites to volatile flavour compounds. Comparisons have been made between *S. phureja* and established varieties of the more familiar *S. tuberosum*. The latter are assessed as having a blander flavour by specialist taste panels.

Volatile compounds generated during cooking of tubers of both species by boiling, were entrained on porous polymers and analysed by automated thermal desorption coupled to gas chromatography – mass spectrometry (ATD GC-MS). Tuber metabolites from both raw and cooked tubers were analysed by time of flight (TOF) GC-MS. Data analysis shows that the species can be clearly differentiated on the basis of their volatile profiles. We are currently investigating the extent to which tuber metabolites can similarly differentiate between species and may also be related to desired organoleptic properties.

P1.27 Abstract not supplied

P1.28 Towards a standard representation for metabolomics experiments and their results — ArMet

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We aim to produce a standard representation of the data associated with metabolomics research. Such a represen-

tation can be used to develop data-handling systems to support a range of activities including: statistical analysis and data-mining of the results in the context of an experiment; reanalysis of data in new contexts; design of new experiments. It should also promote the adoption of Standard Operating Procedures, encouraging international integration between laboratories.

An extensive requirements analysis exercise has been undertaken in the context of a UK Food Standards Agency project investigating a plant metabolome technology platform. This exercise resulted in a detailed description of experiments in plant metabolomics, particularly for profiling controlled environment and field-grown material using GC-MS analysis. This was used to create a data model using the Unified Modeling Language (UML). Work in other areas was taken into account to facilitate extension to microbiology and a range of other analytical techniques e.g. NMR, FT-IR. The data model encompasses the entire time line of a metabolomics experiment. We have identified the data associated with the procedures involved so that the sources of variability are captured. We have developed a prototype implementation of our UML model, using Oracle as the underlying database management system with a Microsoft Access interface. The model can be implemented in other database systems

P1.29 Hierarchical plant metabolomics for gene function and mode of action discovery

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HiMet, the acronym for ‘‘Hierarchical plant Metabolomics for gene function and mode of action discovery’’ is a large collaborative project funded by the BBSRC as part of the Exploiting Genomics initiative, which aims to perform gene function analysis on putative *Arabidopsis* metabolic mutants through high-throughput analysis of plant metabolites and the development of machine learning methodologies.

Here we provide an introduction to HiMet, describing the key elements of this metabolomics project from plant cultivation and harvesting, through to sample analysis using a range of technologies including LC-MS, GC-MS, NMR, FT-IR and ES-MS, and from data acquisition to data analysis and interpretation using multivariate statistical methods and machine learning. The wealth of metabolomic data generated via high-throughput studies

all needs to be described and stored. To meet this demand a database named ArMet (An Architecture for Metabolomics) was developed under a Food Standards Agency contract (G02006) and is being extended and applied in this project. ArMet provides a standard for describing metabolomics experiments by way of a series of related components which cover the experimental timeline from specification of biological source material, through cultivation, preparation and storage to chemical analysis and its output.

A project of this complexity requires a wide range of expertise spanning the disciplines of biological sciences and computer sciences; hence in HiMet there is close collaboration between researchers from five institutes all of whom provide the necessary skills.

P1.30 Tissue preparation and storage affects metabolic fingerprints of *Arabidopsis thaliana*?

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Metabolic fingerprinting involves the analysis of a crude metabolite mixture for biological discrimination and classification, providing a method by which to study relationships between samples. Fourier-Transform Infra-Red (FT-IR) spectroscopy is an ideal technology for fingerprinting as the spectra produced, from the measurement of the vibrations of functional groups and highly polar bonds, are biochemical fingerprints of the samples. The resultant spectra are complex making visual interpretation difficult; hence chemometric and machine learning methods are used to investigate these multivariate data.

Here we describe how the approach of metabolic fingerprinting through the use of FT-IR spectroscopy and chemometrics was adopted to study the effect of different sample preparation methods, storage times and storage conditions on the metabolic fingerprint of wild type Col-O *Arabidopsis thaliana* and the ethylene signal transduction mutants, *etr1-1* and *ctr1-1*.

Every step of the experimental procedure from design, sample preparation and handling through to chemical analysis and data acquisition introduces variability, hence introducing error in the output data and resultant models. In order to limit this variability, so minimising sources of error, all experimental protocols must be robust, reproducible and representative. The effect of sample preparation and storage on the quality of discrimination between the above genotypes will be assessed and the implications that these changes in

experimental protocol have on the interpretation of large biological data sets will be discussed.

P1.31 Metabolic fingerprinting and profiling discriminates between resistant and susceptible interactions involving the rice blast pathogen *Magnaporthe grisea* and *Brachypodium distachyon*

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Currently, functional genomic approaches are focused at the levels of transcriptome and proteome. However, the accurate discrimination of the metabolome, although technologically demanding, has a broader range of application and is more information rich. Two metabolomic approaches were employed to elucidate and identify the biochemical changes occurring within the pathogen and host during the interaction of *Magnaporthe grisea* – (rice blast) – and the emerging Pooid model species *Brachypodium distachyon* ecotypes ABR1 (susceptible) and ABR5 (resistant).

Fourier-transform Infrared spectroscopy (FT-IR) was employed for high-through put metabolomic fingerprinting (generation of metabolome signatures with no regard for individual metabolites). Discriminant function analysis (DFA), allowed the discrimination between developing disease symptoms and the hypersensitive response (HR). The data was validated with DFA again showing tight clustering indicating a high degree of agreement between data and indicating that the experimental approach used produced highly reproducible data.

Metabolomic profiling allows the detection, quantification, and identification of pre-defined target compounds within the metabolome, for which we employed electrospray ionisation mass spectrometry (ESI:MS). Subtraction spectra revealed the ions that separated healthy from infected material, those also present in the DFA loadings, were attributed as substances involved in the fungal disease reprogramming or the plants HR. These ions were identified, and are presently being confirmed via Tandem-MS. A ten replicate data validation showed tight clustering in DFA indicating a highly reproducible system for metabolomics. Our data will undoubtedly reveal novel insights, particularly into in planta fungal development and illustrate the potential of a metabolomic approach to investigate plant-pathogen interactions.

P1.32 Use of Gas Chromatography-(Time-Of-Flight) Mass Spectrometry for Analysis of Metabolites from Solanum Species

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Metabolite profiling of plant species using high throughput GC-MS techniques, linked to automated data processing, is becoming a highly useful tool for the study of metabolite distribution within plants, and can address a range of issues. We are using this approach to study substantial equivalence and unintended effects of genetic modification in *Solanum* species. In addition, metabolite variation within *Solanum* germplasm collections is being measured with the objective of exploring phytochemical diversity and relating metabolite distribution to phenotypic characteristics.

We have developed a metabolite profiling technique based on the use of GC-Time-Of-Flight (TOF) MS for data acquisition, followed by data analysis using a combination of the AMDIS and Xcalibur software programmes. Subsequent data reduction is accomplished using a variety of multivariate analysis techniques. Separate polar and non-polar extracts are prepared from freeze-dried or fresh potato tubers in the presence of internal standards, and are analysed alongside retention index markers following appropriate derivatization. During method development particular attention was given to optimization of the derivatization conditions. Analysis times are relatively short and PTV injection is used to minimise sample degradation. Ions characteristic of each metabolite are selected using AMDIS and are then used as the basis for automatic data analysis using Xcalibur. Metabolite detection and quantitation relative to the internal standard are based on the presence and relative abundance of the selected ions within defined time windows adjacent to the retention index markers.

P1.33 Investigating and exploiting the metabolomic richness of plant germplasm

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The majority of the world's medicinal compounds have derived from compounds obtained from plants. In the UK we have a tremendous store of traditional knowledge of the use of plants, and projects such as *Flora Celtica* at the RBGE have documented this for future generations. However such traditional uses probably only sample a small fraction of the potential medicinal

benefit from the richness in metabolite constitution of native plants. We are exploring the prospects for discovering new uses for plant germplasm, from both native and non-native *ex situ* material. For UK germplasm, a focus is being made on bryophytes, a group which is under-explored in scientific and medicinal terms and for which the UK possesses a broad range of diversity. We are actively applying state-of-the-art LC-MS (and to a lesser degree) GC-MS profiling technologies to analyse and explore a broad range of UK bryophytes, higher plants identified by Flora Celtica, and tuber-bearing *Solanum* species. This data is being used to identify key interesting compounds for beneficial biological activity against targets such as Alzheimer's, tumour growth, inflammation and analgesia, amongst others. Preliminary data suggests that bryophytes possess an unexpected richness at both the biochemical and screening levels, and examples of progress towards understanding the diversity in bryophytes will be presented.

P1.34 Plant-pathogen interactions; Implications for the plant metabolome

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During the interaction between plant endoparasitic nematodes and their hosts, changes in both localized and systemic gene expression occur leading to either compatible or incompatible interactions. Little is known about the changes to cellular metabolism during host-pathogen interactions, how these profiles are affected by the interaction and how these relate to changes in gene expression. Comparisons of metabolite profiles from uninfected and infected tomato and potato roots from compatible and incompatible interactions with plant parasitic nematodes are being made to determine if these profiles can be used to differentiate compatible and incompatible interactions and to characterize the molecular processes underlying these interactions.

An extraction protocol is being established to perform metabolite profiling on roots from tomato and potato plants from soil grown and pouch grown roots. Metabolite profiles from tomato (*Lycopersium esculentum*) roots from a susceptible (Money maker) and resistant (Rossol Mi) cultivars to the root knot nematodes (*Meloidogyne incognita*, *M. javanica* and *M. arenaria*) are being made and from potato genotypes which differ in susceptibility to the potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*).

P1.35 A Model of Carbohydrate Metabolism in Tubers of *Solanum tuberosum*

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A crucial feature of the post-harvest physiology of tubers of the potato plant (*S. tuberosum*) is a process called cold-induced sweetening, *i.e.* the accumulation of the sugars fructose, glucose and sucrose during tuber storage at low temperatures. It results from partial starch breakdown. High levels of these sugars cause discoloration, changes in flavour and loss of essential amino acids in processed products made from stored tubers. Thus, it is very desirable to reduce cold-sweetening, *e.g.* by rationally manipulating the carbon fluxes in potato tubers.

Due to the flexibility and complexity inherent in plant primary metabolism, genetic manipulation of the potato plant has so far failed to produce plants which exhibit decreased cold-sweetening in their tubers while maintaining the same tuber yield and starch content. Instead of a gene-by-gene manipulation strategy, a mathematical model can be employed to investigate the underlying mechanisms causing and regulating cold-sweetening.

A kinetic model of the carbohydrate metabolism of potato tubers was built specifically to search for enzymes crucial for cold-sweetening, and to guide further transgenic experiments. The model includes all reactions believed to be directly involved in the conversion of sucrose through the hexose phosphate pool to starch, as well as the glycolytic pathway. The unique compartmentation of sink tissue cells, with their special organelles for starch synthesis and storage, is accounted for in the model by considering the volumes of cytosolic and amyloplastidic fractions and the distribution of metabolites between these two compartments.

Steady state evaluation of this kinetic model exhibited the occurrence of starch turnover, futile cycling through sucrose, and net breakdown of starch with channelling of its products into glycolysis. For validation of the model, the calculated fluxes were compared with experimental data obtained from the literature. Undertaking control analysis of this model can identify the reactions that exert the highest control over the concentration of sucrose within the potato tuber.

Here the kinetic model of the carbohydrate metabolism of potato tubers is presented and results of its control analysis are discussed, in particular the concentration control coefficients of the reactions included in the model towards the concentrations of fructose, glucose and sucrose.

P1.36 The National Plant and Microbial Metabolomics Centre

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The National Plant and Microbial Metabolomics Centre, recently funded by BBSRC, aims to build and operate a high-throughput primary and secondary metabolite fingerprinting service for the UK plant and microbial community. The role of the Centre will be to provide both a service, based on ongoing research, and a training facility. It is based at Rothamsted Research where a purpose-built analytical chemistry facility will operate an integrated process of metabolite fingerprinting and high resolution structural annotation, which will serve the needs of both large scale screens and targeted analysis. The core of the facility will be a 600MHz NMR spectrometer configured to collect 'fingerprint' data from crude extracts and to provide structural data on purified samples. Further targeted analysis by MS techniques will then be carried out on selected samples with signals of potential interest. Centre facilities in bioinformatics are located at Aberystwyth University and will provide the expertise for the development of data models and construction and refinement of a metabolomics database. Together with the UMIST team, advanced data analysis methods will be applied to further mine metabolomic data, including that from the FT-MS research instrument at UMIST.

Staff at the Centre will carry out both large and small scale metabolomic analyses of any plant and microbial material. Multi-level access to the facility will allow users to submit, plant material, extracts, or data for analysis and/or visit the Centre for training. The Centre will also seek to develop metabolomic projects with groups having interests in systems biology, metabolic engineering and bioinformatics.

P1.37 Profiling and Characterisation of the flavonoids involved in the interactions between *Lotus japonicus* and its symbionts

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Legume roots can interact with both arbuscular mycorrhizal (AM) fungi and rhizobium in soil. These inter-

actions lead to two different symbiotic relationships, which are beneficial for both partners. The main benefit is the supply of minerals, atmospheric nitrogen and phosphate, to the plant by its symbiotic partners in exchange of carbohydrates.

These symbiotic processes involve a communication between the symbiont and its host plant and a wide range of secondary metabolites seem to be involved as signal molecules. In nitrogen-fixing symbiosis, flavonoids, the major secondary metabolites in legumes, are involved at two different levels. First, some of the root exuded flavonoids (luteolin, liquiritigenin) induce the synthesis of the Nod factor by the *Rhizobium*. Second, others such as kaempferol mediate the Nod factor signal leading to a local and transient auxin transport inhibition within the root cortical cells necessary for the induction of cell divisions to form nodule primordia.

In the model legume *Lotus japonicus*, a species that predominantly accumulates condensed tannins rather than isoflavonoids in its roots, the involvement of flavonoid in symbiosis is still unknown. Therefore, we undertook a series of flavonoid profiling experiments using an HPLC-PDA to assess their implication during the early stages of the rhizobium colonisation. The flavonoid profiles obtained did not allow the observation of new compounds. However, some existing flavonoids were either accumulated or decreased by the symbiont presence indicating the specific symbiont recognition by the plant and the involvement of the flavonoids in the signalling pathways leading to the nodule formation.

P1.38 Alterations in the soluble proteome induced by acclimation of *Synechocystis* sp. PCC 6803 to photoheterotrophic growth

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The unicellular cyanobacterium *Synechocystis* is widely used as a model organism especially in studies of oxygenic photosynthesis. This strain is capable of both autotrophic and heterotrophic growth. In spite of the use of *Synechocystis* in photosynthesis research, its heterotrophic metabolism is poorly characterized. Our aim is to understand metabolic alterations that take place when *Synechocystis* cells get acclimatized to photoheterotrophy. Here we present results of a study of qualitative and quantitative changes in soluble cell proteome, when the mode of growth changes from autotrophy to photoheterotrophy. Soluble periplasmic proteins were extracted by cold osmotic shock and proteins from the cytoplasm were extracted by breaking the cells by using glass beads in Hepes (pH 7.2) buffer. The proteins were separated by isoelectric focusing followed by SDS-PAGE in the second dimension. Protein spots were iden-

tified by MALDI-TOF and LC-MS analysis. Our results led to identification of several proteins that are essential for either the autotrophic or the photoheterotrophic growth mode. In addition, we have identified tens of proteins including novel proteins not assigned on previous published gels. Combined data covering cell ultrastructure, enzymatic activities and identified proteins contribute to our understanding of the photoheterotrophic metabolism of *Synechocystis*.

P1.39 Metabolic profiling of natural variation and wide crosses of tomato

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For many years conventional breeding of tomato cultivars led to highly cultivated, inbred lines with low genetic variance. This generated tomato plants more susceptible to pathogen, drought and osmotic stress. To improve the nutritional value of cultivars and overcome these problems breeders have made use of natural variation in the species *Lycopersicon*. Recently, a metabolic profiling method based on GC-MS was established in our lab. This method allows biochemical phenotyping of plant samples on the metabolite level. In order to show the power of wide crosses for the breeding of tomato plants with agricultural and ecological important traits this technique was applied in this experiment. For this purpose five wide species of *Lycopersicon* (*L. pennellii*, *L. pimpinellifolium*, *L. parviflorum*, *L. hirsutum* and *L. chmielewskii*) have been compared with respect to *L. esculentum* cv. M82. Plants were grown in pots under standard conditions. Fully expanded leaf material was collected from six week-old-plants and fruits were collected 45 days after flowering. Results from the metabolic profiling shows substantial changes in primary and secondary metabolites. First studies of breeding material obtained from crosses of these species revealed that the progeny exhibited changes of similar magnitude. This data therefore allows qualitative and quantitative assessment of breeding natural variation into tomato cultivars.

The obtained dataset from this experiment will be presented.

P1.40 Gas chromatography/mass spectrometry and fast gas chromatography/time-of-flight mass spectrometry in metabolomics: investigating unintended effects in genetically modified tomato

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Metabolic profiling poses unique analytical challenges in terms of the inherent chemical diversity that varies with time and environmental conditions, sample complexity, wide dynamic range of analyte concentration and large number of unidentified compounds present within a single sample. Rigid protocols for growing and harvesting tomatoes together with sample preparation techniques to maintain the integrity of the metabolites have been established. Advances made in developing a gas chromatography/mass spectrometry (GC/MS) protocol, designed to minimize analytical variation, together with the initial results from profiling GM and non-GM tomato fruit are presented. The genetic manipulations involved were developed in-house, relate primarily to carotenoid biosynthesis and have been well characterized using HPLC analysis (Roemer et al., 2002). However, potential unintended effects of genetic manipulations are less well understood. GC/MS and the faster GC/time-of-flight (TOF) MS are used for metabolic profiling of the low molecular weight metabolites to illustrate changes in known biosynthetic pathways in GM tomatoes. The technique is able to 'see' small differences between samples above-and-beyond inherent analytical and biological variations, and data are seen to cluster relative to tomato genotype. The work is intended to aid the health assessment of GM foods.

Roemer et al. (2000) Nature Biotechnol. 18: 666–669