REVIEW DOI: 10.2478/V10133-010-0001-Y E&BS

METABOLOMICS – THE STATE OF ART

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ABSTRACT

Metabolomics is an emerging field of "omics" research that focuses on high-throughput characterization of small molecule metabolites in biological matrices. This review focuses on the recent trends and potential applications of metabolomics. We begin with an overview of metabolomic studies with amphasise on metabolomics workflow. The approaches of metabolomics are applied in the biological systems, including human, plants, and microorganisms. Thus the critical evaluation of the contribution that metabolomics has made to the environmental, plant and animal sciences, microbiology and food technology and science, was established.

Keywords: metabolomics, metabolome, metabolite profiling, metabolite fingerprinting

Biotechnol. & Biotechnol. Eq. 2010, **24**(1), 1537-1543

Introduction

In a turn of the century, environmental, food and energy problems are becoming more inevitable. Now is the time to develop an environmentally friendly and sustainable social system. Biotechnology plays a key role in this challenge. "Metabolomics", which is one of the newest 'Omics', based on exhaustive profiling of total metabolites in organisms, is expected to be a powerful tool for functional genomics. Metabolomics would be also applicable for 'medical', 'diagnostic', 'food', and/or 'industrial microbiology'. The de facto standard methodology of metabolomics has not been established due to several technical problems. Metabolomics is a complicated transdisciplinary science comprising of 'organic chemistry', 'analytical chemistry', 'chemometrics', 'informatics', 'bioscience'. It enables sample classification and discrimination of diverse biological status, origin, or quality in samples, through the use of statistical multivariate pattern recognition methods (chemometrics) in which variation is observed principally on the total chromatographic changes of metabolites (15). The words '*Metabolomics*' and ‚*Metabonomics*' are often used interchangably, though a consensus is beginning to develop as to the specific meaning of each. The goals of Metabolomics are to catalog and quantify the myriad small molecules found in biological fluids under different conditions. Metabonomics is the study of how the metabolic profile of a complex biological system changes in response to stresses like disease, toxic exposure, or dietary change.

Functional analyses (**Fig. 1a**) have thus emphasised analyses at the level of gene expression (transcriptomics), protein translation (proteomics; including post-translational modifications) and more recently the metabolite network (metabolomics), with a view within a systems biology approach of defining the phenotype and finally bridging the genotype-to-phenotype gap, (12). Even the representation in **Fig. 1a** is simplistic, since whilst in our linear conception of the cell the general flow of information goes from gene to transcript, to protein, to metabolite, to phenotype, there are multiple feedback loops from metabolites to proteins and/or transcripts, as well as others. Indeed our traditional view of metabolism (**Fig. 1b**) is no longer true and the cellular processes are in reality networked and should be represented as dynamic protein complexes interacting with neighbourhoods of metabolites (**Fig. 1b**). The construction and visualisation of the metabolic network (1), is certainly a big challenge for the future, as it concerns a full understanding of the fluxes through them and their control, (11).

There are many approaches for metabolomics (**Fig. 2**). They can be roughly classified according to a data quality and a number of metabolites that could be detected. First is the "**metabolite targeted analysis**" that refers to the detection and precise quantification of a single or small set of target compounds. Second is the "**metabolic profiling or metabolite profiling**", which provides the identification and approximate quantification of a group of metabolites associated to specific pathways. Discrimination is observed and the approach produces independent information that can be interpreted in terms of known biochemical pathways and physiological interactions (22). Third is "**metabolite fingerprinting**". It is used for complete metabolome comparison without knowledge of compound identification. Usually, a spectral analysis is used to fingerprint analysis. Metabolite variation is observed principally on the total chromatographic pattern changes without the previous knowledge of the compounds investigated; therefore, metabolite identification is not mandatory. This approach is not driven by a priori hypothesis; therefore it is open to new findings (6).

Fiehn (12) gave the first more detailed definition of metabolomics and its subsections. According to Fiehn (12), metabolomics is multi-functional, making use of different analytical approaches depending on the topic of the study. It

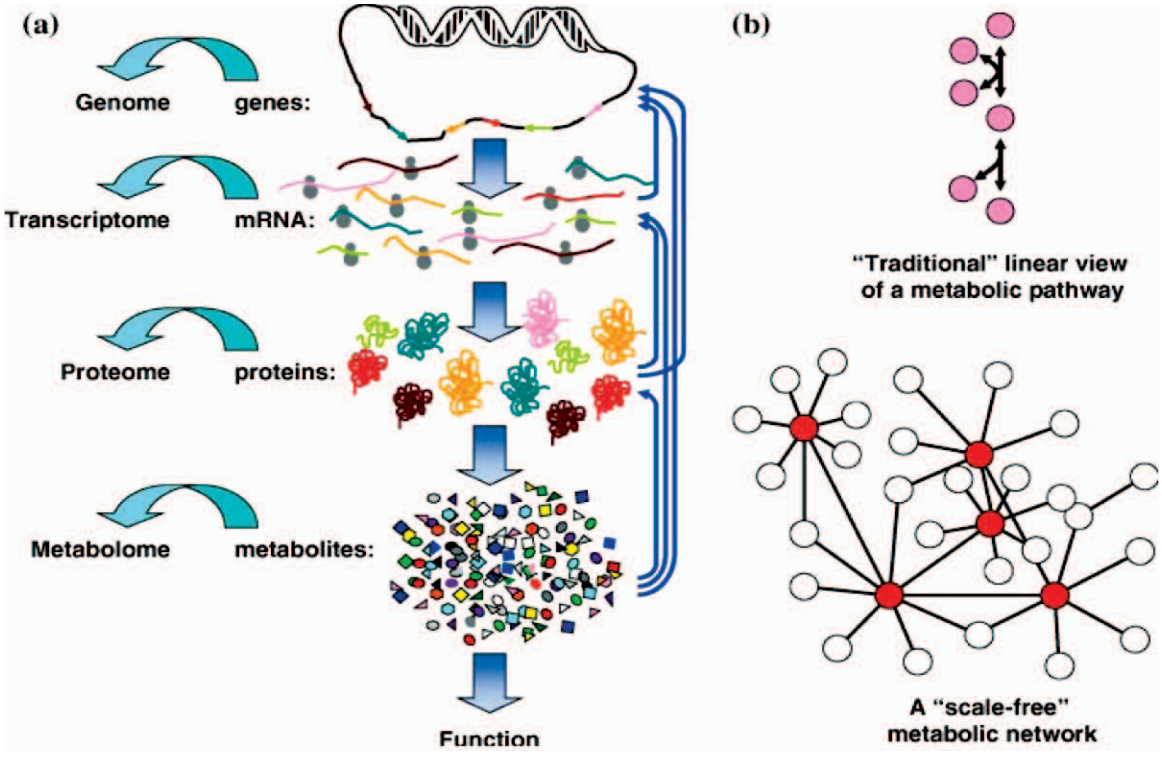

Fig. 1. (**a**) General schematic of the omic organisation. The general flow of information is from genes to transcripts, to proteins, to metabolites, to function (or phenotype); whilst blue vertical arrows indicate interactions regulating respective omic expression. (**b**) Our ''traditional'' linear view of a metabolic pathway and ''scale free'' connections in a metabolite neighbourhood. (Goodacre, 2005)

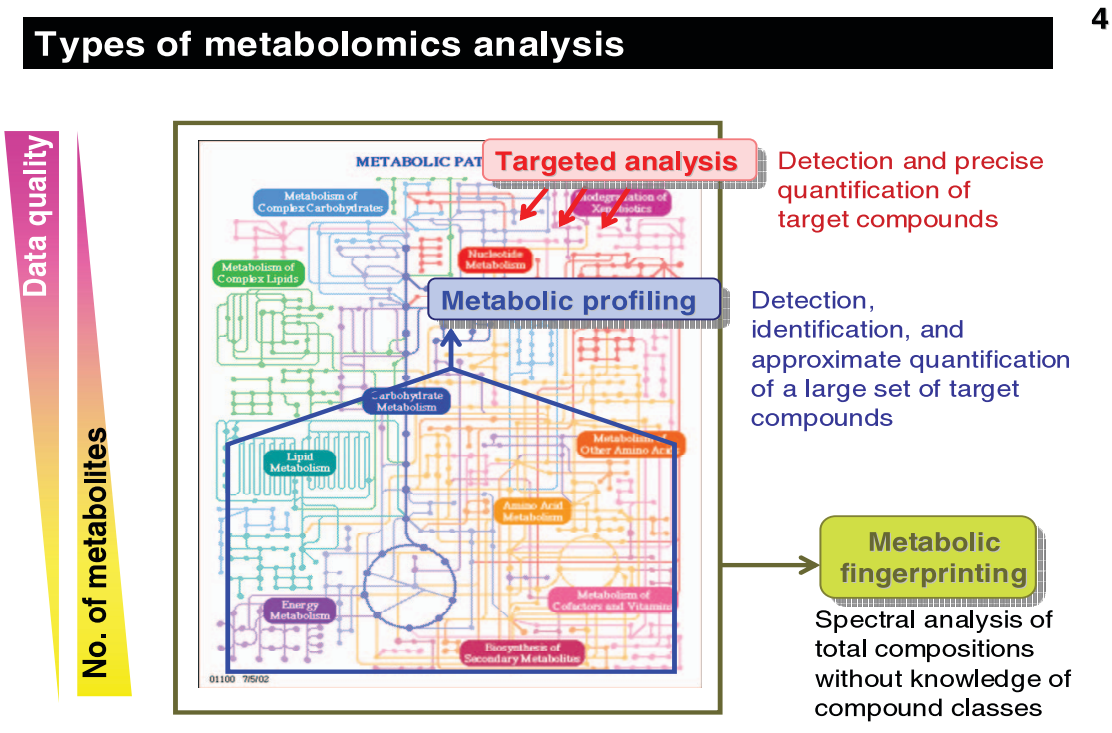

Fig. 2. A genetic manipulations, even at one gene, can affect the downstream intracellular elements, especially the end products of cellular functions, or "metabolites". Metabolite levels can provide information of biochemical status in response to environments or genetic manipulation. With the advance of omic's technologies, we can observe what genes are being expressed, what proteins are present, and now what patterns and concentrations of a large number of cellular metabolites are. Metabolomics aims to detect, identify and quantify a total population of low molecular weight compounds to gain functional information in a biological system

is subdivided into: 1) target analysis, aiming at quantitative analysis of substrate and/or product metabolites of a target protein; 2) metabolic profiling, focusing at quantitative analysis of a set of pre-defined metabolites belonging to a class of compounds or members of particular pathways or a linked group of metabolites; 3) metabolomics, striving for an unbiased overview of whole-cell metabolic patterns. For a more rapid analysis, metabolic fingerprinting (13) can be used, which reduces the analytical effort to the analysis of these intra-cellular metabolites with biochemical relevance.

Metabolic profiling aims at the quantification of preselected metabolic pathways or groups of metabolites with similar chemical properties and promises to be an effective method for investigating microbial metabolism in a quantitative manner. Knowledge about the changes of the intra-cellular metabolite concentrations offers a direct access to the identification of enzymatic kinetics especially to the *in vivo* kinetics of the underlying metabolic network, (4, 7, 28, 33, 44, 45, 46) or may elucidate limiting or inhibiting biosynthetic steps, which can be used for iterative strain optimisation (26).

Metabolomics may also be used to determine the different levels of thousands of molecules in healthy and diseased organisms, the different levels of nutritional value of traditional and GM foods, as well as to define the metabolites synthesised by organisms as a defence mechanism. Additionally, analysing the metabolome of different physiological states of organisms serves as a base for applying the nutrigenomics of interaction of chemical substances in foods, which have the ability to activate or de-activate particular genes linked to various diseases. Thus, metabolomics and nutrigenomics are also a study of the effect of foodstuffs on the totality of genes, proteins and the metabolic processes in the organism. By describing the mechanics of interaction of foodstuffs and the effect of certain diets, metabolomics and nutrigenomics attempt to determine their effect on human health. The joint science explores the idea of personalised nutrition determined by genotype. Its progress is accelerating and it is widely expected that in the next several decades its contribution to public health will increase.

Metabolomics involves the comparison of metabolomes (the full metabolite complement of an organism) between control and test groups to find differences in their profiles. Those differences may be correlated to the disease being studied in biomarker discovery or to changes in metabolic output in toxicology studies when a drug candidate is introduced to a test subject. Environmental metabolomics is also growing in importance where studies are performed to assess chemical risks to wildlife and the environment or to monitor the maintenance of healthy livestock in intensive farming with respect to disease.

Unlike gene-expression studies or proteomics analyses, which only reveal part of what might be happening in a cell, metabolomic profiling can give an instantaneous snapshot of the entire physiology of that cell. More importantly, if data from proteomics, transcriptomics, and metabolomics can be integrated, a more complete picture of a living organism's biology can be obtained.

Metabolomics also represent a useful tool for functional genomic programmes. In order to elucidate an unknown gene function, genetic alterations are introduced in a system. By analysing the phenotypic effect of such a mutation (i.e. by analysing the metabolome), functions may be assigned to the respective gene. Sampling would be performed once for every genotypic mutation and the analysis of the metabolome would optimally include an identification and quantification of all metabolites of a biological system (4).

There are usually several steps involved in metabolomics analysis:

- Profiling (also known as differential expression analysis) involves finding the metabolites of interest with statistically significant variations in abundance within a set of experimental and control samples.
- Identification is the determination of the chemical structure of these metabolites after profiling.
- Validation uses much larger numbers of samples to account for the effect of natural or biological variations to validate the previously identified metabolites. It is quantitative and requires analytical standards.
- Interpretation, the last step in the workflow, makes connections between the metabolites discovered and the biological processes or conditions.

Because of the vast chemical diversity of metabolites and their wide variation in abundance, metabolomics research usually requires multiple techniques; certain classes of samples are more amenable to one analysis technique than others. The workflow of metabolomics consists of sample preparation, analysis, data processing and data analysis.

Sample preparation

It is essential to reduce experimental error to a minimum and to ensure optimal extraction procedures of metabolites from a biological sample prior to analysis. Sampling is especially critical in metabolome analysis due to high exchange rates and small pool sizes of the metabolites of interest (48). Due to this, quenching of the cells during sampling is usually applied. The most popular method that is used for microbial cells, is quenching with cold methanol, maintaining the sample temperature below -20^{o}C, followed by collection of the cells by a centrifugation step prior to extraction (19). This approach has been originally developed and applied for yeast, but has later also been used in studies of different bacteria (4, 5, 21, 23, 26, 49, 50). For animal and plant cells liquid nitrogen is commonly utilized for quenching (43). Also this technique is applied for microbial cells, whereby no separation of culture supernatant and cells is carried out (9). Other approaches for metabolite sampling are based on fast filtration1 (48) or quenching of the cells through fast heating (30). Undoubtedly, metabolome analyses and also the conclusions drawn from the obtained data rise and fall with appropriate sampling. Despite

this, potential problems connected to sampling in metabolome analysis have not been properly considered. In most studies sampling protocols are simply adapted from the literature without critically validating them for the given case and the investigated organism (3).

Samples are usually homogenized or pulverized into smaller particles in order to increase the surface area for exposure to organic solvents. The type of extraction buffer used is dependent on the targeted group of metabolites investigated and should be in accordance to the chemical characteristics (15). This is because the extractant will affect the recovery of different metabolite classes (24). Since metabolomics involves the profiling of all metabolites, selection of an optimum extraction procedure should be investigated in compliance to chosen analytical platform.

Analysis

The analysis of the metobolome looks very attractive from this point of view with fewer numbers of analytes to be identified and quantified. High chemical complexity, analytical and biological variance, and large dynamic range are quite challenging, even for the latest analytical methods. In most cases, analytical methods are based on chromatographic separation techniques like GC and high-performance liquid chromatography (HPLC). In many cases these methods comprise Fourier transform infrared spectroscopy, electron impact ionization– mass spectrometry (EI–MS), electrospray ionization–mass spectrometry (ESI–MS), and nuclear magnetic resonance (NMR) spectroscopy. Mass spectrometers are generally more sensitive and more selective than any other type of detector. Prior to MS detection, the metabolites have to be separated, and separated compounds must be ionized. Ionization techniques may vary, especially for GC–MS and LC–MS couplings. The high-throughput screening with GC– and LC–MS techniques generates large volumes of analytical data that require advanced software for data mining. Metabolomics studies and analysis of the tissue crude extracts cannot be accomplished with the use of a single separation/detection method owing to the high chemical diversity of the analyzed mixture. Hydrophobic components are nicely separated with the use of reversed-phase (RP) chromatography (34, 35, 36), which is very popular and an appreciated method of separation. Hydrophilic and charged small molecules are well separated by the capillary electrophoresis. Hydrophilic and neutral compounds are best suited for hydrophilic (HILIC) separation (37, 38, 39). Tostikov et al. introduced HILIC-ESI–MS analysis for plant-derived samples and demonstrated feasibility of this approach, especially for analysis of the samples taken from hydrophilic compartments like the plant transport system (17, 32, 40, 41, 42). Traditional particles packed columns as well as high-speed monolithic columns can be used for HPLC separations prior to MS detection. Because the metabolomic approach requires large batches of the samples to be analyzed in order to apply statistical methods of the data treatment, micro-HPLC and capillary columns should be mostly used in order to avoid a significant amount of organic solvent evaporated into the atmosphere during this process. Silica-based C18-modified capillary monolithic columns actually offer a new step in micro-HPLC RP chromatography providing up to hundreds of thousands of theoretical plates per column. Therefore, performance of these columns is expected to be superior to conventional ones at the same range of HPLC parameters applied. Contrary to the miniaturisation of chromatography in time and space, Staack et al. (31) introduced the potentials of "freezing" an HPLC separation in time. They combined fraction collection and simultaneous on-line MS monitoring. The mobile phase was divided post-column into the mass analyser and to a micro-fraction collector. Applying software for metabolite identification, selected fractions where re-infused by off-line, chip-based nanoelectrospray infusion. This setup allowed the accomplishment of many different types of MS and MS/MS experiments in a "single" chromatographic run.

Data processing

A major challenge of metabolomics involves data processing and analysis; a full range of software programs is needed to turn raw metabolomics data into useful biological results. A typical metabolomics experiment requires large numbers of samples to generate results that are statistically rigorous. Aside from the need for highly sensitive and accurate instrumentation, powerful software tools are essential to address the vast amounts of data generated by these experiments. Analytical capabilities include deconvolution programs for processing GC/MS and LC/MS files, an array of statistical analysis tools to find significant metabolites, a metabolite database to identify metabolites, and finally, bioinformatics software for visualizing molecular interaction networks. Peak alignment with respect to the retention indives is utilized in order to line up peaks in a parallel manner so that chromatographic shifts are reduced to a minimum and variations caused by column aging or column cuts would be eliminated. Normalization with the internal standard is routinely applied in order to decrease experimental error (14).

Data analysis

This final step in the schematic work flow of metabolomics is data analyses by means of chemometrics. There are several statistical tools available, and the selection should be based on the objective of the experiment or the type of problems the research is investigated. These multivariate tools could be applied for: 1) classification and/or discrimination among groups of observations; or 2) regression modeling between two blocks of data (X and Y). Principal component analysis (PCA) is an unsupervised method that effectively reduced the complexity of chromatographic data by creating a dimensional variable space for the X variables, which consists a large number of interrelated observation (metabolites). This is achieved by transforming to a new set of variables called principal components (PCs) where the variation in the origin

dataset is retained in a linear relationship. Partial least square discriminant analysis (PLS-DA) is supervised technique in which sample discrimination is maximized in accordance to their pre-determined category (class). The method is applicable for both discriminating and classifying a set of samples. For reliable PLS-DA analysis the number of classes must be limited to four; this is because if more than four classes were assigned the discrimination may be incomprehensible and difficult to overview. While chemometric approaches like PCA and PLS-DA, on their own, do not permit the direct identification or quantification of compounds they still allow an unbiased (or untargeted), chemically comprehensive comparison to be made among different samples.

Metabolomics approaches are applied in the biological systems, including human, plants, and microorganisms. An example with the discovery of biomarkers in order to develop drugs and new therapies for human may be given. For plants, metabolomics is still developing, but there's the opportunity of metabolomics application in plant biology. It can be used to characterize metabolic and phenotypic alterations between genetic manipulation and WT lines or between healthy and diseased tissues. It can be used to identify regulated key sites in networks and for studies of gene function. Finally, it can be used to investigate food compositions for crop development.

Food Metabolomics

Evaluation of Food Function is generally difficult because food function would be constituted by abundant number of compounds. They influence one another in the mode of non-liner synergetic relationship. Food Metabolomics employ a technique of "metabolic profiling" and "chemometrics" for analyzing relationship between food function and their components. Possible innovative goal would be a development of evaluation system of food function and value based on the analytical results. The technique would be also applicable for improvement of food production, food preservation, and food transportation. In addition, food metabolomics would be useful for evaluation and quality control of folk medicine. The capacity to identify food constituents using metabolic profiling can also be used to assess both food adulteration and food quality. In particular, the detection of adulterated on contaminated food products exploits the fact that certain chemicals or certain concentrations of chemicals are quite characteristic of certain types of juices, extracts and oils (25). Similar chemical composition characteristics can also be exploited to distinguish between food products with desirable characteristics that cannot otherwise be detected by flavor, aroma or color (such as unsaturated fat content, a favorable amino acid profile, increased vitamin content, enriched phytochemical content). Food quality assessment also impacts food quality control. In fact metabolomic techniques may find their greatest use in the food industry in monitoring quality control or batch-tobatch product reproducibility.

Microbial Metabolomics

Microbes are widely used in the industrial fields of fermentation, foods, environmental remediation, biomass fuel production, etc. Microbial Metabolomics analyze inner metabolisms of microbes for understanding of their performances. This technique employs metabolomics and metabolic profiling for evaluation of food microbes, fermentation microbes and metabolomics analysis of transgenic microbes to check their function and performance. Particularly, metabolomics would be a powerful tool to suggest pin-point problems of failure transgenic studies. The application of metabolomics in this area is to improve transgenic microbes for environmental remediation and biomass fuel production.

Animal metabolomics

Fundamental trial to prove the capability of metabolomics as the experimental tactics for explanation of the dynamism of animal development is performed. Concretely, the metabolome analysis in the initial embryo generation stage of the Zebra fish, that is the model fish, is executed, and an accurate prediction system of the generation stage (post-fertilization time) is constructed with the technique of metabolic fingerprinting. Moreover, challenge to obtain a basic finding of the metabolome change as the drug response in minute administration dose in the experimental animal is performed. These series of researches not only contribute to basic biology but also a wide application to a medical field is expected.

Plant metabolomics

The mission oriented plant transgenic studies are conducted for environmental remediation and biomass production on a world wide level. However, few successes are reported in which the desired metabolic modulations are accomplished. Naturally, harmonic modulations of multiple numbers of genes without disorders of any functions are difficult due to a proper technique for evaluation of metabolic balance quantitatively. Metabolomics and metabolic profiling are applied into the studies concerning the transgenic plants and the transplastidic plants to develop the newly tactics. Recently, metabolic fingerprinting has been widely applied for the quality assessment of foods and crops, in which their compositions were investigated and evaluated by multivariate data analysis, (2, 27). One of the interesting applications of metabolomics is the characterization of pathways and metabolite network behaviors in plants that suffered from environmental stress (8, 18). Moreover, metabolomics may be useful for discovery of gene functions and elucidation of regulatory metabolisms in a metabolic network (10).

Environmental metabolomics

Environmental metabolomics is the application of metabolomics to characterise the interactions of organisms with their environment. This approach has many advantages for studying organism–environment interactions and for assessing organism function and health at the molecular level. As such,

metabolomics is finding an increasing number of applications in the environmental sciences, ranging from understanding organismal responses to abiotic pressures, to investigating the responses of organisms to other biota. These interactions can be studied from individuals to populations, which can be related to the traditional fields of ecophysiology and ecology, and from instantaneous effects to those over evolutionary time scales, the latter enabling studies of genetic adaptation.

Final remarks

Metabolomics is a vibrant diverse field on the exponential part of the growth curve. There are many ongoing global metabolomics-based research initiatives. An international society has been formed, The Metabolomics Society (metabolomicssociety.org), the mission of which is to promote the growth and development of the field of metabolomics internationally. There is also a clear and necessary trend to improve comparability of metabolome data sets in literature. In 2005, the call to contribute for a MS database for all available MS data of reference standard was published (20); first data collections were already published in the past (29, 47). All aim at a comprehensive collection of biologically related compounds in plants, microorganisms or other biological systems. In combination with more sophisticated and reliable automated raw data postprocessing, this will improve both high- and low-throughput metabolome applications.

Today there are a number of research projects in the field of nutrigenomics and metabolomics financed by the governments of EU, USA, Japan and other countries. The current national policy of Bulgaria with regard to the research, development and application of metabolomics is yet to be clearly stated. The declared EU view is that while Bulgaria's policy for R&D in this area is relatively well formulated, the country is still lagging behind in the practical application of this science. Metabolomics is an ultra-modern multi-disciplinary science encompassing areas of nutrition, endocrinology, genetics, biotechnology, cytology, neurology, paediatric gastroenterology, chemistry, biochemistry and public health, as well as modelling, risk assessment, agriculture and ecology. A clearly stated national policy for the development of this science will bring about the flourishing of this entire range of sciences in Bulgaria and will be a major boost to human health. It is necessary to encourage the Bulgarian scientists to continue developing their research in nutrigenomics and especially in metabolomics with a reliable scientific and communication strategy.

REFERENCES

1. **Barabasi A.L. and Oltvai Z.N.** (2004) Nature Reviews Genetics, **5**, 101-113.
2. **Beckman M., Enot D.P., Overy D.P., Draper J.** (2007) J. Agricultural Food Chem., **55**, 3444-3451.
3. **Bolten C.J., Kiefer P., Letisse F., Portais J-C, Wittmann C.** (2007) Anal. Chem., **79**, 3843-3849.
4. **Buchholz A., Hurlebaus J., Wandrey C., Takors R.** (2002) Biomol. Eng., **19**, 5-15.
5. **Buchholz A., Takors R., Wandrey C.** (2001) Anal. Biochem., **295**, 129-137.
6. **Carraro S., Giordano G., Reniero F., Perilongo G., Baraldi E.** (2009) J. Pediatr., **154**, 638-644.
7. **Chassagnole C., Noisommit-Rizzi N., Schmid J.W., Mauch K., Reuss M.** (2002) Biotechnol. Bioeng., **79**, 53-73.
8. **Cook D., Fowler S., Fiehn O., Thomashow M.F.** (2004) Proc. National Academy of Sciences of the USA, **101**, 152343-15248.
9. **Dominguez H., Rollin C., Guyonvarch A., Guerquin-Kern J. L., Cocaign-Bousquet M., Lindley N.D.** (1998) Eur. J. Biochem., **254**, 96-102.
10. **Ducruix C., Junot C., Fievet J-B., Villers F., Ezan E., Bourguignon J.** (2006) Biochimie, **88**, 1733-1742.
11. **Fell D.A.** (1996) Understanding the control of metabolism, Portland Press, London.
12. **Fiehn O.** (2002) Plant Molecular Biology, **48**, 155-171.
13. **Fiehn O.** (2001) Compar. Funct. Genom., **2**, 155-168.
14. **Fiehn O., Kopka J., Dormann P., Altman T., Trethewey R.N., Willmitzer L.** (2008) Nat. Biotech., **18**, 1157-1161.
15. **Fukusaki E. and Kobayashi A.** (2005) J. Biosci. Bioeng., **100**, 347-354.
16. **Goodacre R.** (2005) Metabolomics, **1**(1), 1-2.
17. **Ikegami T., Kobayashi H., Kimura H., Tolstikov V.V., Fiehn O., Tanaka N.** (2005) In: Metabolomics. The Frontiers of Systems Biology, Springer Verlag, Tokyo, Japan, pp. 107-126.
18. **Kaplan F., Kopka J., Haskell D.W., Zhao W., Schiller K.C., Gatzke N., Sung D.Y., Guy C.L.** (2004) Plant Physiology, **136**, 4159-4168.
19. **de Koning W. and van Dam K.** (1992) Anal. Biochem., **204**, 118-123.
20. **Kopka J., Schauer N., Krueger S., Birkemeyer C., Usadel B., Bergmuller E., Dormann P., Weckwerth W., Gibon Y., Stitt M., Willmitzer L., Fernie A.R., Steinhauser D.** (2005) Bioinformatics, **21**, 1635-1638.
21. **Li M., Ho P. Y., Yao S., Shimizu K.** (2005) J. Biotechnol., **119**, 356-362.
22. **Lokhov P.G. and Archakov A.I.** (2009) Biomed. Chem., **3**, 1-9.
23. **Moritz B., Striegel K., De Graaf A.A., Sahm H.** (2000) Eur. J. Biochem., **267**, 3442-3452.
24. **Nordstrom A. and Lewensohn R.** (2009) J. Neuroimmune Pharmacol., DOI 10.1007/s11481-009-9156-4.
25. **Ogrinc N., Kosir I.J., Spangenberg J.E., Kidric J.** (2003) Analytical and Bioanalytical Chemistry, **376**, 424-430.

26. **Oldiges M., Kunze M., Degenring D., Sprenger G. A., Takors R.** (2004) Biotechnol. Prog., **20**, 1623-1633.
27. **Pongsuwan W., Fukusaki E., Bamba T., Yonetani T., Yamahara T., Kobayashi A.** (2007) J. Agricult. Food Chem., **55**, 231-236.
28. **Rizzi M., Baltes M., Theobald U., Reuss M.** (1997) Biotechnol. Bioeng., **55**, 592-608.
29. **Schauer N., Steinhauser D., Strelkov S., Schomburg D., Allison G., Moritz T., Lundgren K., Roessner-Tunali U., Forbes M.G., Willmitzer L., Fernie A.R., Kopka J.** (2005) Febs Lett., **579**, 1332-1337.
30. **Schaub J., Schiesling C., Reuss M., Dauner M.** (2006) Biotechnol. Prog., **22**, 1434-1442.
31. **Staack R.F., Varesio E., Hopfgartner G.** (2005) Rapid Commun. Mass Spectrom., **19**, 618-626.
32. **Tanaka N., Tolstikov V.V., Weckwerth W., Fiehn, O., Fukusaki E.** (2003) In: Frontier of Metabolomic Research, Springer- Verlag, Tokyo, Japan, pp. 85-100.
33. **Theobald U., Mailinger W., Baltes M., Rizzi M., Reuss M.** (1997) Biotechnol. Bioeng., **55**, 305-316.
34. **Tolstikov V.V., Lommen A., Nakanishi K., Tanaka N., Fiehn O.** (2003) Anal. Chem., **75**, 6737-6740.
35. **Tolstikov V.V., Fiehn O., Shulaev V.** (2005) 53rd ASMS Conference, San Antonio, TX, June 5-9, 2005.
36. **Tolstikov V.V., Lorence A., Cortes D.F.** (2005) 28th International Symposium On Capillary Chromatography and Electrophoresis, Las Vegas, NV, May 22-25, 2005.
37. **Tolstikov V.V. and Fiehn O.** (2002) Anal. Biochem., **301**, 298-307.
38. **Tolstikov V.V., Tanaka N., Fiehn O.** (2003) 2nd Plant Metabolomics Conference, Potsdam, Germany, April 25-28, 2003.
39. **Tolstikov V.V., Tanaka N., Fiehn O.** (2003) Cereal Chemistry Symposium, Adelaide, Australia, September 8-10, 2003.
40. **Tolstikov V.V., Zhang B., Weckwerth W., Fiehn O.** (2001) 49th ASMS conference on Mass Spectrometry and Allied Topics, Chicago, IL, May 27-31, 2001.
41. **Tolstikov V.V., Costisella B., Weckwerth W., Zhang B., Fiehn O.** (2002) 50th ASMS Conference on Mass Spectrometry and Allied Topics, Orlando, FL, June 2-7, 2002.
42. **Tolstikov V.V., Tanaka N., Fiehn, O.** (2003) LCMS Montreux Symposium, Savannah, GA, October 15-17, 2003.
43. **Viant M.R., Pincetich C.A., de Ropp J.S., Tjeerdema R.S.** (2005) Metabolomics, **1**, 149-158.
44. **Visser D., van Zuylen G.A., van Dam J.C., Oudshoorn A., Eman M.R., Ras C., van Gulik W.M., Frank J., van Dedem G.W.K., Heijnen J.J.** (2002) Biotechnol. Bioeng., **79**, 674-681.
45. **Wiechert W.** (2002) J. Biotechnol., **94**, 37-63.
46. **Wiechert W. and Takors R.** (2003) In: Metabolic engineering in a post genomic era (H.V. WesterhoffB, N. Kholodenko, Eds.) Horizon Bioscience, Wymondham.
47. **Wagner C., Sefkow M., Kopka J.** (2003) Phytochemistry, **62**, 887-900.
48. **Wittmann C., Kromer J. O., Kiefer P., Binz T., Heinzle E.** (2004) Anal. Biochem., **327**, 135-139.
49. **Zhao Y. and Lin Y. H.** (2002) Process Biochem., **37**, 1455-1461.
50. **Zhu J. and Shimizu K.** (2004) Appl. Microbiol. Biotechnol., **64**, 367-375.