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METABOLOMICS AND ITS APPLICATIONS IN THE FIELD OF PHARMACEUTICAL SCIENCE

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INTRODUCTION

In a post genomic era, it is crucial to perform unbiased metabolic analyses and eventually define the biochemical functions of plant primary and secondary metabolic pathways (Tretheway et al., 1999). The "metabolome" has been used to describe the observable chemical profile or fingerprint of the metabolites in whole tissues (Ott et al., 2003). Many analytical methods have been used to profile the metabolome, such as infrared spectroscopy, nuclear magnetic resonance spectroscopy (NMR), LC/UV/PDA, CE/UV, CE/MS), GC/MS, LC/MS, and LC/NMR/MS. In metabolite profiling, it would be preferable to use a wide spectrum chemical analysis technique, which is rapid, reproducible, and stable in time while needing only a very basic sample preparation. NMR is one of the techniques that could meet those demands. The last few decades, a number of techniques have been devised to develop NMR spectroscopy as a fingerprinting tool for the interpretation and quality assessment of industrial and natural products and multivariate or pattern recognition techniques such as the well-described

principal component analysis (PCA) and hierarchical cluster analysis (HCA) have been specifically designed to analyze complex data sets (Sumner et al., 2003).

Key words: metabolomics, transgenic plants, deer's antler, pine-mushroom

METABOLOMICS OF TRANSGENIC PLANTS

The introduction of *entC* gene from *Escherichia coli* and the *pmsB* gene from *Pseudomonas fluorencens*, encoding two enzymes involved in the bacterial biosynthetic pathway of salicylic acid (SA), into tobacco (*Nicotiana tabacum* 'Samsun' NN) resulted in constitutive salicylic acid producing plants (CSA). SA is known as an important signal compound in systemic acquired resistance (SAR), and CSA-tobacco plants showed less damage after viral infection. Metabolite profiling using various chromatographic methods of the CSA plants was conducted in the fields of phytoalexins, alkaloids, and flavonoids. However, those trials used a targeted approach on a specific group of compounds. It was reported that ^{1}H-NMR spectroscopy method, coupled with multivariate analysis could be used for the metabolomic analysis of wild type and transgenic (CSA) tobacco. Tobacco mosaic virus (TMV) inoculated leaves and systemic leaves were also analyzed to determine the difference in metabolite profiles in wild type and CSA plant (Choi et al. 2004).

METABOLOMICS OF DEER'S ANTLER

Deer antlers are widely used as a traditional oriental medicine in many Asian countries such as Korea and China, with their consumption being associated with anti-stress, anti-aging, anti-inflammatory, and phagocytic effects. Various types of

antlers from various countries including Russia, New Zealand, and China are sold commercially for use in traditional medicines in Korea. Determining the origin of deer antlers has conventionally relied upon visual inspection, which is unreliable. Chemical components analysis has also been attempted, with many biochemical components including lipids, peptides, carbohydrates, and inorganic substances reportedly being present. The chemical classification or discrimination of each species based on classical chromatographic or spectrometric methods was still unclear. This study has proven that it is possible to discriminate various types of deer antler by using PCA of ^{1}H NMR spectra of crude extracts. The per-weight price of deer antler used for oriental medicine varies according to the origin, and hence the technique for discriminating antlers developed in this study can be used for commercial quality-control purposes, especially since it is simple, efficient, and does not require any pre-purification steps. Furthermore, the method is easily applicable to the metabolomic fingerprinting of other medicinal sources such as herbs (Choi et al. 2006).

METABOLOMICS OF PINE-MUSHROOM

Mushrooms have been used widely since ancient times not only as a source of foods and to flavor food but also for medicinal and functional purposes. The pine-mushroom (*Tricholoma matsutake* Sing.) is the most valuable species of mushroom throughout the world; this mushroom exhibits several useful biological activities in humans, such as lowering cholesterol concentrations, antioxidant effects, immunomodulation, and antitumor effects. Pine-mushrooms that are cultivated in the pine forests of the Republic of Korea are particularly highly valued, mainly due to the unique environment and climate of this region. Pine-mushrooms can be classified according to their appearance, and a standard for the classification of pine-mushrooms was developed by the National Forestry Cooperatives Federation

of the Republic of Korea. Metabolomic analysis of raw and cooked pine-mushrooms (*Tricholoma matsutake* Sing.) of different grades was performed using ^{1}H nuclear magnetic resonance (NMR) spectrometry and principal component analysis (PCA). PCA of the ^{1}H NMR spectra of aqueous fractions allowed different grades of raw pine-mushroom to be discriminated by a combination of principal component (PC) 1 and PC 2, which accounted cumulatively for 94.1% of the variation in all variables. The major peaks in the ^{1}H NMR spectra that contributed to discrimination of raw mushrooms were assigned to choline, trehalose, threonine, leucine/isoleucine, succinic acid, alanine, and fumaric acid. The combination of PC 1 (70.8%) and PC3 (7.5%) allowed different grades of cooked pine-mushroom to be discriminated, and the major peaks in the ^{1}H NMR spectra that contributed to discrimination of cooked mushrooms were assigned to succinic acid, trehalose, and fumaric acid. This metabolomic analysis-based method allows different grades of pine-mushroom to be distinguished without any prepurification (Choi et al. 2007).

CONCLUSIONS

Metabolomics seems to become of major importance in pharmaceutical and food sciences with following applications. First, it can be used for the development of early biomarker for specific disease for prevention. A biomarker concept can be exploited with metabolomics, where fingerprints of patterns of many metabolites together are indicative for early changes in physiology or onset of pathology. Second, the metabolome can be regarded as an useful tool for safety assessment for genetically modified organisms by comparing the metabolome with wild type organisms. Third, different resources of medicinal foods with different origins can be readily discriminated using metabolomics.

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Table 1. Comparison of 'omics' technology

	Genomics	Transcriptomics	Proteomics	Metabolomics
Target material	Gene, chromosome (genetic code)	mRNA (genetic code)	Protein (function of the protein)	Low molecular weight metabolites
MW	100,000-120,000	100,000-120,000	5,000-20,000	100- 5000
Characteristics	Context independent	Context dependent	Context dependent	Context dependent
Analysis	Mapping, sequencing	Sequencing	Separation, characterization	Separation, characterization
Methods	DNA sequencer	Hybridization	2D gel, Maldi TOF	NMR, MS, GC, LC
Human	30,000 genome		>1,000,000,000?	~2,500

Table 2. SWOT analysis of metabolomics (Lindon et al. 2006)

Strength ➢ Robust and stable analytical platforms ➢ Minimally invasive ➢ Real biological endpoint ➢ Whole system integration	Weakness ➢ Analytical sensitivity ➢ Analytical dynamic range ➢ Complexity of data sets ➢ High capital cost
Oppurtinities ➢ Much experience from mammalian system studies (e.g. pathways) ➢ Potential of multi-omics integration ➢ Web-based diagnotics	Threats ➢ Skepticism of non- hypothesis led studies ➢ Conservatism ➢ Lack of well trained scientists