

Hirata Corporation  
Research and Development Headquarters

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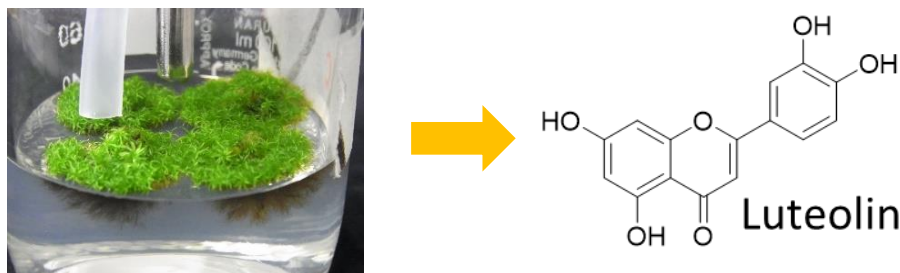
## Hirata Co., Ltd. R&D progress report on the exploration of metabolic pathways of functional secondary metabolites from plants using metabolome analysis technology\*.

The Research and Development Headquarters of Hirata Co., Ltd. (Hirata R&D) is implementing a new business plan with a view to commercializing biological resources (mainly plant resources) for the healthcare industry. We recognize liquid chromatography coupled mass spectrometers (LCMS) utilizing high mass accuracy and high mass resolution as important metabolome analysis technologies [1] for the comprehensive detection of functional components derived from plant resources.

We introduce three published research papers as application examples utilizing LCMS metabolome analysis technology differently from conventional targeted approaches to provide unique research achievements.

### (1) Metabolome analysis for model moss plants (*Physcomitrella patens*)

By combining comprehensive LCMS metabolome analysis technology and trace analysis technology for labeling stable isotope elements with stable isotope oxygen administration ( $^{18}\text{O}_2$ ), we succeeded for the first time in detecting luteolin (flavone), a type of flavonoid (generic name) [2] from model moss plants (*Physcomitrella patens*) (Fig. 1). Luteolin is a functional component that is expected to have anti-inflammatory properties and suppress uric acid levels. This result illustrates how LCMS metabolome analysis has made it possible to systematically discover compounds in well researched plants.



**Fig. 1** Detection of luteolin (flavone) from model moss plants (*Physcomitrella patens*)

The above research results can be found at the Kazusa DNA Research Institute's website ([https://www.kazusa.or.jp/news/210118\\_2/](https://www.kazusa.or.jp/news/210118_2/)) and are published in the following paper:

“Metabolome analysis using multiple data mining approaches suggests luteolin biosynthesis in *Physcomitrella patens*”; Yasuhide Hiraga, Takeshi Ara, Yoshiki Nagashima, Norimoto Shimada, Nozomu Sakurai, Hideyuki Suzuki, Kota Kera; *Plant Biotechnology* **37**, 377–381 (2020); DOI: 10.5511/plantbiotechnology.20.0525b

## (2) Metabolome analysis for model Legume plant (*Lotus japonicus*)

Two gossypetin 3-*O*-glycosides (gossypetin 3-*O*-glucoside and gossypetin 3-*O*-galactoside), a type of flavonoid, were identified using LCMS metabolome analysis technology focusing on the yellow pigmentation of petals and the distribution between tissues of the model Legume plant (*Lotus japonicus*) (Fig. 2). The results were successfully applied to the isolation and functional analysis of genes related to the yellow pigment of *Lotus japonicus* petals using the EST data mining method [3]. Importantly, the metabolome analysis obtained from the tissue-to-tissue comparisons allowed the research to identify the genes involved in the biosynthetic pathways of functional components (yellow flavonol) (Fig. 2). By using this knowledge of the genetic code, it is expected to be possible to make ornamental plants with petals of various colors. This result is an example of how LCMS metabolome analysis has made it possible to predict biosynthetic pathways, leading to gene isolation related to the metabolic pathways of functional components.



**Fig. 2 Prediction of the biosynthetic pathway of glycoside at position 3 of gossypetin (flavonol) and isolation of flavonoid 8-hydroxylase with gossypetin synthase activity from model Legume plant (*Lotus japonicus*).**

The above research results can be found at the Kazusa DNA Research Institute's website ([https://www.kazusa.or.jp/news/210118\\_3/](https://www.kazusa.or.jp/news/210118_3/)) and are published in the following paper:

“Identification of a flavin monooxygenase-like flavonoid 8-hydroxylase with gossypetin synthase activity from *Lotus japonicus*”; Yasuhide Hiraga, Norimoto Shimada, Yoshiki Nagashima, Kunihiro Suda, Tina Kanamori, Kanako Ishiguro, Yuka Sato, Hideki Hirakawa, Shusei Sato, Tomoyoshi Akashi, Yoshikazu Tanaka, Daisaku Ohta, Koh Aoki, Daisuke Shibata, Hideyuki Suzuki, Kota Kera; *Plant & Cell Physiology*, in press 2021; DOI: 10.1093/pcp/pcaa171

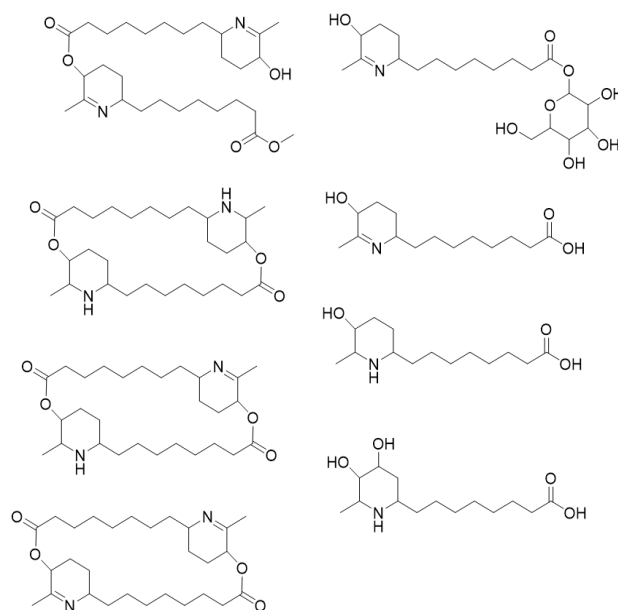
### **(3) Metabolome analysis for papaya (*Carica papaya* L.)**

Around 2019, we started a joint research project looking at functional fruits with Kogakuin University in Hachioji City, Tokyo. In order to promote the utilization of unripe papaya (called green papaya) produced as a vegetable in Japan, we examined the potential of green papaya as a functional food using LCMS metabolome analysis technology. It was suggested that green papaya contains more carpaine-related derivatives, a type of alkaloid (compounds containing nitrogen) [4] reported to have cardiovascular effects, than ripe papaya (Fig. 3). In addition, it was also suggested that the functional components (carpaine-related derivatives) are contained in the peel (unripe and ripe) discarded in the cooking process in large amounts when compared to the pulp (unripe and ripe). These results suggested that various carpaine-related derivatives and detected intermediates can offer clues to their metabolic pathways, leading to the prediction of biosynthetic pathways and the discovery of new functional components (Fig. 3). Furthermore, it was found that even after processing green papaya fruit into an easy-to-use dry powder, proteolytic enzyme activity [5] used in food processing, the Maca [6] functional component: benzyl glucosinolate [7], and polyphenols [8] with antioxidant properties were detected. In addition, examination of the use of stems and roots that are usually discarded, with LCMS metabolome analysis techniques suggesting that stems and roots can be used as raw materials for benzyl glucosinolates (Fig. 3). This result is an example of how LCMS metabolome analysis has made it possible to discover new use and value of discarded foods.

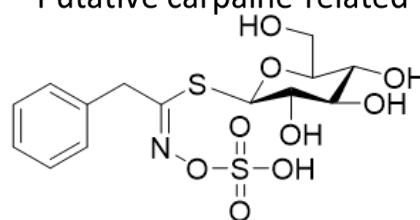
The above research results can be found at the Kazusa DNA Research Institute's website (<https://www.kazusa.or.jp/news/210130/>) and are published in the following paper:

“Metabolic analysis of unripe papaya (*Carica papaya* L.) to promote its utilization as a functional food”; Yasuhide Hiraga, Takeshi Ara, Nao Sato, Nayumi Akimoto, Kenjiro Sugiyama, Hideyuki Suzuki, Kota Kera; *Bioscience, Biotechnology, and Biochemistry*, in press 2021; DOI: 10.1093/bbb/zbab01

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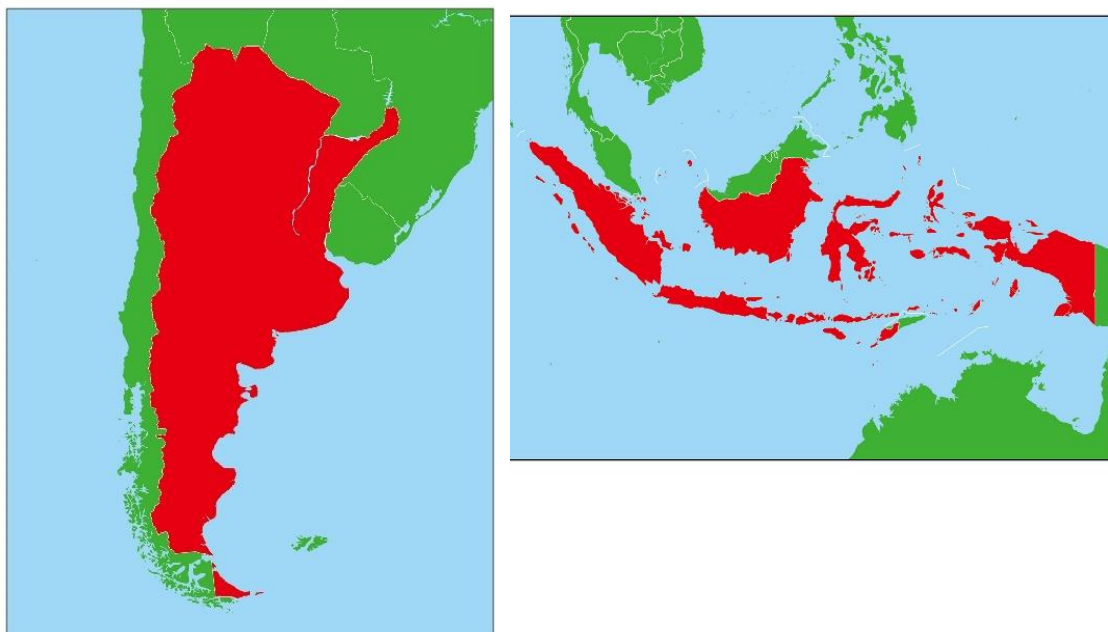
Putative carpaine-related derivatives



Benzyl glucosinolate

**Fig. 3 Detection of various carpaine-related derivatives from unripe papayas (fruit, top) and benzyl glucosinolates from roots (bottom)**

Hirata R&D has signed agreements with the Indonesian Agency for Agricultural Research and Development (IAARD) and the Instituto Nacional de Tecnologia Agropecuaria (INTA) to explore and use plant genetic resources for functional food, cosmetics, toiletries and drug development (Fig. 4). We have continued to negotiate joint research and development, paying close attention to the Republic of Indonesia [9], which has a vast number of islands, covering large range of longitude and latitudes, and the Republic of Argentina [10], which has a wide variety of biological resources and is rich in diverse climates and soils (Fig. 4). The rare plant resources of the two countries are ideal for bioprospecting, and the continued development and use of the LCMS metabolome analysis technology introduced here to search and identify the metabolic pathways of functional secondary metabolites derived from plants will form the core technology of Hirata R&D as illustrated by the three results described here.



**Fig. 4 Map: Republic of Argentina (left) and the Republic of Indonesia (right)**

We are confident that these metabolome analysis technologies will accelerate the development of advanced and innovative products that are accepted in the global market including new functional food materials, cosmetic materials, toiletry materials, and pharmaceuticals.

※The information in the press release is as of the date of publication. Please note that at this time, it may differ from the information as of the date of announcement.

## Glossary

- (1) ‘Metabolome analysis technology’ is a technology that analyzes and processes the entire metabolite contained in living organisms. Here, we show a method for comprehensive non-targeted analysis of biological components.
- (2) ‘Flavonoids’ are a group of naturally occurring organic compounds and is a generic term for plant secondary metabolites derived from chalcone, which can be formed from Coumaroyl-CoA and malonyl-CoA. It is a polyphenol known to show antioxidant activities.
- (3) ‘The EST data mining technique’ is the construction of an Expressed Sequences Tags (EST) library. RNA extracted from a living organism is reverse transcribed from mRNA (necessary for protein translation) to cDNA (complementary DNA) which is then sequenced to create a database. These short EST fragments are sequence tags for gene expression, allowing information to be extracted for the identification of gene transcription/gene discovery/gene sequencing with automated pattern recognition.



- (4) ‘Alkaloids’ is a generic term for naturally derived organic compounds that contain nitrogen atoms and, in most cases, show basicity. Many alkaloids are toxic to other organisms, but are often used as pharmaceuticals because they show pharmacological effect(s).
- (5) ‘Proteolytic enzymes’, also known as proteases, is a generic term for hydrolytic enzymes that break protein into smaller polypeptides or single amino acids. Green papaya contains a protease called papain, which softens meat.
- (6) ‘Maca’ is a perennial of the Brassicaceae family that is found in Peru, South America. Maca is called "Andean carrot" locally, and the highly nutritious roots are used also as a medicinal herb.
- (7) ‘Benzylglucosinolate’ is a type of aromatic glucosinolate. It is reported to have anti-fatigue and anti-obesity properties. Glucosinolate is a functional component found in many spicy Brassicaceae such as mustard, cabbage, and wasabi, and has been reported to have antioxidant and anticancer properties. Since benzyl glucosinolates contain nitrogen atoms and can also be classified as alkaloids, other novel physiological activities have also been investigated.
- (8) ‘Polyphenols’ are found in most plants. It is a generic term for plant components having a number of phenolic hydroxy groups in the molecule. It is found in most plants and has free radical scavenging ability as well as many physiological properties including antioxidant activity.
- (9) ‘The Republic of Indonesia’ is located in the southern part of Southeast Asia and consists of about 13,400 large and small islands. It is located in the tropical climate region (dry season and rainy season) across the equator and is one of the Megadiverse cities (i.e., those rich in biological resources).
- (10) ‘The Republic of Argentina’ is a treasure trove of genetic resources blessed with a variety of climates, from tropical regions in the north to the cold regions of Patagonia and the Andes. It is one of the most important regions for exploring new plant resources.

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