Impact Objectives

- Analysis of proteins in plant remains to simulate interactions between plant and phytopathogens
- Contribute to our understanding of the foods and cuisines of ancient civilizations
- Unravel the foods and cuisines of Japonesian through understanding more about ancient Proteomics

Insights into ancient civilizations

__Associate Professor Takumi Nishiuchi__ leads a project attempting to reconstruct the eating habits of civilizations by analysing ancient proteins found at archaeological sites

You are a plant biologist and plant pathologist. Can you talk a little about this field of research?

I use transcriptomics and proteomics to study plant and pathogen interactions at the molecular level. I am fortunate to be based at the Advanced Science Research Center which forms part of Kanazawa University in Japan; we have all the equipment needed for such omics studies. My team collaborates with researchers to support their omics studies, but we also work with archaeologists.

We began using proteomics to identify the amino acid sequence of collagen proteins of excavated animal bones to identify the biological species. Then, we tried to perform proteomic studies using some different types of excavations. Among them, we identified many proteins derived from not only plant themselves but also microorganisms such as phytopathogens in the plant remains. From there, we became interested in the analysis of proteins in the plant remains to simulate the interaction between plant and phytopathogens.

Part of your research involves extracting and purifying proteins from plant remains such as carbonised rice from the different archaeological sites. What are the main goals of this research?

Ancient food cultures are spread over many places. In particular, cereals were a staple food and an important step in reading the habits of ancient people. Rice is a staple food in east Asia and the archaeological sites along the Chang Jiang river are thought to be the origin of rice cultivation (rice cultivation in Japan and Korea is thought to have been assimilated from China). We are therefore studying carbonised rice and food crusts from different archaeological sites in east Asia. We have already identified some candidates for rice marker protein in plant remains and food crusts, and from there have tried to identify marker proteins from other excavated cereals such as common millet. These analyses are useful for the elucidation of materials in the food crusts and will contribute to our understanding of the foods and cuisines of ancient civilizations.

What methods or tools have you used in your analysis?

In this study, we usually use shotgun proteomics, which refers to bottom-up proteomics techniques where we combine high performance liquid chromatography with mass spectrometry. The protein preparation step is very important for this particular study. After that, ancient proteins are digested by trypsin and digested peptides are purified using stage chips. Then, purified peptides are separated by nanoLC, ionised by electronspay ionisation and then resulting fragment ions are analysed by Orbitrap QE plus.

How are you investigating the Sungitani Chanobatake ruins?

Many students are interested in the lifestyles of ancient civilizations and this includes the foods they ate. With that in mind, I will introduce our research about proteomic studies of plant remains and food crust into my classes. In addition, I sometimes guide the student experiments of identification of collagen proteins in the excavated animal bones. The oldest rice balls were excavated from the Sungitani Chanobatake ruins near our university. These collaborations are very important for us and our university and the results of our research are of practical use to the local community and wider society. ●

Proteins of foods from the past

*A team based at **Kanazawa University** are attempting to shed some light on the lifestyles of ancient Japanese civilizations by understanding the food they ate. The findings will highlight the importance of proteomics and may help to restore ancient foods of the Japonesian*

Studying ancient civilizations is an effective means of understanding how people, societies and cultures have changed over thousands of years, thereby providing important context to present-day civilizations. Then there is the idea of determining similarities between humanity today and humanity back then which, if nothing else, can generate a raft of ideas concerning what is common to all homo sapiens.

There are many ways of studying ancient civilizations, from reviewing the literature and other texts that remain from civilizations such as the Ancient Greeks, to excavating patches of land to uncover pottery, building materials, bones and plant remains. Indeed, these remains (coupled with modern analytical technologies), enable scientists to gain insight into the foodstuffs that ancient civilizations grew and ate, and shed further light onto what life was really like for people all those years ago.

One method that has gained traction in recent years is proteomics, which is the large-scale study of proteins. By identifying proteins in materials that have been found during excavations, researchers are hoping to uncover more information on ancient civilizations.

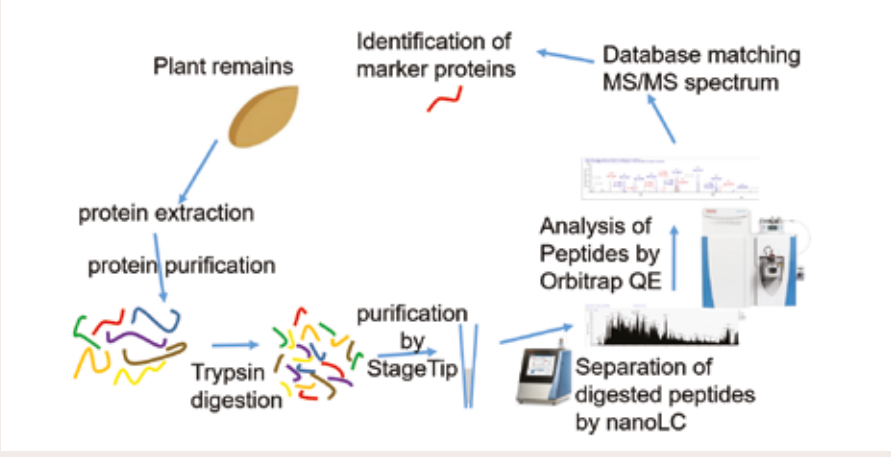
FASCINATING CONTEXT

Associate Professor Takumi Nishiuchi is a researcher based within the Advanced Science Research Center at Kanazawa University in Japan. He leads a team which is analysing the ancient proteins in animal bones, plant remains, dental calculus and food crusts that have been found at archaeological sites. 'By identifying proteins in the excavations, we want to uncover important clues that enable us to simulate ancient foods, such as meat, fish and cereals,' explains Nishiuchi. 'Based on the results we attain, coupled with archeological data, we will be able to envisage the lifestyles of ancient civilizations.'

His team has been working at ruins in China and South Korea, as well as two Japanese ruins located at Fukuoka prefecture. It is believed that rice cultivation and related food cultures that surfaced in Japan thousands of years ago were introduced from China via South Korea. Thus, by focusing their studies at these locations, the team are intent on analysing samples that can provide an understanding of the propagation of rice food culture. Gaining such an understanding will provide fascinating context to a food culture that continues to exist across Asia to this day.

PROVING THE EFFICACY

The methods of protein identification in animal bones and dental calculus have already been long established, but many people (including scientific researchers), believe that plant remains do not have enough stable proteins to perform effective proteomic studies. However, this is not a belief that Nishiuchi subscribes to and some of his team's research has already attested to this. 'Plant remains, such as seeds, definitely do have useful proteins which we can use to ▶



Schematic flow of ancient proteomics

identify plant species,' says Nishiuchi. 'The innovation of the Orbitrap mass spectrometry has made significant contributions to our ancient proteomic studies and by using the appropriate protein preparation from various types of excavations enables us to collect many important clues into what life was really like back then.'

One of the team's greatest achievements to date has been to perform protein analysis in plant remains and food crusts found at various sites. This has not been done many times before, which not only provides fascinating insight, but helps to demonstrate the efficacy of the tools and methods they are using. By highlighting the usefulness of their methods, the team opens up the possibility of similar studies at various sites in countries around the world, which can provide further insight about other civilizations.

> *By identifying proteins in the excavations, we want to uncover important clues that enable us to simulate ancient foods, such as meat, fish and cereals*

PROTEINS OF CARBONISED RICE

Among food materials, cereals are often a staple part of any diet and so it was felt that by uncovering more about them, the team would be able to better understand the food of ancient people. They therefore set about trying to identify proteins of carbonised rice at some of the archeological sites they were located at. 'We tested some methods of protein preparation and then improved one selected method enough to identify more than 100 proteins by shotgun proteomic studies,' outlines Nishiuchi. 'From there, we found that proteins are commonly observed in the excavated carbonised rice from different sites. In particular, some proteins are commonly detected with their many trypsin-digested peptides by nano LC-MS/MS analysis.'

'In addition, these particular proteins were thermostable (meaning they are generally unaffected by heat and are therefore more resilient than other proteins),' confirms Nishiuchi. 'So, they were candidates as marker proteins which the team could use to identify plant species in plant remains and food crusts.' Highlighting these markers were important steps for the future of the team's research, as it gave them a reliable starting point from which other studies at other sites could begin from.

SECURING FUTURE FOOD

As well as the obvious importance to understanding which foods and plants were cultivated in ancient civilizations, it is possible that the team's findings could be translated into important applications within industry and healthcare. 'By identifying the microorganisms around excavated plant seeds, the team are able to gain an understanding of their condition - whether it is diseased or healthy,' notes Nishiuchi. 'It is possible that the team will find useful microorganisms that can help plants retain a healthy condition by activating plant immunity or suppressing phytopathogen growth.' If this can be achieved, the findings could lead to innovations such as the development of microbial pesticides that can help to prevent crop disease. It would present a situation where the food that ancient civilizations grew would secure the future of food for modern civilizations.

The next steps for the study will build on the work that has already been done by the team. 'The proteomic study does not contain the amplification step, such as polymerase chain reaction of DNA,' explains Nishiuchi. 'We are therefore hopeful that by using these methods in the future, we can use ancient proteomics to provide accurate information about specific biological species, including the plant itself and the microorganisms that existed in the excavated samples.'

There is also the exciting possibility that by employing the team's methods across many different excavations in archaeological ruins, we will be able to understand the specific ecological systems that existed back then. Elucidating the ancient biome could have significant positive benefits to protect current ecosystems.

Project Insights

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