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CONTENTS

Note

Wood selection for Japanese wooden Komainu Suyako Tazuru-Mizuno	1
Plavonoids and lignins: Dathway elucidation and modification for improved biomass properties Pui Ying Lam	3
Recent research activities	
Computer vision-based wood anatomy Sung-Wook Hwang and Junji Sugiyama	7
Determination of the cross-linking structure of lignocellulose, and evaluation of anti-viral active substances through biomass convert reaction	8
Structure, biosynthesis, and bioengineering of lignocellulose and phenylpropanoid metabolites for auture biorefinery	9
Yeast production of plant-derived bioactive prenylated phenolics	10
Strateole-2 (long-duration balloon flights at the tropical tropopause layer) project	11
A proposal for satellite observation of the whole atmosphere Superconducting Submillimeter-Wave Limb-Emission Sounder (SMILES-2) Masato Shiotani	12
Master Plan 2020: Study of coupling processes in the solar-terrestrial system	13
nfluence of electron beam irradiation as pretreatment on wood flow forming	14

Update on the yellow crazy ant project: global invasion, myrmecophile and
host-microbial interactions
Chin-Cheng Scotty Yang
UV grafting: surface modification of cellulose nanofibers
Utilization of <i>p</i> -toluenesulfonic acid for a sucrose adhesive
Cyclic loading tests of 3-storey CLT structures
Production of useful substances from tropical wood by fast pyrolysis
Simulations and modeling of geospace environment
Wireless power transfer for flying drone with novel beam forming
Exploration of space environments and developments of scientific instruments
Prize

Abstracts (Ph.D. thesis)

Studies on lignocellulose supramolecular structures and deconstruction properties in
lignin-altered rice mutants
Andri Fadillah Martin
Lipid secretion mechanism in Lithospermum erythrorhizon using shikonin as
a model of hydrophobic metabolites
Kanade Tatsumi
Development of software-defined multichannel receiver for
Equatorial Atmosphere Radar (EAR)
Nor Azlan bin Mohd Aris

Strong cellulose nanofiber composite hydrogels via interface tailoring
Optically transparent nanocellulose-reinforced composites via pickering emulsification
Study on structural analysis model for generalization of CLT panel method
Response control and seismic design method by applying oil damper for timber structure 40 Masatoshi Shinohara
Evolutionary history of a global invasive ant, <i>Paratrechina longicornis</i>
Study on novel rectifiers for microwave wireless power transfer system
Abstracts (Master thesis)
Local tracheid structure of <i>Cryptomeria japonica</i> and its potential for dendrochronology

Identification of two anatomically similar Cupressaceae species using two-dimensional			
MFA mapping	47		
Yusuke Kita			

Screening and identification of aldehyde dehydrogenases in a white-rot fungus,	
Ceriporiopsis subvermispora	48
Junseok Lee	

Screening and characterization of candidate genes involved in the biosynthesis of ceriporic acid B from *Ceriporiopsis subvermispora*, a selective lignin-degrading fungus 49 Akito Morita

Stepwise enzymatic fractionation and structural analysis of lignin-carbohydrate complex 50 Saho Kashima

Production of antiviral compounds from sugarcane bagasse by microwave solvolysis	
Tatsuya Miyazaki	

Characterization of an O-methyltransferase involved in antitumor lignan biosynthesis
in Thujopsis dolabrata
Yu Matsuura
Analysis of shikonins in the rhizosphere of <i>Lithospermum erythrorhizon</i>
Ko Sato
Establishment of virus-induced gene silencing (VIGS) system by use of domestic plant viruses in
<i>Lithospermum erythrorhizon</i>
Yuki Izuishi
Secretion of tomatine from tomato roots and analysis of tomatine in the field
A transport engineering approach to synthetic biology for artepillin C production in yeast 56 Koki Yanagihara
In silico search of candidate genes involved in shikonin production
in Lithospermum erythrorhizon
Hao Li
Development of a low noise RASS using a parametric array
Development of low-latitude ionosphere observation technique with dual-band
beacon experiment from COSMIC-2 and TBEx satellites
Artificial lignification improves mechanical property of cellulose microfibril gel
Tsubasa Yonekawa
Development of cellulose nanofiber/collagen sponge for 3D cell culture device
Effect of kraft pulping on cellulose nanofibrillation
The structural performance of reinforced concrete flames with CLT infills
Full-scale shaking table test of high-strength CLT structure with framework and verification of performance by analysis

The development of optimal CLT floor with two-by-four
Distribution, morphology, and phylogenetic analysis of the genus <i>Coptotermes</i> Wasmann (Rhinotermitidae) in Sumatra and West Java, Indonesia
Microplastics in a forest soil environment: interactions with termites
Relativistic acceleration of protons by EMIC waves in Jovian magnetosphere
A method for estimation of cold plasma density from whistler mode waves observed by Magnetospheric Multiscale mission spacecraft
Study on efficiency improvement of microwave power transfer system for stratospheric platform
Study on microwave power transmission systems in multipath environments
Study on high efficiency wireless power transfer method for the In-Line Inspection robot
Development and integration of the high-speed current detection circuits in particle sensors dedicated to Wave-Particle Interaction Analyzer
A study on data processing for the Wave-Particle Interaction Analyzer onboard the Arase satellite
Study of Electron Cyclotron Harmonic waves in the Earth's inner magnetosphere
Publications

Wood selection for Japanese wooden Komainu

(Laboratory of Biomass Morphogenesis and Information, RISH, Kyoto University)

Suyako Tazuru-Mizuno

Introduction

Wood identification of wooden cultural assets provides significant information of wood selection and a new interpretation of the role that these wood species played in ancient periods [1]. In Japan, wood anatomist Jiro Kohara and art historian Takeshi Kuno, performed wood identifications in the 682 Buddhist sculptures from all over Japan in the 1950s [2]. Further scientific wood identification of wooden statues from the 8th century in Japan has been systematically conducted by several researchers [3-5], a hypothesis was proposed that the selection of *Torreya nucifera* for the 8th century statues is referring to an ancient sacred text that mentions the use of the so-called Hakuboku, as a substitute for *Santalum album*, that sacred wood indigenous in India. Compared to the Buddhist statues, wood identification of wooden Komainu statues which were often placed closed to Buddhist statues and Deity statues has not been done on a large scale. Our approach is focusing on wood selection of Komainu statues preserved in Kyoto and Shiga prefectures, where many religious sculptures are preserved. For the wood identification, conventional microscopic method and synchrotron Xray micro-tomography (SRX-ray μ CT) [6] was applied.

Materials and methods

In this study, wood identification was performed on wooden Komainu, 14 samples from Shiga prefecture and 4 samples from Kyoto prefecture. These statues were all assumed to be made from the Kamakura period to Muromachi period (Table 1). Small samples were collected by curators or taken from the underside of the sculpture and cracks without harming the integrity of the sculptures. For wood identification, the samples were brought to Kyoto University and preparation were made. First, the samples were soaked in water for softening. For the following preparation of the microscopic slides, thin sections were taken, either by hand, in cross, radial and tangential directions ($20\mu m$ to $30\mu m$ thick). Sections were heated on a hot plate for removing the air-bubbles inside, and enclosed by the Gum-chloral. The slides were studied under the optical microscope (BX51), and photos were taken by a digital camera. Since 4 samples from Shiga prefecture and 1 sample from Kyoto prefecture were too fragile and tiny for making preparation, synchrotron Xray micro-tomography (SRX-ray μ CT) was applied at BeamLine20XU in SPring-8 located in Hyogo Prefecture, Japan. This method allows us to see the high resolution (0.472 μ m/pixel) 3-D image of the wood's anatomical micro-structure and to identify wood species.

Results

Of the 17 wooden komainu statues and one pedestal of Komainu statue located Shiga and Kyoto prefecture, the following wood species were identified:

Chamaecyparis obtusa (14 samples)

Torreya nucifera (4 samples)

Table. 1 show the details of samples and identified results. \square indicate the Komainu statue open its mouth and \square close its mouth. No. 14 is the pedestal of Komainu statue.

Discussion and conclusion

In Japan, a pair of guardian dogs Komainu is put in front of shrines and temples. They have a role to protect holy places because of its dignity. They are made of stone nowadays, however before Meiji period they were mainly made of wood and placed in front of sanctuary or set with Buddha and Deity statues. In fact, the systematic researches for the wooden Komainu have been delayed. The reasons include its difficulty in morphology classification, regional bias and lack of opportunity for investigation. Recently, as mentioned above, wood identification for Buddhist statues in Japan brought significant results concerning about the wood selection in Buddhism to Art historical field. On the other hand, wood selection criteria of other Buddhist art assets and Shintoism art assets has not been revealed.

Our study showed that some Komainu in Kyoto and Shiga prefecture were also made from Torreva nucifera which hold important meanings in making Buddhist sculpture in 8th century in Japan. Wood species used for Buddhist statues had mainly changed from Torreya nucifera to Chamaecyparis obtusa with their carving style had changed from "Ichiboku" statues carved out of a single bole to "Yosegi" statues separately carved various part and assembled. In any case, we may ask the question why the Japanese selected Torreva nucifera and Chgamaecyparis obtusa as a material for sacred wooden statues such as Buddha statues and Komainu. This is an initial step that opens the way for

Serial Number	Prefecuture	Shrine/Temple name	阿/吽	Date	Identified species	
1		Kasuga shrine	冏		Chamaecyparis obtusa	
2		Nonomiya shrine	冏	Kamakura	Chamaecyparis obtusa	
3			吽		Chamaecyparis obtusa	
4		Tamao shrine	ßन)		Chamaecyparis obtusa	
5			吽		Chamaecyparis obtusa	
6	Shiga	Shina shrine	吽	Nanboku cho	Chamaecyparis obtusa	
7		higa Mikami shrine	冏	Muromachi (1420)	Torreya nucifera	
8			吽		Torreya nucifera	
9		Syokakuji temple	吽		Chamaecyparis obtusa	
10		Mochihi shrine	阿	Muromachi	Chamaecyparis obtusa	
11			吽		Chamaecyparis obtusa	
12		Lionii shrino	阿		Chamaecyparis obtusa	
13		Jonji snine	吽		Chamaecyparis obtusa	
14		Nonomiya shrine	Pedestal	Muromachi (1552)	Torreya nucifera	
15	Kyoto		Sudai shrine	吽		Chamaecyparis obtusa
16		Tenman shrine	ßन)	Muromachi	Chamaecyparis obtusa	
17			吽		Chamaecyparis obtusa	
18		Yotsutsuji Hachiman shrine	吽	Unknown	Torreya nucifera	

further investigation for Komainu statues. For further investigation, identification, dating of many statues and constructing database would be necessary. So far, I found some Komainu statues with Buddha statues and Deity statues are preserved in foreign Museum through my resent study in the USA. I hope my study would be one of a strong call to all museums in all over the world to focus on wood species of Wooden Buddhism assets such as Buddhist statues, Komainu and other heritages.

*This research had partly presented in Bulletin of Shiga Prefectural Azuchi Castle Archaeological Museum (2020) [7] and Seizonken kenkyu in Kyoto University (2016).

Acknowledgements

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Table. 1 Details of statues and identification results.

Flavonoids and lignins: pathway elucidation and modification for improved biomass properties

(Laboratory of Metabolic Sciences of Forest Plants and Microorganisms, RISH, Kyoto University)

Pui Ying Lam

Introduction

Flavonoids and lignins are two major classes of plant secondary metabolites widespread across plant kingdom. They are not only essential for plant growth and physiology, but also important for human activities. Understanding the occurrences, structures and biosynthetic mechanisms of flavonoids and lignins therefore could facilitate our knowledge of plant development and evolution, and also biotechnology approaches to improve plant growth performance as well as biomass-orientated applications.

Flavonoids confer plant fertility, resistance towards biotic and abiotic stresses and pigmentation in flowers and fruits. They are also an important part of human diet due to their antioxidant, anti-cancer and anti-inflammatory properties. In grasses, the predominant flavonoids in vegetative tissues are tricin *O*-conjugates and flavone *C*-glycosides, while anther additionally contains flavonols [1]. The biosynthesis of flavonoid skeleton from *p*-coumaroyl-CoA in the phenylpropanoid pathway requires two highly conserved enzymes, chalcone synthase (CHS) and chalcone isomerase (CHI) (Figure 1). However, the downstream enzymes, in particular those involved in tricin biosynthesis, remained largely uncharacterized.



Figure 1. Flavonoid and lignin biosynthetic pathway in rice. Grey arrow: previously proposed tricin biosynthetic pathway.

On the other hand, lignins, which mainly deposit in the secondary cell walls of vascular plants, provide plant mechanical support, conductivity of water and nutrients and resistance towards pathogen attack. Lignins are considered a major component of plant biomass invaluable for the sustainable production of biofuels and biochemicals. However, lignins have long been viewed as a major recalcitrance for chemical pulping and production of fermentable sugars for polysaccharide-derived biofuels and biochemicals. Therefore, modification of lignin content and composition by bioengineering to improve plant biomass utilization properties has become a major research focus [2]. In general, lignins are generated in plant cell walls by polymerization of monolignols, i.e. coniferyl alcohols, sinapyl alcohols and small

amount of *p*-coumaryl alcohols, which respectively generate guaiacyl (G), syringyl (S) and *p*-hydroxyphenyl (H) units of lignins (Figure 1). With *p*-coumaroyl-CoA as a substrate, monolignols were generated after undergoing aromatic hydroxylations, *O*-methylations as well as side chain reductions (Figure 1). In grasses, a portion of the monolignols are acylated by *p*-coumarate (Figure 1) [3]. Interestingly, it was discovered that flavonoid tricin is also a canonical lignin monomer in grasses [4,5]. However, not much is known about the physiological functions and biosynthesis of such tricin-integrated lignins (tricin lignins). It is also intriguing to know whether manipulating tricin lignins could improve biomass utilization properties of grasses.

Here, the author's recent research activities related to the biosynthesis and bioengineering of flavonoids and lignins are summarized [6-12]. My research focus was particularly directed to elucidation of the tricin biosynthetic pathway, as well as analysis of tricin-depleted rice mutants with emphasis on their cell wall properties and biomass utilization properties. Other flavonoid- and lignin-related studies which the author conducted with many collaborators are also presented here.

Cytochrome P450 (CYP) enzymes involved in tricin biosynthesis

Tricin biosynthetic pathway in rice was elucidated by the identification of two grass-specific CYP enzymes. The first step of tricin biosynthesis involves the formation of flavone skeleton. Several enzymes in rice have been shown to harbor such *in vitro* activities, but our results suggested that CYP93G1 is the sole flavone synthase II (FNSII) enzyme catalyzing this reaction step, leading to the formation of soluble and lignin-bound tricin (Figure 1) [7,8].

It was long believed that the hydroxylation steps in tricin biosynthesis are catalyzed by flavonoid 3',5'-hydroxylase (F3'5'H), an enzyme that catalyzes the same reaction steps with other classes of flavonoids as substrates [15]. However, in contrast to this general belief, we have identified CYP75B4 as a novel enzyme that function as apigenin 3'-hydroxylase/chrysoeriol 5'-hydroxylase (A3'H/C5'H) catalyzing hydroxylations specifically in tricin biosynthetic pathway [9,10]. The identification of CYP75B4 as an A3'H/C5'H has reconstructed the tricin biosynthetic pathway with chrysoeriol instead of tricetin as the intermediate (the previously proposed pathway that utilizes F3'5'H instead of A3'H/C5'H is indicated in grey in Figure 1) [9,10]. On the other hand, we have also found out that the canonical flavonoid 3'-hydroxylase (F3'H) does not play a significant role in hydroxylation step in tricin biosynthesis, but it is the predominant hydroxylase involved in flavone *C*-glycoside biosynthesis [10]. Collectively, our work suggested that grasses have recruited two closely related hydroxylases for two parallel pathways leading to the formation of tricin type metabolites and flavone *C*-glycoside [10].

Improvement of biomass utilization properties by manipulating tricin biosynthesis

Tricin was discovered as a canonical lignin monomer in grasses but not much is known about its *in planta* functions and how it affects biomass utilization properties. To address these issues, we have carried out in-depth cell wall analysis using rice mutants deficient in CYP93G1 or CYP75B4. Both CYP93G1 and CYP75B4 mutants generated altered lignins depleted in tricin and incorporated with novel flavonoids, naringenin and apigenin, respectively (Figure 1) [8,10]. Biomass digestibility was improved in both CYP93G1 and CYP75B4 mutants compared with wild-type controls without any growth penalty, suggesting molecular manipulation of these tricin biosynthetic genes could increase the production of glucose from rice biomass for further biorefinery applications [8,10].

Bifunctional enzyme involved in both tricin and monolignol biosynthesis

5-hydroxyconiferaldehyde *O*-methyltransferase (CAldOMT) is well known for its function in the biosynthesis of S lignins (Figure 1) [16]. We have further shown that CAldOMT is also involved in the biosynthesis of soluble and lignin-bound tricin (Figure 1) [9,11]. Rice CAldOMT mutant was depleted in tricin lignins and S lignins, and produced atypical 5-hydroxyguaiacyl (5H) lignins [11]. Together with the results of *in vitro* enzymatic assays of recombinant CAldOMT, our results suggested that CAldOMT is a bifunctional enzyme catalyzing the key methylation steps in the two parallel pathways leading to the formations of S lignins and tricin-type metabolites including tricin lignins (Figure 1) [11].

Tricin biosynthesis in Medicago species

Although tricin is ubiquitously accumulated in grass species, it is only accumulated in isolated and unrelated dicot lineages including *Medicago* species. The tricin biosynthetic pathway in *Medicago* species was explored by the identification of novel *Medicago*-specific flavonoid B-ring hydroxylases which harbor similar *in vitro* and *in planta* A3'H/C5'H activities as rice CYP75B4 (a study led by Dr. Clive Lo's research group of the University of Hong Kong, Hong Kong, China) [12]. This finding also suggests that *Medicago* species have evolved and recruited another set of enzymes for tricin biosynthesis independent from those in grass species [12].

Involvement of flavonoids in fertility in rice

Flavonoids, more specifically those belong to the flavonoid class flavonols (Figure 1), were reported to be essential for fertility in some but not all plant species. We have found out that flavonoids were also essential for fertility in rice; rice chalcone synthase (CHS; entry enzyme for flavonoid biosynthesis as indicated in Figure 1) mutant which was completely depleted in the accumulation of all kinds of flavonoids was sterile [1]. However, in contrast to the essential roles primarily played by flavonols for fertility in other plant species, our finding suggested that a combination of different classes of flavonoids (flavonols, flavones and flavone *C*-glycosides) confer fertility in rice [1].

Improve Populus biomass properties by regulating lignin biosynthesis in fibre cells

Wood biomass is widely used for generating fuels, timber products, chemicals and papers. A new strategy to improve *Populus* biomass properties without any growth penalty was developed by fibre-specific suppression of lignification through down-regulating lignin-biosynthesis related transcription factor 1 (LTF1) (a study led by Prof. Laigeng Li's research group of the Shanghai Institutes for Biological Sciences, China) [13]. The cell-type specific suppression of lignification employed in this study also revealed that fibre cells are more enriched in S lignins, whereas vessel cells are more enriched in G lignins [13].

Lignin-inspired modification of nanocellulose surface properties

A new strategy to improve surface properties of nanocellulose was proposed as inspired by lignification in plant cell walls (a study led by Prof. Takuya Kitaoka's research group of Kyushu University, Japan) [14]. Using pickering emulsion system, lignin was uniformly polymerized on the surface of nanocellulose, modulating the hydrophilicity of nanocellulose [14]. In addition, the presence of nanocellulose at oil-water interface in the pickering emulsion system also influenced the structures of the synthetic lignins generated [14]. This work has provided new insight on modifications of surface properties of nanocellulose which could be useful for industrial applications.

Conclusions

In the author's recent research activities, flavonoid biosynthetic pathway, especially tricin biosynthetic pathway, in rice was elucidated. These works also suggested that manipulating tricin lignins in rice could improve biomass utilization properties that could be beneficial in cellulose-oriented biorefinery applications. The author's studies were also extended to explore tricin biosynthesis in non-grass species, functions of flavonoids in fertility in rice as well as other lignin-related research including improving biomass utilization properties of *Populus* and modifying surface properties of nanocellulose by *in vitro* lignification. In RISH, Kyoto University, the author is currently conducting studies aiming to bioengineer grass biomass plants by introducing new flavonoid and lignin features for further improving their biorefinery applications.

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Computer vision-based wood anatomy

(Laboratory of Biomass Morphogenesis and Information, RISH, Kyoto University)

Sung-Wook Hwang and Junji Sugiyama

Training a sufficient number of frontline workers to perform wood identification has always been a big challenge in the wood industry. In recent years, the remarkable advancement in the field of artificial intelligence (AI) has created a new research field in wood science called computer vision (CV)-based wood identification. Since most of the CV-based wood identification studies focused only on the development of automated wood identification systems, understanding of the wood itself was neglected, and consequently their achievements were difficult to extend to various subfields encompassed by wood science. For the further expansion of AI technologies into wood science in this context, we aim to understand CV based on the domain knowledge of wood anatomy.

Local features, which are distinct structural elements such as points, corners, and edges in an image, are suitable for approaching the microscopic scale image used in established wood anatomy. From clustering analysis of local features detected in cross-sectional micrographs of wood, it was confirmed that the features can be classified into major anatomical elements of interest in conventional wood anatomy [1]. In addition, it has been found that local features encoded by clustering can be used as anatomical predictors for quantitative wood anatomy, as well as improving discriminative power for wood identification [2]. Combining this method with an image partitioning strategy, especially radial partitioning from pith to bark

helps to distinguish wood porosity. Feature importance measures based on the rarity of image features detected by CV provides clues to predict speciesspecific features. In Fig. 1, both species in Lauraceae have vessels as informative features, but in more detail, the speciesspecific features are large earlywood vessels for Sassafras tzumu, whereas inter-vessel pitted walls for Lindera communis. As such. anatomical approaches to CV may provide clues and new insights into species-specific morphology that have not yet been identified in established wood anatomy.



Figure 1. Species-specific features of *Sassafras tzumu* (left – large vessels in earlywood) and *Lindera communis* (right – inter-vessel pitted walls) determined by computer vision.

As a digitized database of the wood collection of the Kyoto University Xylarium database, the recently released Xylarium Digital Database (XDD) for wood information science and education covers the widest biodiversity among the open wood digital databases currently available. Based on the anatomical studies mentioned above, XDD was built with two pixel resolutions and consists of a total of 16 micrograph datasets (https://repository.kulib.kyoto-u.ac.jp/dspace/handle/2433/250016?locale=en). In the historical context where there was no large wood database annotated by wood experts, the emergence of XDD could make an important contribution to the advancement of wood science with deep learning techniques as well as conventional machine learning.

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Determination of the cross-linking structure of lignocellulose, and evaluation of antiviral active substances through biomass convert reaction

(Laboratory of Biomass Conversion, RISH, Kyoto University)

Hiroshi Nishimura, Chihiro Kimura, Ruibo Li, and Takashi Watanabe

Promoting the utilization of plant biomass for a sustainable society is an urgent task. Plant biomass is the most abundant organic resource on earth. The secondary plant cell wall is mainly composed of cellulose, hemicellulose, and lignin. Hemicellulose surrounds cellulose microfibrils and is filled with lignin. Each component is properly packed, and there are covalent linkages between the lignin and hemicellulose. However, direct evidence of those linkages has been under the question for many years. We have established a highly efficient separation/extraction method for the lignin-carbohydrate complex (LCC) and succeeded in the first direct proof of lignin-polysaccharide linkages by using the multi-dimensional NMR spectroscopy (Figure 1). 2D-NMR, such as ¹H-¹³C HSQC, HMBC, and 3D TOCSY-HSQC, clearly demonstrated the covalent bond between glucomannan and lignin and elucidated the entire cross-linked structural unit. [1]

We have been researching to obtain bioactive substances from lignocellulosic biomass. By conducting an artificial conversion reaction of lignocellulose and fractionating, we have found the anti-viral active ingredient. Catechol, 3-methyl-, 4-methyl-, 4-ethyl-, and 3-methoxycatechol, and 2-methyl-1,4-benzenediol were identified as the main anti-viral compounds from pyroligneous acid of pine and clarified structure-activity correlations. [2]

Twenty-five phenolic derivatives were identified from pyroligneous acid of hardwood, softwood, and bamboo and evaluated as anti-viral active ingredients. [3]

Recently, we have found a lignin-derived anti-viral substance from sugarcane bagasse by microwave heating at 200 °C in aqueous glycerol containing 0.5 % H_2SO_4 . It has no cytotoxicity and showed potent inhibition towards encephalomyocarditis virus (EMCV) replication as a result of cell experiments. [4]

These results will contribute to protecting infections and human health care in our globalized society, Sustainable Humanosphere.



Figure 1.

Chemical structure of the lignin-carbohydrate linkage and typical NMR correlation spectra and future prospects [1]

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Structure, biosynthesis, and bioengineering of lignocellulose and phenylpropanoid metabolites for future biorefinery

(Laboratory of Metabolic Science of Forest Plants and Microorganisms, **RISH, Kyoto University)**

Toshiaki Umezawa, Yuki Tobimatsu, and Masaomi Yamamura

It is becoming increasingly important to establish a sustainable society by reducing our excessive reliance on fossil resources and mitigate global climate change. As lignocellulosic biomass represents the most abundant renewable and carbon-neutral resources on earth, technologies to improve their utilizations are key for realizing the goal. In this context, we investigate the structure, biosynthesis and bioengineering of lignocellulose using various model plants and biomass crops. In addition, we are interested in understanding the biosynthesis of plant-derived phenylpropanoid metabolites with useful biological activities. Our program typically integrates research ideas and approaches based on chemistry, biochemistry, and plant molecular biology. Among a wide variety of biomass feedstocks, grass biomass crops, such as Erianthus, Sorghum, sugarcane and bamboo, have attracted particular attention due to their high biomass productivity and superior environmental adaptability. To explore new breeding strategies to improve the production of useful fuels and materials from grass biomass, we seek to develop transgenic rice plants that produce biomass with improved utilization properties. Our research particularly focuses on manipulating lignin, a phenylpropanoid polymer accounting for 15-30 wt% of lignocellulosic biomass. We have developed various rice transgenic lines in which specific genes encoding enzymes and transcription factors functioning in the lignin biosynthetic pathway are down- and/or up-regulated. Some of our developed transgenic lines display notably improved biomass utilization properties. In parallel, we are also working on selective breeding of grass crop varieties, such as *Erianthus* spp. and *Sorghum* spp., with superior lignins suited for bioenergy and biomaterial productions. In addition, aiming at biological production of useful phytochemicals, we have been characterizing plant and microbial enzymes/genes involved in formations of bioactive phenylpropanoids such as lignans and norlignans. Our recent projects include elucidation of the biosynthesis of antitumor podophyllotoxin in Anthriscus sylvestris, unravelling crystal structures of hinokiresinol synthases, unique enzymes responsible for the enantioselective formation of bioactive norlignans, and identification of enzymes/genes involved in the formation of estrogenic mammalian lignans (enterolignans) via human intestinal bacteria.

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Yeast production of plant-derived bioactive prenylated phenolics

(Laboratory of Plant Gene Expression, RISH, Kyoto University)

Ryosuke Munakata, Akifumi Sugiyama, and Kazufumi Yazaki

Plant-derived metabolites have supported human life for a long time as medicines, nutrients, and so on. However, considering the assumption that plants produce 200,000–1,000,000 metabolites, much majority of plant metabolites that potentially show beneficial bioactivities for humans are remained to be exploited yet, which is mainly due to the generally low and variable quantity of metabolites in plants. For such problems, synthetic biology recently opens a way to highly and stably produce plant-derived bioactive compounds in microorganisms by introducing related biosynthetic genes.

Honeybees prepare propolis from resinous plant tissues to physically and chemically reinforce their hives. This honeybee product shows a variety of pharmaceutical bioactivities which are attributed to plant-derived metabolites. Among different propolis types by production sites, Brazilian green propolis is a major propolis type and globally commercialized. A main bioactive of this propolis type is artepillin C (di-prenylated *p*-coumaric acid) derived from an Asteraceae bush, *Baccharis dracunculifolia*. Artepillin C shows various bioactivities beneficial for human health such as anti-oxidation and anti-obesity, while the concentration of this metabolite in Brazilian green propolis is highly variable depending on natural factors such as climates and performance of honeybees, being a serious problem in quality control of propolis.

We tried synthetic biological production of artepillin C by introduction of its biosynthetic pathway in yeast. To reconstruct artepillin C pathway, we conducted identification of a gene encoding a prenyltransferase (PT) for *p*-coumaric acid which has not been reported so far. A promising gene source is *B. dracunculifolia*, the major habitat of which is restricted in southwestern part of Latin America, meaning that utilization of this species requires consideration of Nagoya protocol. Therefore, we selected *Artemisia capillaris* (kawara-yomogi in Japanese), which is native to Japan, as an experimental sample.

A transcriptome analysis of *A. capillaris* leaves that accumulate artepillin C found *A. capillaris PT1* (*AcPT1*) as a fine candidate. Biochemical characterization showed that its gene product solely catalyzes a sequential di-prenylation of *p*-coumaric acid to synthesize artepillin C via drupanin, the mono-prenyl intermediate (Fig. 1). Together with *p*-coumaric acid biosynthetic genes, *AcPT1* is introduced in budding yeast strain which is engineered to highly accumulate DMAPP, the prenyl donor substrate of AcPT1 [1]. The resulting yeast transformant was shown to produce artepillin C. Improvements of the artepillin C-producing strain toward higher productivity would provide a stable source of artepillin C alternative to propolis.



Fig. 1 The enzymatic function of AcPT1

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Strateole-2 (long-duration balloon flights at the tropical tropopause layer) project

(Laboratory of Atmospheric Sensing and Diagnosis, RISH, Kyoto University)

Hiroyuki Hashiguchi

The tropical tropopause layer (TTL) is a key region of the atmosphere in the tropics as it interfaces the moist, quickly mixed troposphere below with the dry, stratified stratosphere above. The TTL constitutes the gateway for air entering the stratosphere, and the processes occurring in the TTL contribute to setting the composition of the whole stratosphere. Strateole-2 is an international project led by Laboratoire de Météorologie Dynamique (LMD), France [1, 2]. Strateole-2 is aimed at providing observations of the TTL (16-20 km altitudes in the tropics) to better understand dynamical and transport processes in this region. The originality of the project comes from the use of long-duration superpressure (SP) balloons developed by Centre National d'Etudes Spatiales (CNES), France, which are able to fly for several months at targeted altitudes. During the flight campaigns, each balloon is carried by winds and circumnavigate the Earth a few times. By moving with the air along a quasi-Lagrangian trajectory, the SP balloon measurements provide unique data of chemical and physical processes evolving over time in a given air mass. The long duration of the flight is of particular interest for characterizing the dynamics and chemistry of the TTL.

There are, however, difficulties and limitations, since the SP balloons follow trajectories at a constant density level above the cold point tropopause. Complementary observations such as radiosondes (Lagrangian measurements in vertical direction) and the Equatorial Atmosphere Radar (EAR) (Eulerian measurements) at Kototabang, Indonesia (100.3E, 0.2S) are essential to interpret and understand the SP balloon measurements, which provide the information on the local dynamics of the atmosphere (wave field and occurrence of turbulence), and eventually the vertical profile of water vapor or ozone. About the meso-scale dynamics, radar and radiosonde measurements provide complementary information about the vertical structure of the wave field, especially of the large-scale Kelvin waves. Ozone sondes allow to describe the ozone gradients at the height of the SP balloon flights and to characterize the signature of large wave activity. Such observations may prove crucial in order for the Strateole-2 data to be conclusive regarding vertical transport and dehydration issues.

The first Strateole-2 preparatory campaign was conducted in November 2019-February 2020. Eight SP balloons were launched (20 SP balloons will be launched during two main campaigns scheduled in late 2021 and late 2024). SP balloons were launched at the Seychelles islands (55E, 4S) and the quasi-biennial oscillation (QBO) was westerly in this period. So the balloon crosses the Indian Ocean and reaches Indonesia in about one week after launch. (The second passage was two months later, but the dispersion in latitudes of the balloon was quite large.) The EAR was continuously operated in the troposphere/stratosphere standard observation mode with temporal and vertical resolutions of 1 min and 150 m, respectively, in November 19-December 6 to measure the wind field, wave activity and turbulence. In this period, 20 GPS radiosondes with 11 ozone sondes were launched at the EAR site. They bring complementary observations about the temperature and ozone field (mean state and wave activity) as well as about the turbulent regions.

Acknowledgements

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A proposal for satellite observation of the whole atmosphere - Superconducting Submillimeter-Wave Limb-Emission Sounder (SMILES-2)

(Laboratory of Atmospheric Environmental Information Analysis, RISH, Kyoto University)

Masato Shiotani

The Superconducting Submillimeter-Wave Limb-Emission Sounder (SMILES) launched by an H-IIB rocket with the H-II Transfer Vehicle (HTV) in September 2009 and attached to the Japanese Experiment Module (JEM) on the International Space Station (ISS) demonstrated high-sensitivity submillimeter limb-emission sounding of atmospheric observations. Though the observation period is limited to about 6 months (October 12, 2009- April 21, 2010) owing to the failure of a critical component, outstanding results have been achieved such as an identification of diurnal cycle of the stratospheric ozone, which had not been examined yet because of poor data quality in the previous satellite measurements (Sakazaki et al., 2013). In addition, by comparing SMILES data with existing observation data and results from the chemistry climate models, it has been recognized that we need a reference data for temperature, wind, and minor species with high precision to constrain the models. After the launch there has been discussions about the possibility to derive wind information from the Doppler shift of observed lines, and the necessity to have temperature information from adequate line selection. Thus, we now recognize the potential of high-sensitivity observations about temperature and wind fields up to 150 km to cover the uncharted territory, the mesosphere and the lower thermosphere (MLT).

Based on the SMILES heritage, we propose a satellite mission to observe temperature and wind fields, and distributions of atmospheric minor species for the full diurnal cycle from the middle atmosphere (stratosphere and mesosphere) to the upper atmosphere (thermosphere and ionosphere) for a period of five years. SMILES-2 observations will enable us to obtain global information with unprecedented accuracy on the whole atmosphere including upper mesosphere and lower thermosphere where observation data have been lacking. This mission has three specific science objectives.

- To investigate the effects of lower atmosphere on the MLT via upward propagating atmospheric waves such as atmospheric tides
- To understand atmospheric variations due to energy inputs from the magnetosphere (particle precipitation and magnetic storm)
- To understand the solar-terrestrial system by providing benchmarks for whole atmosphere models

Using observation data from the middle atmosphere to the upper atmosphere as a whole, we will be able to grasp the 4-D dynamical structure of diurnal variations (atmospheric tides) which are one of the most essential variabilities in the earth's atmosphere. For understanding climate change in view of chemical processes affecting the ozone layer, we will be able to utilize high-sensibility measurements of the atmosphere, a transition layer between the atmosphere and the outer space, we will be able to clarify a role of energy inputs from the magnetosphere. The observation data will provide unprecedented benchmark data to comprehensive whole-atmosphere simulation models whose development is necessary for accurate seasonal weather forecasts, future climate projections, and space weather forecasts. For more detailed description about the proposal, see Shiotani et al. (2019).

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Master Plan 2020: Study of coupling processes in the solar-terrestrial system

(Laboratory of Radar Atmospheric Science, RISH, Kyoto University)

Tatsuhiro Yokoyama and Mamoru Yamamoto

Outline

"Coupling processes in the solar-terrestrial system" aims to study the solar energy inputs into the Earth and the response of Geospace (magnetosphere, ionosphere, and atmosphere) to these energy inputs[1]. Solar energy can mainly be divided into two parts: solar radiation and solar wind. The former involves infrared, visible, ultraviolet and X-ray, while the latter is the high-speed flow of plasma particles. Solar radiation is maximized at the equator. Atmospheric disturbances are actively generated near the Earth's surface and further excite various types of atmospheric waves, which propagate upward carrying energy and momentum. On the other hand, the energy associated with solar winds converges into the polar regions where disturbances are generated. Part of the energy is transported toward lower latitudes and lower atmospheric regions. This project was selected as an important project in the Master Plan 2014 and 2017 by the Science Council of Japan. Recently, it was again selected in the Master Plan 2020.

We propose to establish large atmospheric radars with active phased array antennas at the equator and the arctic region. In the equatorial region, we focus on the Indonesian region where atmospheric disturbances are most intense. We strive to establish a comprehensive observatory in Indonesia with the Equatorial MU (EMU) radar as the main facility. Additionally, we are part of an international collaboration to construct a state-of-the-art radar, called EISCAT_3D, in northern Scandinavia. We also develop a global observation network of portable equipment from the equator to both polar regions. With these radars and global network, we will study the flow of the energy and materials in the whole atmosphere.



Figure 1. Large atmospheric radars and global observation network

Equatorial Fountain

Cumulonimbus convection is active in the equatorial atmosphere. It generates various types of atmospheric waves that propagate upward to transport energy and momentum into the upper atmosphere, including the ionosphere. In addition, different kinds of materials (atmospheric minor constituents) originating at low- and mid-latitude regions that converge into the equatorial region are blown upward through the tropopause; they eventually reach the middle atmosphere and spread around the globe. In the upper atmosphere, there are plasma disturbances, and the equatorial ionization anomaly is generated around the equator. We will capture the energy and material flow occurring in all height ranges of the equatorial atmosphere as the "Equatorial Fountain" using the Equatorial MU (EMU) radar. In 2001 we established the Equatorial Atmosphere Radar (EAR) in West Sumatra, Indonesia and continue our observations as part of an international collaboration. In this project, we propose developing the EMU radar, which is 10-times more sensitive than the EAR. Master Plan 2020 may help the realization of the EMU in the near future.

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Influence of electron beam irradiation as pretreatment on wood flow forming

(Laboratory of Fiber Multiplication, RISH, Kyoto University)

Satoko Okubayashi

Wood flow forming is a technique, in which products of various shapes can be obtained by hot-pressing wood in a metal mold to cause detachment and movement of cells and then rejoining. Currently, the problem is that the load required for flow is high. The purpose of this study is to examine the influence of electron beam irradiation as a pretreatment on mechanical properties of flow formed wood with and without containing resin

Flat-sawn specimens of hinoki (Chamaecyparis obtuse) were used as a test material in oven-dry condition. Irradiation was carried out using a EBC-300 (NHV Corporation) at an accelerating voltage of 300 keV and an average absorbed dose of 0 to 263 kGy. Free compression tests were performed on the irradiated specimens at moist and heated conditions to obtain stress-strain curves. Young's modulus, proportional limit stress, and flow starting stress obtained from the curves were decreased by increasing dosage, which suggests the electron beam decomposed the cellulose chains. The proportional limit strain,

flow starting strain, and maximum strain in the curves indicated that the EB irradiation promoted the flow deformation, though it did not affect the elastic deformation and densification deformation. The change in shape of specimens during the compression test was also remarked. The deformation along the tangential direction which was also observed for the non-irradiated specimens, was accelerated by the electron beam irradiation. The irradiation also induced deformation along the fiber direction which was not observed in the non-irradiated specimens, and this deformation enhanced with increase of the dosage. It was indicated that the deformation along the fiber direction was caused by fracture of the wood fiber due to the EB irradiation by a scanning electron microscopic observation [1].



Figure 1. Influence of absorbed dose on Young's modulus and MOR of molded specimen

On the other hands, the specimen after irradiation was heat-treated in a blow dryer and soaked into a phenol resin aqueous solution under reduced pressure until the weight increase rate became about 180%. After conditioning the specimen at 30°C, the molding was performed using a materials testing machine (INSTRON5582) and a disk molding die. A strip of specimen was cut from the molded material and used for a three-point bending test that was performed using a materials testing machine (INSTRON4411). Young's modulus and modulus of rupture (MOR) decreased with increasing absorbed dose. However, even with the lowest breaking stress, the strength was about 200 MPa as shown in Figure 1. A boiling test was also examined to check water resistant of the molded specimens. The dimensional stability of irradiated specimen against water reduced, though its effect was small.

Acknowledgements

This study was carried out in a collaboration with Prof. Kozo Kanayama, Prof. Kenji Umeura, Dr. Soichi Tanaka, Mr. Hideaki Sugino in Laboratory of Sustainable Materials and Mr. Yuga Kasamatsu, a cooperated student of Kyoto Institute of Technology.

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Update on the yellow crazy ant project: global invasion, myrmecophile and host-microbial interactions

(Laboratory of Urban Pestology, RISH, Kyoto University)

Chin-Cheng Scotty Yang

Overview of the yellow crazy ant project

Yellow crazy ant, *Anoplolepis gracilipes* is an invasive species posing a major threat to native ecosystem and global biota. This article is to provide an update on the progress of ongoing projects concerning this invasive ant in the Laboratory of Urban Pestology.

Global invasion

Using population genomic approach, we identified peninsular Southeast Asia as the most likely origin of the yellow crazy ant, and also unraveled two major historic introduction pathways: one into East Asia and the other into Indonesia/Papua New Guinea. Most Pacific islands received colonization by the ants in the East Asia clade, whereas ants in the other clade are responsible for the invasion into Oceania. The invasion process of this ant is consistent with a biogeographic pattern typical of other introduced ants where most of the invasions tend to originate from neighboring introduced populations.

Myrmecophile

Myrmecophiles refer to organisms that live in association with ants. We assessed diversity and taxonomy of myrmecophilous ant crickets (Fig. 1) associated with the yellow crazy ant across Indo-Pacific regions, and identified the presence of at least eight species. This finding represents to date the highest ant cricket diversity associated with a single ant species. The molecular data indicate that these ant crickets have spread as a hitchhiker with the yellow crazy ant. Our preliminary data also suggest that co-introduction of both the yellow crazy ant and ant cricket has pushed out native ant crickets most likely via host competition.

Host-microbial interactions

Myrmecophiles are generally distantly related to ants (i.e., as in different insect orders), offering a unique opportunity to study how microorganisms are circulating between ants and myrmecophiles. We thus analyzed *Wolbachia* diversity in two species of crazy ants and their associated ant crickets, and showed that sharing the same *Wolbachia* strains between the two parties is rather common (Fig. 2), and this most likely results from frequent horizontal transmissions.

Acknowledgements

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Figure 1. Interactions between an ant cricket and a yellow crazy ant worker.



Figure 2. Maximum likelihood phylogeny of ant crickets and their corresponding *Wolbachia* strains.

UV grafting: surface modification of cellulose nanofibers

(Laboratory of Active Bio-based Materials, RISH, Kyoto University)

Xianpeng Yang, Kentaro Abe, and Hiroyuki Yano

Polymer grafting provides cellulose nanofibers (CNFs) with new features. However, the process of polymer grafting, which generally involves a large amount of organic solvent, is environmentally unfriendly.

In the 1970s, ultraviolet (UV) irradiation was used to generate radicals on the surfaces of cotton cellulose fabric in neutral aqueous conditions, initiating graft polymerization without an external initiator. This method is called UV grafting. Subsequently, UV grafting has been reported in native cellulose from the yellow poplar, cellulose derivatives, and lignocellulose fibers.

CNFs have a much higher specific surface area than cotton fabrics, and hemicellulose has a similar photochemical behavior to cellulose. Therefore, UV grafting is expected to be an effective means of grafting polymer from the surfaces of CNFs. In the current study, we try to utilize the UV to generate radicals on CNFs in aqueous conditions, aiming to graft polymers on CNFs with an organic solvent-free process. We present the new features of polymer grafted CNFs and confirm the versatility of UV grafting by using various vinyl monomers.

We placed a CNF aqueous suspension and the monomer in a Schlenk tube (Figure 1). This was followed by non-strict deoxygenation by bubbling nitrogen gas. We then irradiated the suspension with a UV lamp at constant temperature and stirring speed at a maximum wavelength of 365 nm. As a result, poly (methyl methacrylate) (PMMA) was readily grafted from the CNFs, without destroying the crystalline region of cellulose. As well as PMMA, various other polymers can be grafted from CNFs without using organic solvents.



The CNFs dispersed well in water owing to their

hydrophilic surfaces. Increased hydrophobicity resulted in the aggregation of CNF-g-PMMA in water. After drying and re-dispersing in DMF, we observed severe aggregation of the raw CNFs. On the other hand, CNF-g-PMMA re-dispersed readily in DMF (r. The water contact angles (CAs) of the CNF-g-PMMA film were $99.7 \pm 0.7^{\circ}$, much larger than the CA of the raw CNFs. It should be noted that the CAs of CNF-g-PMMA films were higher than the intrinsic CA of PMMA (around 70°), which arose from the roughness generated by hot pressing. The aggregation of CNF-g-PMMA in water and the re-dispersibility of dried CNF-g-PMMA in an organic solvent might facilitate dewatering for storage and supply of CNF materials. Increased hydrophobicity might also improve the compatibility of the CNFs with a hydrophobic matrix.

Surprisingly, CNF-g-PMMA had a unique nanofiber–nanoparticle structure in water. We assume that the primary free radicals were generated on the surfaces of CNFs and that the polymerization process of PMMA was similar to soap-free emulsion polymerization. Our green process may provide CNF materials with new advantages. For example, the aggregation of CNF-g-PMMA in water facilitated the dewatering process, which is important for storage and supply of CNF materials. We can use polymer grafted CNFs in the form of powders owing to their redispersibility after drying. Furthermore, the versatility of UV grafting can improve the compatibility between CNFs and various polymer matrixes.

We believe that this technique, called UV grafting, may provide a greener method for producing CNF materials with novel features.

Utilization of *p*-toluenesulfonic acid for a sucrose adhesive

(Laboratory of Sustainable Materials, RISH, Kyoto University)

Shunsuke Sakai and Kenji Umemura

Introduction

Commercial wood adhesives are generally synthesized using chemicals derived from fossil resources. Therefore, there are concerns about the dependency on fossil resources and environmental impact. To solve these problems, researches on bio-based adhesives such as protein-, tannin-, and lignin-based adhesives have been conducted. We have been focused on sucrose as a raw material of bio-based adhesive. According to our previous paper¹, sucrose was changed to matter insoluble in boiling water by adding ammonium dihydrogen phosphate (ADP) and heating. Actually, particleboards manufactured by using ADP added sucrose as an adhesive exhibited good mechanical properties and water resistance²). However, high adhesive content and high hot-pressing temperature were required when manufacturing the particleboards. To improve these disadvantages, we are trying to use p-toluenesulfonic acid (PTSA) which is widely known as an acid catalyst. The effect of PTSA content on the insolubility of sucrose in boiling water was measured.

Experiment

Sucrose and PTSA were dissolved in distilled water at various weight ratios: 100:0, 95:5, 90:10, and 85:15. The concentration of the solution was adjusted to 50wt%. Each solution was dried at 60 °C for 24 h, and the dried samples were heated at 120, 140, 160, and 180 °C for a 10 min. The heated samples were immersed in boiling water for 4h, and each insoluble matter rate was calculated. In addition, thermal analyses and FT-IR were measured.

Results and discussion

Figure.1 shows the effect of heating temperature on insoluble matter rate at various sucrose/PTSA ratios. The insoluble matter rate of sucrose (100:0) was not observed at all, indicating that sucrose was soluble irrespective of heating temperature. The





insoluble matter rate of PTSA added sucrose tended to increase with increasing heating temperature. The effective heating time and the mixture ratio at 180 °C were 10 min and 95:5, respectively. Compared with the result of ADP¹, PTSA was able to insolubilize sucrose by a small amount of addition and a low heating temperature. According to the results of FT-IR, it was suggested that a furan ring and carbonyl group was contained in the heated mixture. This indicated that sucrose was changed to a high water-resistant substance containing furan compounds by the addition of PTSA and heating treatment. Furthermore, thermal analyses revealed that the thermal change of PTSA added sucrose occurred at a temperature lower than that of ADP added sucrose. Sucrose with added PTSA would be expected as a wood adhesive.

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Cyclic loading tests of 3-storey CLT structures

(Laboratory of Structural Function, RISH, Kyoto University)

Hiroshi Isoda and Xiaolan Zhang

1. Introduction

For the sake of promoting cross laminated timber (CLT) structure, Japanese government notifications (GN) on the structural design of CLT panel buildings and definition of standard strength of CLT were issued on 2016. Following the issue of the GN, the guidebook on the regulations of the GN and the manual on design and construction of CLT panel buildings were published on Jun. and Oct. 2016 respectively. In the GN and manual, three kinds of platform framing CLT structures were classified. Besides the platform framing structure, balloon framing structure (Figure 1) is the other type in CLT buildings. However, there is little information about the balloon framing CLT structures and compared with the seismic performance of balloon framing structures and compared with the standard model specified in GN.



Figure 1. Platform and balloon framing structures

2. Research subjects

Seismic performance of balloon framing CLT structures

Static cyclic loading tests were carried on four full-scale 3-story CLT structures: ① platform framing with small size shear walls; ② balloon framing with continuous shear walls; ③ platform framing with broad panels; ④ balloon framing with continuous shear walls coupled with glulam beams. The photos of 4 specimens were shown in Figure 2.

Feasibility of design method specified in GN

Since the balloon framing structures have not been specified in current standard, the seismic design should follow limited strength design method, which is much complex than allowable design method. According to the experiment results, the seismic performance of (2) and (3) are similar with standard model of GN. It is possible to apply the allowable design method to these two types of structures.

3. Future plan

In order to verify the results, numerical models will be built and analyzed. Corresponding element tests will be conducted to get the structural properties of CLT members and joints, such as the embedment tests, compression tests and beam-wall tests. In addition, the results of static tests will be contrasted with that of corresponding shaking table tests (as shown in Figure 3).



Figure 2. Photos of 4 specimens



Figure 3. Shaking table test

Production of useful substances from tropical wood by fast pyrolysis

(Laboratory of Innovative Humano-habitability, RISH, Kyoto University)

Toshimitsu Hata, Aya Yanagawa, and Tsuyoshi Yoshimura

A stable supply of fossil fuels is expected to be difficult in the future. The conversion from unused biomass to the production of useful products has the potential to create a society with sustainable production. The production of useful products from unused biomass, is expected to stimulate the local economy. Based on the above research background, the authors have been investigating the optimal conditions for producing useful products from various tropical fast-growing woods. Here, we focused on the solid residues produced by fast pyrolysis. We then attempted to analyze the nanopore structure of the solid residues, which has an effect on carbon dioxide and ammonia adsorption. The size of nanopores is an important factor in gas adsorption. The microporous structure of the pyrolysis residue was observed using transmission electron microscopy (TEM) and the image analysis of the TEM images was conducted.

A schematic of the fast pyrolysis system is shown in Fig.1. The fast pyrolysis system is an enclosed system consisting of an L-shaped reaction tube, a liquid recovery tube, and a gas recovery bag. The graphite dye in which the reaction tube is inserted between the two electrodes of the pulsed current-heating device, and a load and a current are applied. Sengon (*Paraserianthes falcataria*) and Abies sachalinensis (*Abies sachalinensis*) were powdered and inserted into the graphite dye in the L-shaped reaction tube shown on the left side of Figure 1. The materials of the reaction tube are quartz, Cu, and Ti. A thermocouple was inserted near the reaction tube in the graphite dye, and the temperature was controlled by monitoring the temperature with a PC. The temperature of pyrolysis was 300°C to 800°C, the pyrolysis time was 3 minutes, and the



Fig. 1. Schematic of fast pyrolysis system¹

heating rate was 15°C to 20°C/s. The TEM was used to observe and process the fast pyrolysis residues.²: For the electron microscope observation, the images were processed in the image being observed on a PC monitor connected to the TEM, and then taken into the PC. The image analysis consists of two processes: spatial frequency analysis and measurement processing. In the spatial frequency analysis, a two-dimensional fast Fourier transform (2D-FFT) was performed.

Different spacing of the hexagonal carbon layers was obtained depending on the species, treatment temperature, and reaction tube material. Pyrolysis of Abies sachalinensis in a quartz reaction tube showed that the spacing of the hexagonal carbon layer was smaller at 500°C, with the spacing increasing in the order of quartz, Cu, and Ti. When compared under the same non-species conditions, the face spacing of the carbon in Sengon tended to be smaller than that in Abies sachalinensis. The surface spacing is very important for gas adsorption because it reflects the potential of gas molecules that can be stored or adsorbed in the nanopores.

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Simulations and modeling of geospace environment

(Laboratory of Computer Space Science, RISH, Kyoto University)

Yoshiharu Omura and Yusuke Ebihara

Efficient acceleration of relativistic electrons at Landau resonance with obliquely propagating whistler-mode chorus emissions is confirmed by theory, simulation, and observation. The acceleration is due to the perpendicular component of the wave electric field. We have derived formulae of inhomogeneity factors for Landau and cyclotron resonances to analyze nonlinear wave trapping of energetic electrons by an obliquely propagating chorus element [1]. We conduct test particle simulations to examine the acceleration mechanism of relativistic electrons through interaction with multi-subpacket chorus waves. We develop the wave model with rapidly fluctuating amplitude and phase discontinuities across each subpacket in order to examine how these features of multi-subpacket chorus wave influence the nonlinear trapping processes in efficient acceleration (URA) [2]. Applying test particle simulations with a pair of whistler mode chorus emissions, we traced a large number of electrons in various initial conditions along an L = 4.5 magnetic field line to build a set of Green's functions for analyzing evolution of the electron distribution under the chorus emissions propagating obliquely to the magnetic field. Employing convolution integral for the Green's functions, we tracked the formation of relativistic electron fluxes from an injected energetic electron through interaction with consecutive chorus emissions [3].

The pathway of energy from the solar wind to the ionosphere is not fully understood. We performed the global magnetohydrodynamics (MHD) simulation, and showed two pathways. One comes from the solar wind directly, and the other one comes from the solar wind by way of the ionosphere. The field-aligned current (FAC) flowing in the inner magnetosphere is called a Region 2 FAC. The distribution of the Region 2 is shown to depend on altitude [4]. By combing the MHD simulation with the advection simulation, we showed the region where the whistler mode chorus waves can grow nonlinearly. The whistler waves can first grow due to the linear mechanism, followed by rapid, nonlinear mechanism accompanied with rising-tone chorus elements. When the solar wind speed is high, the whistler waves grow more efficiently due to linear and nonlinear mechanisms over a wider area. For slow solar wind, the linear growth is mostly suppressed, but the nonlinear growth can still take place when external seed waves are present [5].

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Wireless power transfer for flying drone with novel beam forming

(Laboratory of Applied Radio Engineering for Humanosphere, RISH, Kyoto University)

Naoki Shinohara, Tomohiko Mitani, Yohei Ishikawa, and Junji Miyakoshi

Our laboratory joins a Cabinet Office Cross-ministerial Strategic Innovation Promotion Program (SIP) named "Energy system of an Internet of Energy (IoE) society" from FY2018[1]. This subject realize an Internet of Energy(IoE) society with Society 5.0 era energy systems. We are involved in sub-theme of "R&D for application/practical implementation of IoE" presents the form of a super-smart, resilient IoE society. It develops wireless power transfer (WPT) systems for indoor sensor networks and mobile information equipment as a practical example of implementation. We are developing a drone WPT systems via microwave. In FY2019, we showed the following novel research results; 1) novel beam form to maximize total WPT efficiency which includes a beam efficiency between a transmitting antenna array to a receiving antenna array and microwave-electricity conversion efficiency which has input microwave power dependence[2], 2) new diode parameter estimation method with only S₁₁ (reflection) parameter for a receiving alterna 3) estimation of effect of the microwave beam to conventional wireless communication system. We will apply the WPT technologies for future IoE society toward a sustainable humanosphere.



Fig. 1. Grand design for the energy system of an IoE society[1]

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Exploration of space environments and developments of scientific instruments

(Laboratory of Space Electromagnetic Environment Exploration, RISH, Kyoto University)

Hirotsugu Kojima, Satoshi Kurita and Yoshikatsu Ueda

Exploration of electromagnetic space environments

The dominant phenomena in space plasmas are electromagnetic. The medium that transfers kinetic energies of plasma particles in space plasmas is plasma waves, because space plasmas are essentially collisionless. The energy transfer process taking place in space is so-called wave-particle interaction. Exploring electromagnetic environments in space is to investigate wave-particle interactions. The present research focuses on plasma wave observations via scientific satellites. The Exploration of the terrestrial radiation belts by the Arase satellite is the most recent activity. Plasma waves are believed to have significant roles for the generation and loss of high energy particles in the radiation belts. We have conducted statistical investigations of plasma wave data obtained by the Arase satellite to reveal propagation characteristics of plasma waves in the radiation belts. This work will have significant contributions to modeling studies on the dynamic variation of radiation belt electrons. Plasma waves observed by the Arase satellite are also extensively analyzed consulting particle measurement data. The results have been showing the detailed and quantitative processes of wave-particle interactions in the radiation belts. Importance of nonlinear wave-particle interactions on particle acceleration has been suggested based on simultaneous particle and plasma wave measurements by the Arase satellite. Another exploration got started in October 20, 2018. It is the BepiColombo mission targeting the Mercury. Plasma wave instruments onboard the spacecraft will reveal the wave-particle interactions that no one has seen before around the Mercury after 6years' cruising.

Miniaturization of plasma wave receiver system

Plasma wave receiver is one of the essential instruments for space environment exploration; however, conventional receiver has a problem in its large weight and size. In order to overcome this problem, we have been miniaturized plasma wave receiver by developing Application-Specific Integrated Circuits (ASIC) for plasma wave receivers. We succeeded in developing miniaturized plasma wave receiver by realizing analog circuit, which is especially large part of the receiver, using ASIC. This miniaturized receiver will be onboard the SS-520-3 sounding rocket, which will launch in the not-too-distant future to resolve the cause of ion outflow phenomena at the cusp region. In addition, we aim to develop a mixed-signal ASIC chip for one-chip plasma wave receiver. The mixed-signal ASIC chip includes all analog and digital circuits for plasma wave receiver. One-chip plasma wave receiver allows to reduce weight and size of the instruments drastically, and it will contribute for increasing opportunities of plasma wave observation.

Theoretical study of fine bubble and its application research

Fine bubble (FB, less than 1 micro meter) technology is standardized as ISO/TC 281 and its basic and application research is conducted by many researchers. Basic properties and assumed generation mechanisms are now making clear. There is still remained problems of integrated theory of FB such as generation and stabilization. And we also need to apply FB technology to various application field with its detailed theory. As for integrating basic properties, we conduct various measurement such as ultrasonic attenuation of FB water, as measuring electrical potential of FB and as comparison with nano-particles in water. We also try to do application experiment in agricultural field as international collaboration study.

Professor Junji Sugiyama was awarded the Japan Prize of Agricultural Science and Yomiuri Prize of Agricultural Science 2019

Japan Prize of Agricultural Science and Yomiuri Prize of Agricultural Science is awarded to researchers who have produced excellent results in the field of agriculture by the Association of Japanese Agricultural Scientific Societies (AJASS) and Yomiuri Shinbun. The award of this prize, which is considered the honor among researchers of agriculture in Japan, is a validation of his achievements in the "Studies on hierarchical structural diversity from cellulose to wood".

Drs. Makiko Imai, Asako Mihashi, Tomoya Imai, Satoshi Kimura, Tomohiko Matsuzawa, Katsuro Yaoi, Nozomu Shibata, Hiroshi Kakeshita, Kazuaki Igarashi, Yoshinori Kobayashi & Junji Sugiyama were awarded the Journal of Wood Science Best Paper Award by Japan Wood Research Society on 17 March 2020.

Drs. Makiko Imai, Asako Mihashi, Tomoya Imai, Satoshi Kimura, Tomohiko Matsuzawa, Katsuro Yaoi, Nozomu Shibata, Hiroshi Kakeshita, Kazuaki Igarashi, Yoshinori Kobayashi & Junji Sugiyama were awarded the Journal of Wood Science Best Paper Award by Japan Wood Research Society on 17 March 2020 for their paper titled "Selective fluorescence labeling: time-lapse enzyme visualization during sugarcane hydrolysis" published in Journal of Wood Science 65, 17 (2019).

Dr. Hiroshi Nishimura received New Chemical Technology Research Encouragement Award, The Japan Association for Chemical Innovation (JACI)

Dr. Hiroshi Nishimura received New Chemical Technology Research Encouragement Award, The Japan Association for Chemical Innovation (JACI) for the Research on innovative materials and technologies of biomass-derived products for the sustainable society on Jun. 6th 2019.

Mr. Chihiro Kimura was awarded the Best Poster Award by the 4th Asia Research Node Symposium on Humanosphere Science

On Dec. 28th 2019, Mr. Chihiro Kimura was presented with the Best Poster Award at the 4th Asia Research Node Symposium on Humanosphere Science on production of antiviral compounds from sugarcane bagasse by microwave reaction.

Dr. Akifumi Sugiyama was awarded JSBBA Award for Young Scientists by Japan Society for Bioscience Biotechnology, and Agrochemistry

On March 25, 2020, Dr. Akifumi Sugiyama was awarded JSBBA Award for Young Scientists by Japan Society for Bioscience Biotechnology, and Agrochemistry for his research on the dynamics and functions of plant metabolites in the rhizosphere.

Kyoto University group with Professor Hiroyuki Yano was awarded EcoPro Award Incentive 2019

The kyoto university group led by Prof. Yano received "EcoPro Award Incentive 2019" for significant contribution to develop the process for fabricating cellulose nanocomposites "STARCEL®".

Professor Hiroyuki Yano was awarded Special Jury Prize of Japan Open Innovation 2019

The joint group led by Prof. Yano received Japan Open Innovation Prize 2019 for significant contribution to industry-academia-government collaboration and social implementation of cellulose nanofibers.

Dr. Subir Kumar Biswas received the Best Poster Award in the 4th Asia Research Node (ARN) Symposium on Humanosphere Science

Dr. Subir Kumar Biswas was awarded the Best Poster Award in the 4th ARN Symposium organized jointly by Nanjing Forestry University (China) and Kyoto University's Research Institute for Sustainable Humanosphere (RISH), held in December 2019 in Nanjing, China. His poster title was "Nanocellulose reinforced hierarchical nanocomposites for high thermal performing flexible and transparent electrodes".

Professor Hiroshi Isoda and Associate Professor Takafumi Nakagawa received Awards for Science and Technology by the Minister of Education, Culture, Sports, Science and Technology

On 17 April 2019, Professor Hiroshi Isoda and Associate Professor Takafumi Nakagawa received Awards for Science and Technology by the Minister of Education, Culture, Sports, Science and Technology for contribution to promotion and public awareness of wooden houses with resilience and sustainability against extremely severe earthquakes.

Professor Hiroshi Isoda received the 32nd First Division Award of Research Fund for Timber Materials and Timber Structure Technology (Sugiyama Hideo Prize) by Japan Timber Engineering Society

On 25 June 2019, Professor Hiroshi Isoda received the 32nd First Division Award of Research Fund for Timber Materials and Timber Structure Technology (Sugiyama Hideo Prize) by Japan Timber Engineering Society for contribution to improvement of seismic performance of timber structures based on the experimental research.

Associate Professor Takafumi Nakagawa received the Best Presentation Awards for Robust Quality Engineering by The Precise Measurement Technology Promotion Foundation 2019

On 29 June 2019, Associate Professor Takafumi Nakagawa received the Best Presentation Awards for Robust Quality Engineering by The Precise Measurement Technology Promotion Foundation 2019

Ms. Zherui Li (graduate students at Laboratory of Timber Science and Engineering) received the Excellent Presentation Awards in Timber Engineering Division at 2019 AIJ Annual Meeting by Architectural Institute of Japan

On 17 October 2019, Ms. Zherui Li (graduate students at Laboratory of Timber Science and Engineering) received the Excellent Presentation Awards in Timber Engineering Division at 2019 AIJ Annual Meeting by Architectural Institute of Japan

Mr. Kotaro Sumida (graduate student at Laboratory of Timber Science and Engineering) received the Excellent Presentation Awards in Timber Engineering Division at 2019 AIJ Annual Meeting by Architectural Institute of Japan

On 17 October 2019, Mr. Kotaro Sumida (graduate students at Laboratory of Timber Science and

Engineering) received the Excellent Presentation Awards in Timber Engineering Division at 2019 AIJ Annual Meeting by Architectural Institute of Japan

Mr. Akira Takise (graduate student at Laboratory of Timber Science and Engineering) received the Excellent Presentation Awards in Timber Engineering Division at 2019 AIJ Annual Meeting by Architectural Institute of Japan

On 17 October 2019, Mr. Akira Takise (graduate students at Laboratory of Timber Science and Engineering) received the Excellent Presentation Awards in Timber Engineering Division at 2019 AIJ Annual Meeting by Architectural Institute of Japan

Mr. Rui Li (graduate student at Laboratory of Timber Science and Engineering) received the Best Student Poster Award at the 4th Asia Research Node Symposium on Humanosphere Science in Nanjing, China

On 27 December 2019, Mr. Rui Li (graduate students at Laboratory of Timber Science and Engineering) received the Best Student Poster Award at The 4th Asia Research Node Symposium on Humanosphere Science in Nanjing, China

Dr. Toshimitsu Hata received The Wood Carbonization Research Society Award

Dr. Toshimitsu Hata received The Wood Carbonization Research Society Award for study on the microstructural analysis of carbonized wood on June 6th, 2019.

Professor Yoshiharu Omura was honored as an AGU outstanding reviewer of 2018

On May 30, 2019, Professor Yoshiharu Omura was recognized as an outstanding reviewer of 2018 by editors of Geophysical Research Letters published by American Geophysical Union.

Professor Yoshiharu Omura has been appointed as a Member of IAA

On October 20, 2019, Professor Yoshiharu Omura has been appointed as a member (basic science) of International Academy of Astronautics (IAA) for his significant contribution to wave and particle interactions in the space environment.

Mr. Jie Chu was awarded WTC (Wakate Technical Committee) Best Rookie Award at 12th Kansai Microwave Meeting for Young Engineers by IEEE Microwave Theory and Technique Society (MTT-S) Kansai Chapter

On June 22, 2019, Mr. Jie Chu was awarded WTC (Wakate Technical Committee) Best Rookie Award for his presentation titled "Development of a 5.8-GHz Magnetron Phased Array for High-Power Microwave Power Transmission" at 12th Kansai Microwave Meeting for Young Engineers

Mr. Hiroyuki Matsubara's team with international university's members was awarded the Student Design Competition Award 1st Place at Thailand Japan Microwave 2019 (TJMW2019)

On June 26-28, 2019, Mr. Hiroyuki Matsubara's team with international university's members was awarded the Student Design Competition Award for development of microwave filter at Thailand Japan

Microwave 2019 (TJMW2019)

Mr. Jie Chu was awarded the Young Researcher Encouragement Award at Thailand Japan Microwave 2019 (TJMW2019)

On June 26-28, 2019, Mr. Jie Chu was awarded the Young Researcher Encouragement Award for his poster presentation titled "Development of a 5.8-GHz Magnetron Phased Array" at Thailand Japan Microwave 2019 (TJMW2019)

Mr. Hiroyuki Matsubara was awarded the Best Presentation Award at Thailand Japan Microwave 2019 (TJMW2019)

On June 26-28, 2019, Mr. Jie Chu was awarded the Best Presentation Award for his poster presentation titled "Study on Estimation Method of Directions and Distances for Microwave Power Transmission to Moving Targets" at Thailand Japan Microwave 2019 (TJMW2019)

Mr. Bo Yang was awarded the Best Student Award, at 2019 Asia Wireless Power Transfer Workshop

On October 31 - November 2, 2019, Mr. Bo Yang was awarded the Best Student Award for his presentation titled "A 5.8GHz Magnetron Phased Array System" at 2019 Asia Wireless Power Transfer Workshop.

Mr. Bo Yang was awarded the WiPoT Award, at 2019 Asia Wireless Power Transfer Workshop

On October 31 - November 2, 2019, Mr. Bo Yang was awarded the WiPoT Award for his presentation titled "A 5.8GHz Magnetron Phased Array System" at 2019 Asia Wireless Power Transfer Workshop.

Mr. Nobuyuki Takabayashi was awarded the Student Award, at 2019 Asia Wireless Power Transfer Workshop

On October 31 - November 2, 2019, Mr. Nobuyuki Takabayashi was awarded the Student Award for his presentation titled "Study on the Practical Use of Microwave Power Transfer to Mini-drones Using Flat-topped Beams at 5.74 GHz" at 2019 Asia Wireless Power Transfer Workshop.

Mr. Nobuyuki Takabayashi was awarded the Best Presentation Award at IEEE AP-S Kansai Joint Chapter Workshop

On December 7, 2019, Mr. Nobuyuki Takabayashi was awarded the Best Presentation Award for his presentation in Japanese titled "Study on Novel Beam Forming of Microwave Power Transfer for Flying Mini-Drone" at IEEE AP-S Kansai Joint Chapter Workshop.

Mr. Yuta Nakamoto was awarded the Best Presentation Award at IEEE AP-S Kansai Joint Chapter Workshop

On December 7, 2019, Mr. Yuta Nakamoto was awarded the Best Presentation Award for his presentation in Japanese titled "Study on a Microwave Power Transfer System to a Stratospheric Platform Airship" at IEEE AP-S Kansai Joint Chapter Workshop.

Mr. Jie Chu was awarded the Best Poster Award at IEEE AP-S Kansai Joint Chapter Workshop

On December 7, 2019, Mr. Jie Chu was awarded the Best Poster Award for his poster presentation in Japanese titled "Development of a 5.8-GHz Magnetron Phased Array" at IEEE AP-S Kansai Joint Chapter Workshop.

Ms. Airi Shinjo was awarded the Student Presentation Award (Aurora Medal) by the Society of Geomagnetism and Earth, Planetary and Space Sciences (SGEPSS)

Ms. Airi Shinjo received the Aurora Medal for her excellent oral presentation titled "Consideration of electron cyclotron harmonic waves and the environment observed by the Arase satellite with linear analysis" at the fall meeting of the SGEPSS on October 26th, 2019.

Mr. Motoyuki Kikukawa was awarded the Best Poster Award by the 4th Asia Research Node (ARN) on Humanosphere Science

Mr. Motoyuki Kikukawa received the Best Poster Award for his poster presentation titled "Development of the new device implementing high-speed current detection circuits dedicated to particle sensors on board space missions" at the 4th ARN symposium on December 27th, 2019.

Ms. Tomoe Taki was awarded the Best Poster Award by the 4th Asia Research Node (ARN) on Humanosphere Science

Ms. Tomoe Taki received the Best Poster Award for her poster presentation titled "Isolated electrostatic potential structures observed by the Arase satellite" at the 4th ARN symposium on December 27th, 2019.

Mr. Ryotaro Isoyama was awarded the Best Poster Award by the 4th Asia Research Node (ARN) on Humanosphere Science

Mr. Ryotaro Isoyama received the Best Poster Award for his poster presentation titled "Characteristics of the BroadBand Extremely Low Frequency waves through the Akebono observations with high time resolutions" at the 4th ARN symposium on December 27th, 2019.

ABSTRACTS (PH D THESIS)

Studies on lignocellulose supramolecular structures and deconstruction properties in lignin-altered rice mutants

(Graduate School of Agriculture, Laboratory of Metabolic Science of Forest Plants and Microorganisms, RISH, Kyoto University)

Andri Fadillah Martin

Lignocellulosic biomass represents abundant and renewable carbon sources that can be exploited for the sustainable production of bio-based energy and chemicals. Lignocellulose is majorly produced in plant cell walls and mainly composed of three structural polymers, i.e., cellulose, hemicelluloses and lignin, which intricately interact with each other through both non-covalent and covalent linkages. Lignin, a phenylpropanoid polymer typically accounting for 15%-30% of raw lignocellulose feedstocks, has long been recognized as a key recalcitrant factor limiting the efficiency of lignocellulose deconstruction and downstream processing in polysaccharide-oriented biomass utilization processes, for example, those used in the production of pulp and paper and the generation of fermentable sugars for biomaterials and biofuels. To mitigate such lignin-associated biomass recalcitrance, numerous mutants and transgenic plants that produce lignocellulose with reduced lignin contents and/or lignins with altered chemical structures have been produced and characterized. However, largely because of technical challenges in characterizing the highly complex and heterogeneous structure of lignocellulose, it is not fully understood how altered lignin chemistry affects the supramolecular structure of lignocellulose, and consequently, its utilization properties. This study aimed to dissect the impacts of genetic modifications of lignin on the supramolecular structure and deconstruction properties of lignocellulose. Particular focus was directed to the characterization of rice mutants deficient in CINNAMYL ALCOHOL DEHYDROGENASE (CAD)and 5-HYDROXYCONIFERALDEHYDE O-METHYL-TRANSFERASE (CAldOMT), both of which encode key enzymes in lignin biosynthesis and represent major gene targets in lignin bioengineering research.

Altered lignocellulose chemical structure and molecular assembly in CAD-deficient rice

In the first part of this study, the author conducted comprehensive chemical and supramolecular structural analyses of lignocellulose produced by a *CAD*-deficient mutant rice, which harbors a *Tos17* retrotransposon insertion in *OsCAD2*, a major CAD gene involved in lignin biosynthesis of rice. The solution-state two-dimensional NMR and complementary wet-chemical methods elucidated the structural details of the altered lignins enriched with unusual hydroxycinnamaldehyde-derived subunits produced by the *CAD*-deficient mutant rice. In parallel, lignocellulose supramolecular structure was investigated by solid-state NMR, X-ray diffraction and Simon's staining approaches. The obtained data indicated that cellulose assembly and mobility were notably disrupted in the *CAD*-deficient lignocellulose. In particular, both solid-state NMR and X-ray diffraction data suggested that *CAD*-deficient lignocellulose has less well-defined cellulose alignment compared to that in the wild-type control lignocellulose, which may account for the improved saccharification performance of lignocellulose produced by the *CAD*-deficient mutant rice [1].

Insights into lignocellulose molecular assembly and its deconstruction from lignin-altered rice mutants deficient in *CAldOMT* and *CAD*

In the second part of this study, impacts of lignin-modifications induced by deficiencies of *CAldOMT* and *CAD* were comparatively investigated. Rice mutants deficient in either or both *OsCAldOMT1*, a major OMT gene involved in lignin biosynthesis of rice, and *OsCAD2* genes investigated in the earlier part of this study were generated in part by using the Cluster Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR associated 9 (CRISPR/Cas9) system. Isolated homozygous mutant lines and wild-type

ABSTRACTS (PH D THESIS)

control rice plants were grown side-by-side and subjected to in-depth analyses of lignocellulose structures and enzymatic saccharification efficiency. In line with the proposed functions of CAldOMT and CAD in the lignin biosynthetic pathways, CAldOMT-deficient mutant lines produced altered lignins largely depleted in syringyl and tricin subunits and partially incorporated with atypical 5-hydroxyguaiacyl units, whereas CAD-deficient mutant produced lignins incorporated lines with unusual hydroxycinnamaldehyde-derived subunits. Solid-state NMR and X-ray diffraction analyses suggested that, whereas disruptions of CAldOMT and CAD both prominently affects the lignocellulose supramolecular structure, the disruption of CAldOMT more prominently affects lignocellulose supramolecular structures than does the disruption of CAD, resulting in higher cellulose mobility as primarily gauged by nuclear magnetic relaxation. Partly in line with this observation, CAldOMT-deficient mutant lignocellulose showed significantly greater glucose release upon enzymatic saccharification compared with those of the wild-type control and CAD-deficient mutant lignocellulose [2].

Comparative analysis of lignocellulose chemical degradability and enzymatic saccharification performance of *CAD*- and *CAldOMT*-deficient rice mutants

In the last, third part of this study, the author investigated the deconstruction properties of *CAD*- and *CAldOMT*-deficient rice mutant lignocellulose in terms of their chemical reactivities in typical biomass processing reactions. 2D NMR and chemical structural analyses on the rice lignocellulose samples before and after dilute alkaline, dilute acid and liquid hot water treatments revealed different reactivities of lignin and polysaccharide components comprising *CAD*- and *CAldOMT*-deficient mutant cell walls. Saccharification efficiency of the *CAD*- and *CAldOMT*-deficient mutant lignocellulose was differently improved by using the three chemical reactions as pretreatments to facilitate dissociations of lignocellulose prior to enzymatic polysaccharide hydrolysis. In particular, dilute alkaline treatment was effective to promote saccharification of both *CAD*- and *CAldOMT*-deficient mutant lignocellulose, whereas dilute acid and liquid hot water treatments were effective for *CAldOMT*-deficient mutants but apparently not for *CAD*-deficient mutants. Overall, the use of biomass processing reactions in combination with genetic lignin alterations based on manipulations of *CAD* and *CAldOMT* can be strategic to boost lignocellulose deconstructions [3].

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Lipid secretion mechanism in *Lithospermum erythrorhizon* using shikonin as a model of hydrophobic metabolites

(Graduate School of Agriculture, Laboratory of Plant Gene Expression, RISH, Kyoto University)

Kanade Tatsumi

Introduction

Plants synthesize a large number of metabolites for adaptation to their environmental stresses. Among them, lipophilic secondary metabolites often accumulate in extracellular spaces and function as barrier to biotic and abiotic stresses. For instance, in Laminaceae plants, monoterpenes are produced by secretory cells of glandular trichomes and accumulate in the epicuticular cavity [1]. Furanocoumarins are biosynthesized in the peels (flavedo) of citrus species and accumulate specifically in oil cavities [2]. Moreover, all land plants secrete cutin, lipophilic polymers, to protect the plant tissues from external aggressions and passive water loss [3]. However, it is largely unknown how these lipophilic metabolites are secreted and accumulated in extracellular spaces. To investigate the transport of plant lipophilic secondary metabolites, I have used in this study shikonin derivatives as a model compound of lipophilic endogenous substances. This red pigment is produced in the root epidermal cells of *Lithospermum erythrorhizon* and secreted from these cells into the apoplastic spaces. The shikonin production system is an excellent model system because of three major benefits; 1) shikonin is a visible pigment, 2) production of shikonin is clearly regulated as 0 to 100% manner by medium composition and illumination, 3) there are culture system of shikonin-producing cells and hairy root cultures. These properties may lead to greater understandings of the intracellular trafficking of lipophilic metabolites [4]. This Ph.D thesis provides several biochemical evidences relevant for the excretion of lipophilic metabolites from plant cells.

Results

Establishment of L. erythrorhizon transformation using domestic Rhizobium rhizogenes strain A13

An effective transformation method is required to conduct the researches mentioned above. However, standard *L. erythrorhizon* transformation methods have not yet been established. To overcome this problem, I established a methodology of effective stable transformation of *L. etyrhrorhizon* using a domestic *Rhizobium rhizogenes* strain A13 as a carrier (Fig. 1). This protocol achieved high transformation efficiency (50 - 70%) [5]. Furthermore, an inducible transcriptional regulation system was introduced into the hairy root culture to prevent a large clonal variation that obstructs the evaluation of gene functions of interests. In conclusion, our protocols allowed *in-planta* functional analysis of candidate genes from *L. erythrorhizon*, which may contribute to understanding the molecular mechanisms of shikonin biosynthesis and secretion.

Dedifferentiation



Figure 1. Scheme of the transformation method of L. erythrorhizon. (sited from [5])

Analysis of subcellular events accompanied with shikonin production using hairy roots

The biosynthesis of shikonin derivatives has been intensively studied. A key regulatory reaction in its biosynthetic pathway is the conjugation of a geranyl moiety to *p*-hydroxybenzoic acid, yielding *m*-geranyl-*p*-hydroxybenzoic acid. This reaction is catalyzed by a membrane-bound prenyltransferase, LePGT [6]. To identify the organelle involved in shikonin synthesis, I analyzed the subcellular localization of this enzyme. It was shown that the LePGT localized at endoplasmic reticulum (ER). Moreover, in electron microscopic analysis, highly developed ER was observed in shikonin-production epidermal cells. Next, I searched for inhibitors of shikonin secretion in hairy root cultures, and identified two compounds, cytochalasin D, an inhibitor of actin filament polymerization, and brefeldin A, an inhibitor of the adenosine diphosphate (ADP)-ribosylation factor/guanine nucleotide exchange factor (ARF/GEF) protein system, which are often used in membrane traffic studies. These results suggested that the secretion of shikonin derivatives into the apoplast utilizes the pathways common to the ARF/GEF system and actin filament polymerization, at least in part [7].

Identification of the matrix lipid involved in shikonin transport mechanism

As shikonin is a very lipophilic substance, it is rapidly crystallized in water and cytosol as well as in the medium, while it is accumulated as red droplet/granules at the surface of the living cells and root tissues of *L. erythrorhizon*. It is therefore presumed that a matrix lipid plays an important role in solubilizing and secreting shikonin safely out of the cells. In an attempt to identify the lipids, we analyzed secreted shikonin derivatives by electron microscopy and chemical analysis of secreted lipids, suggesting that shikonin derivatives form oil droplet structures with matrix lipids and polar lipids.

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Development of software-defined multichannel receiver for Equatorial Atmosphere Radar (EAR)

(Graduate School of Informatics, Laboratory of Atmospheric Sensing and Diagnosis, RISH, Kyoto University)

Nor Azlan bin Mohd Aris

Equatorial Atmosphere Radar (EAR) is a very high frequency (VHF) Doppler radar operated with an active phased-array antenna system, approximately 110 meters in diameter which consist of 560

three-element Yagi antennas, located at the equator in Kototabang, West Sumatra, Indonesia (0.20°S, 100.32°E, 865 m above sea level) [1]. Each antenna is driven bv а solid state transmitter-receiver module (TR module). The array antenna is divided into 24 groups for ease of signal distribution and maintenance where each group consists of 24 antennas except for eight groups along the periphery which contain one to three fewer antennas. These design is purposely constructed to produce a quasi-circular array pattern as shown in Figure 1. It uses active phased-array system similar to the Middle and Upper atmosphere radar (MU radar) due to its fast beam steerability where phase shift and signal division and combination operations are carried out at a low signals level by electronic devices. The EAR had originally been equipped with a single receiving channel system. This research presents development of a multichannel receiver system for the EAR using a combination of the Universal Software Radio Peripheral X300 (USRP X300) and GNU Radio software. There are a number of advantages to have multichannel receiver system such as to enable spaced-antenna (SA) method and spatial domain interferometry.

First test observation was conducted on November 2017 to analyze the system reliability, followed with subsequent tests in March and July 2018. The configuration of the test observation is shown in Figure 2 USRP [2]. Two X300 devices. corresponding to four receiving channels, were synchronized using 10 MHz reference clocks and a pulse per second (1 PPS) signal. The standard observation system of the EAR is retained by splitting the received echo signals through directional which enabled coupler observation of the two different techniques,



Figure 1. EAR antenna array



simultaneous Figure 2. The configuration of the EAR multichannel receiver for test observation.

SA and Doppler beam swinging (DBS). Each receiving antennas for the EAR SA method formed by a combination of two antenna array groups (aperture size is approximately one twelfth of the whole EAR). The signal for SA application is fed to the USRP X300s for digital conversion, and then stored on a Hard Disk Drive (HDD). The ranging of the data is carried out by taking advantage of the leaked transmitted pulse [3], before demodulated and coherently integrated. The initial results show the existence of noticeable fluctuations in the estimated horizontal wind.

Further, performance analysis using multiple receiving antennas orientation for the application of SA method on the EAR has been carried out through multiple experiments over the duration between April 2019 and September 2019. Phase correction is applied to all channels for a single spectrum in the real time signal processing for improving the phase synchronization. Then, a comparison of the EAR SA performance using five different orientations taking into consideration the aperture size of receiving antenna and its separation distance has been presented, where the horizontal wind profiles using Full Correlation Analysis (FCA) were estimated and compared with the EAR standard observation data. Based on the results, the configuration with the largest aperture shows slight advantage over the other four configurations but with limited improvement [4].

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Strong cellulose nanofiber composite hydrogels via interface tailoring

(Graduate School of Agriculture, Laboratory of Active Bio-based Materials, RISH, Kyoto University)

Yang Xianpeng

Cellulose, the most abundant biomass, has been commercially available in diverse fields, while the application of cellulose in the nanoscale is not yet widely commercialized. This study is to explore the application of cellulose in the nanoscale. Hydrogels are highly hydrated polymer networks, and have great potential in many areas, such as artificial tissues and soft bioelectronics. In many cases, hydrogels require load-bearing properties, however it is still challenging to obtain simultaneously tough and strong hydrogels. Nanocelluloses possess many advantages to reinforce hydrogels, including good hydrophilicity, impressive strength and stiffness, high specific surface area, and tunable surface chemistry. However, the reinforcement is limited: (1) the concentration of nanocellulose is low; (2) the nanocellulose-matrix architecture is scarcely designed. To solve above problems, cellulose nanofibers (CNFs), mechanically disintegrated from wood powders, were used to fabricate composite hydrogels. First, it is easy to filtrate CNF suspension to form a wet cake with a high CNF concentration. The obtained CNF cake, with layered structures, is expected to be composited with polymer networks. Second, the surfaces of CNFs are covered with hemicellulose which is more reactive than cellulose. The interfacial interactions between CNFs and polymer networks may be precisely fabricated.

In Chapter 2, CNF/poly(vinyl alcohol) (PVA) hydrogels were prepared via direct blending. In brief, a wet CNF cake was immersed into PVA solution with various concentrations to absorb PVA chains, followed by drying-annealing and rehydration processes. The CNF/PVA hydrogels showed comparable elastic modulus and fracture strength to skin and cartilage. In addition, the CNF/PVA hydrogels had a high water content and showed high fracture energy.

In Chapter 3, poly(acrylamide-co-acrylic acid) networks were incorporated into CNF cakes via in situ polymerization, followed by ionic cross-linking with Fe^{3+} . This method also resulted in simultaneously stiff, strong and tough hydrogels. Compared with the blending method in Chapter 2, the method of in situ polymerization was applied to diverse monomers, presenting versatile features. Based on the results of Chapter 2 and Chapter 3, it was proved that a wet CNF cake was an appropriate start material for strong and tough composite hydrogels. The polymer networks should have good compatibility with CNFs and be easily cross-linked.

In Chapter 4, the surfaces of CNFs were modified to enhance the interfacial interactions between CNFs and matrix. The reaction of CNFs and maleic anhydride, using a CNF cake as a reactor, was examined. The water in the CNF cake was readily replaced by filtrating a mixture of solvent and reactant through the cake. At the same time, the reaction between the CNFs and reactant mixture occurred. This new modification method, inspired by the fluid in a plug flow reactor, was effective and convenient. Then, the polymer networks, which were the same as Chapter 3, were fabricated in the modified CNF cake. The composite hydrogels were indeed stiffened owing to the enhanced interfacial interactions, although the toughness was reduced.

In Chapter 5, a new method of grafting polymers from CNFs was developed. Various vinyl polymers, including hydrophobic and hydrophilic polymers, were grafted from CNFs via UV irradiation in the absence of an initiator. It is assumed that the primary free radicals were generated on the surfaces of CNFs (Figure 1). The hydrophilic-hydrophobic properties of CNFs were readily adjusted after polymer grafting while the crystalline structure of CNFs was retained. This green and effective method was called UV grafting.

In Chapter 6, polyacrylamide was grafted on CNFs via UV grafting to form a composite hydrogel, which resulted in toughened property in contrast to the initiator-based sample. It was confirmed that UV grafting was a powerful tool prepare CNF composites with a nanofiber/grafted matrix architecture and

excellent features. In addition, the surface chemistry of CNFs played an important role in the process of grafting.

In Chapter 7, poly(2-hydroxyethyl methacrylate) was grafted on CNF via UV grafting to form a composite hydrogel which presented strain-stiffening, strong and tough properties. The stain-stiffening behavior was ascribed to the synergistic alignment of CNFs and rearrangement of polymer networks. Double grafting was used to enhance the stain-stiffening behavior. It is believed that multiple UV grafting can result in diverse CNF/matrix architectures.

The main findings of this study are summarized as follows: (1) incorporating polymer networks into a CNF cake was an effective approach to prepare stiff, strong and tough composite hydrogels; (2) interfacial interactions between CNFs and matrix were tailored by surface modification and polymer grafting; (3) the plug flow reactor method simplified the chemical modification of CNFs; (4) UV grafting was a powerful method to graft vinyl polymers on CNFs and prepare composite hydrogel with excellent features.



Figure 1. Possible process of UV grafting

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Optically transparent nanocellulose-reinforced composites via pickering emulsification

(Graduate School of Agriculture, Laboratory of Active Bio-Based Materials, RISH, Kyoto University)

Subir Kumar Biswas

Introduction

Cellulose nanofibers (CNFs), ~15–50-nm-wide semi-crystalline long fibers made up of 3–5-nm-wide nanofibrils of extended cellulose chains, are one of the marvelous materials produced in the plant cell walls. With their strength seven times stronger than steel and an elastic modulus of 100-130 GPa (of the crystalline part called cellulose nanocrystals, CNCs; 70–90% of the CNF), CNFs not only support the huge body of a tree, but also offer an incredible potential as the reinforcement material for man-made nanocomposites. Also, CNFs have a very high thermal-dimensional stability (coefficient of thermal expansion, CTE: 0.1 ppm K⁻¹), and therefore, are expected to reduce the high thermal expansion of the polymers. However, the processing of CNF-reinforced nanocomposites suffers from the difficulty of dispersing native hydrophilic CNFs in a hydrophobic resin matrix (most resins are hydrophobic). Therefore, many surface modification techniques have been employed to produce hydrophobic CNFs, which made the composite fabrication process long. To defeat this difficulty, previously, a simple impregnation (IM) method has been developed to produce nanocomposite by impregnating hydrophobic resin into a nanopaper of the native (non-modified) CNFs. The resulting nanocomposites were several times stronger, stiffer, and thermal-dimensionally stable than the neat polymer. Additionally, because the width of the CNFs is much less than the visible wavelength, the use of a transparent flexible polymer yielded a transparent and flexible nanocomposite. This transparent nanocomposite caught attention as a substrate for flexible optoelectronic devices due the desirable combination of flexibility and low-CTE. However, it was found that the impregnation process is only limited to produce flat nanocomposites, i.e., three-dimensionally (3D)-molded transparent materials are not obtainable. This is because of the high stiffness of the nanopaper bestowed by intensive hydrogen bonding among the CNFs. Therefore, many exciting applications of the CNF-reinforced transparent nanomaterials, such as the substrate for smart contact-lenses and 3D-curved displays, could not be realized.

Aimed to address the above issues, in this study a very simple water-based method was devised by exploiting the idea of emulsification of oil and water. The dual role of the nanocelluloses as the Pickering-stabilizer of the resin-in-water emulsion and the resin reinforcing nano-component was utilized.

Development of pickering emulsification method for facile fabrication of transparent and 3D-molded CNF-reinforced nanocomposites

The detailed development of the facile Pickering emulsification method to obtain novel transparent materials reinforced with native CNFs was presented. In this method, an acrylic resin-in-water Pickering emulsion (PE) stabilized solely by CNFs was successfully prepared by vigorously agitating in a blender. Because of the strong encapsulation of the liquid resin micro-droplets by the CNFs network, the emulsion could be easily dehydrated by vacuum-filtration and subsequently hot-pressed with a negligible loss of resin followed by UV-polymerization to obtain CNF-reinforced transparent materials. The optical transparency of the nanocomposites was as high as 89% at 16 wt% CNFs content, which indicated that a good dispersion of the network of hydrophilic CNFs in a hydrophobic resin can be achieved via PE method without any chemical intervention. Interestingly, the nanocomposites had a unique self-assembled two-tier hierarchical architecture resulted from the aggregation of the CNF-encapsulated resin micro-droplets during dehydration of the emulsion. Because of the hierarchical structure of the nanocomposites, they possessed a rare but desirable combination of high strength, toughness, and mechanical flexibility compared to their counterparts having a similar CNFs content prepared via the IM method. Also, PEs of

various formulations were prepared by only changing the water content while keeping the CNF-to-resin ratio same, which resulted in the emulsions having different resin droplet sizes. It was found that the PEs with smaller sized droplets produced nanocomposites with high mechanical properties owing to the more uniform hierarchical microstructure. However, the optical transparency was similar for all the nanocomposites irrespective of the resin droplet size.

Meanwhile, because of the inclusion of liquid resin micro-droplets in the CNF-network, which also reduced the H-bonding among the nanofibrils, the nanocomposites could easily be molded into 3D-shaped transparent materials during the hot-pressing. Such a 3D molded transparent material could not be achieved via IM method. The surface of the nanocomposites could even be nano- or micro-molded easily by simple hot-pressing.

Conclusions

A simple Pickering emulsification method to prepare nanocomposites of immiscible nanocelluloses and resins was devised. The potential of this new method in order to fabricate structurally hierarchical, highly transparent, strong, tough, super thermally-stable, and macro/micro/nano-moldable nanocomposites of hydrophilic 'native' nanocelluloses and hydrophobic resins for application in the next-generation optical devices was explored. The super thermal stability of the nanocomposites could allow their application in the devices those frequently undergo extreme changes in the temperature. The successful fabrication of 3D-molded transparent nanocomposites could open new application areas of the nanocellulose reinforced materials. Example of the application could be the substrate for micro-electronic contact lens devices, substrate for curved and flexible displays, data storage devices, microlens arrays for high-resolution 3D-imaging, or anti-reflection substrate for the improved light-trapping in a thin-film solar cell.

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Study on structural analysis model for generalization of CLT panel method

(Graduate School of Agriculture, Laboratory of Structural Function, RISH, Kyoto University)

Motoshi Sato

1. Abstract

The purpose of this research is to construct a structural analysis model that can trace experimental results for the purpose of generalizing the CLT panel method in the future. The CLT structure targeted in this study uses a CLT panel with a width of 1 to 2 m for the walls, the hanging walls, and the waist wall that make up the vertical structure, as shown in Fig. 1. The panels are connected to each other and the base frame with pull bolts and screw metal fittings so that they are oriented in the same direction. (hereinafter, "narrow panel frame") It is a frame that transmits bending moment by connecting pull bolts between the upper and lower ends of the wall and between the wall and the hanging wall. The main purpose of this research is to construct an analytical model that can trace experimental results including local stress concentration in such a frame. In addition, conducting a study on a test for the purpose of confirming the influence on the yield strength and rigidity when a small opening is provided, targeting a single panel.

1-M24

Plywood

This paper described three types of experiments for the purpose of constructing a structural analysis model of the CLT structure, and explained the construction of the structural analysis model and comparison with the test results in five chapters including the introduction and conclusion.

Chapter 1 is an introduction, and describes the background and purpose of the research.

Chapter 2 targeted the 1.5-story structure test of the narrow panel frame, and

described the construction method and accuracy verification of the structural analysis model.

Chapter 3 targeted the shaking table test of a 5-story CLT structure with a narrow panel frame, and described the construction method and accuracy verification of the structural analysis model, and the seismic design using marginal strength calculation and its validity.

Chapter 4 report the test results and consideration of bending and shearing tests of CLT panels with small openings, which were conducted to confirm the influence of the presence and position of small openings on the yield strength.

Chapter 5 concludes and concludes this research.

2. Proposal of analysis model of CLT structures for narrow panel and accuracy verification intended

Discussed the seismic performance to examine the structural design method of CLT building structures, and conducted a vibration table test to obtain basic data. Compressive failure occurred at corner of CLT wall panel in shaking table test Indication of the construction of the analysis model, tracking behavior when exceeding the test results, as aid in the proposed formula, the construction of the analysis model is useful. In this chapter, we report the construction method and accuracy verification of the analysis model, and subject of the analysis model is 1.5 story of plane specimen.

Using the FEM shell model for the construction of the structural model. It reason for using FEM shell model is capable of confirmation of the distribution of compressive stress in the shaking table in the cause in

b) Target building



Figure 1. Target building

L type

which the considered panel plane of the crushing of the panel leg seen in destructive nature of the test, also, the contact surfaces of the joint and the panels to each other by making substitutions are arranged on the spring element, because we consider an element comparison of the test results and the analysis results can be achieved.

In this model, CLT panel is an elastic shell elements, the junction is a non-linear spring element. Structural performance of CLT panels and joints were set based on the history of the experimental results. Based on the elements test results, set the properties of each element, you create a structure model of the 1.5-story plane test by the FEM shell model. To compare the analysis and test results of the structural model, we performed the accuracy verification of the structural model, to confirm the validity.

3. A seismic behavior and numerical model of narrow paneled cross-laminated timber building

This chapter presents results of shaking table testing for full-scaled CLT building and a design procedure. The three different systems examined are buildings composed of narrow panels, wide panels with an edge tensile connection for each no-window shear part. The focus of this chapter is the building of narrow panels. This building system is suitable for midrise CLT building with high ductility produced through rocking. The structure was shown to behave well during severe strong motion as specified in the Japanese building standard law and to have survived the 1995 Kobe earthquake despite the occurrence of a compressive rupture in shear walls which are support elements against the vertical load. Story shear capacity calculated from a numerical model and element tests were safely evaluated; but to evaluate the capacity correctly, further research is required in the element and system levels. Though a variety of undetermined issues and challenges remains, the Building Standard Law and Notification for three different CLT construction systems was enforced in April 2016 to ensure the construction of safe CLT buildings.

4. A study on the reduction of proof stress by small opening of equipment for CLT panel

In the case of both narrow panels and large panels, the CLT panel, which is the load bearing wall, may be provided with a small opening for building equipment. In this chapter, in order to confirm the influence of the presence and the position of the small opening on the yield strength and stiffness under the bending test and the shear test of the CLT panel with the small opening were conducted.

Test results and calculated values were compared for the value of the moment of inertia when the specimen was considered as a uniform cross section over the entire length under the bending test. Although the moment of inertia calculated by calculation substantially matches the one obtained by the test result, the moment of inertia I' of the notched portion of the specimen having a shape in which either upper or lower of the specimen is cut out Is overrated. They matched when I' was set to 0.6 times when there was a notch. Although it is necessary to study the adjustment coefficient in the future. If there is a notch on the top and bottom, calculating as a simple cross-sectional defect may result in a dangerous side.

The bending strength was calculated from the bending test results. Although there was no significant difference in bending strength depending on existing or no opening and the position of the opening, the bending strength of the specimen with the opening at the upper end had a value of 1.43 times. In addition, comparing the two specimens in which the opening position is vertically symmetrical, the value of the specimen with the opening on the upper side was somewhat lower. In order to confirm the influence of the fluctuation of the test piece strength on the calculation result, the bending strength was calculated on the assumption that all the test pieces were damaged with the same strain. It almost agreed with the bending strength calculated from the test results. The shear strength calculated from the shear test results does not show large deference due to the existing or no opening and the position of the opening. If the size of the opening this time, it was shown that the shear strength could be obtained by simple calculation without depending on the position of the opening.

5. Conclusion

In this paper, for the purpose of constructing the structural analysis model of CLT structure, mainly reported the construction of the structural analysis model and comparison with the test results for three types of experiments.

Response control and seismic design method by applying oil damper for timber structure

(Graduate School of Agriculture, Laboratory of Structural Function, RISH, Kyoto University)

Masatoshi Shinohara

1. Abstract

Over the past few years, many earthquakes have been encountered in Japan, so high earthquake resistance for the buildings are required. Furthermore, the construction of high-rise timber structures in the world is progressing toward a sustainable society. For nations that have many earthquakes like Japan vibration control structure with high energy absorption performance is expected to be applied for high-rise timber buildings. The purpose of this research is to make clear the applicability of oil dampers for timber structures by full-scale shaking table tests, and to provide the basic concept for constructing a seismic design method considering dampers.

In this paper, for the purpose of proposing a seismic design method considering dampers, we conducted a fullscale shaking table test, proposed an analytical model including a design method, and verified the validity. The content of 6 Chapters are shown below.

Chapter 1 is an introduction and explanations of the background and purpose of this research.

Chapter 2 is an explanation about the



a)Target timber building image b)Tested Damping wall Figure 1. Target timber building and Tested Damping wall

full-scale shaking table test of two-story wooden structure test piece with an oil damper.

Chapter 3 is a proposal of a time history response analysis model for wooden house with an oil damper, and explanation of a seismic design method considering dampers for wooden house.

Chapter 4 is an explanation of a full-scale shaking table test of a semi-rigid timber frame structure with an oil damper.

Chapter 5 is a proposal of ,a vibration damping design method based on the calculation of the limit strength of the semi-rigid timber frame structure with an oil damper, and the verification by numerical experiments and shaking table tests.

Chapter 6 is the conclusion of this research.

2. Shaking table tests of two story wood house with oil damper

In this chapter, we conducted a shaking table test of a full-scale two-story test piece in order to quantitatively understand the effectiveness of the oil damper in a wooden house. The oil damper unit test and the dynamic wall test of the damping wall with oil damper were conducted, and useful data were obtained to analyze the effect of the oil damper in the shaking table test. In the shaking table test, a test piece with an oil damper and a test piece using general structural plywood were manufactured, and vibrations of earthquake were continuously input. Then, the response deformation and energy absorption were compared to quantitatively confirm the reduction effect of the response due to the oil damper.

As a result, compared with general structural plywood, the maximum response deformation of the

damping test piece with oil damper is reduced by about 69 to 25%. Furthermore, it became clear of the energy absorption rate that the damper are increasing with each repetition of the vibration, and even with repeated large earthquakes the oil damper is not damaged and effective for earthquake resistance.

3. Analytical simulation of displacement response for two-story wood house with oil damper shear wall and design value for wood shear wall with damper

In this chapter, the numerical calculation method of nonlinear Maxwell model, which is a series combination of non-linear damper support springs specific to timber and bilinear type oil damper is presented, and shaking table test from Chapter 2 is simulated by time history response analysis using this method.

As a result of the simulation, it was confirmed that not only the maximum response deformation but also the differences of structural behavior in each phase can be precisely reproduced. Next, using this analysis method, the safety of damping wall evaluation method "Equivalent evaluation of shear wall strength" used for seismic retrofitting of Japanese wooden houses was verified. As a result, it was shown that this is mostly valid and safe.

4. Shaking table test of semi-rigid timber frame structure with oil damper

Due to Non-residential timber buildings do not have enough shear walls to keep large and many openings, semi-rigid timber frame were developed and increased. However, semi-rigid timber frame structure is relatively soft. This chapter presents seismic performance of semi-rigid timber frame structure with damper through full-size shaking table tests. We compared response deformations during moderate and severe ground motions among only frame, frame with oil damper and frame with shear wall to verify the damping effect of oil damper.

As a result, the maximum response deformation of "Semi-rigid timber only frame" was 1/106rad for Taft (25 kine) in the middle earthquake and 1/45rad for BSL (80%) in the big earthquake. Although "Semi-rigid timber only frame" has about 2.5 times the actual performance as compared with general performance, it was confirmed that the response deformation may become large depending on the seismic wave. Then, the maximum response deformation of "Semi-rigid timber frame with oil damper" was the smallest for any of seismic wave compared to the other test pieces. Furthermore, it was confirmed that damage to the main structure can be suppressed by damping because the natural frequency almost not changed.

5. Seismic design method based on spectrum capacity procedure for timber semi-rigid frame with oil damper

This chapter is about equivalent linearization method based on calculation of response and limit strength for SDOF model of timber structure with oil damper by assuming slip type resilience characteristic of main frame. By integrating many literatures, the evaluation of oil damper effects in vibration control design by extended limit strength calculation method is shown. By comparing with numerical analysis and full scale shaking table tests the accuracy has been verified. In particular, complying to previous research etc., refer to what current calculation of response and limit strength can do to evaluate the effect of oil damper on "Japanese seismic Isolation Building Design Standards", by replacing Maxwell model with equivalent Voigt Model, the extended method is considered and shown.

As a result, it was confirmed that the tendency of overall seismic response can be predicted by the proposed evaluation method. Furthermore, it was confirmed that the accuracy of evaluation was improved by adding the evaluation based on the existing literature.

6. Conclusion

In this paper, full-scale shaking table test shows the effectiveness of applying an oil damper to a timber structure. Then, the possibility of considering oil dampers based on the current seismic design method was shown.

Evolutionary history of a global invasive ant, Paratrechina longicornis

(Graduate School of Agriculture, Laboratory of Innovative Humano-habitability, RISH, Kyoto University)

Shu-Ping Tseng

One successful management strategy to mitigate the negative impacts of invasive species relies on reconstructing the invasion history, which traces the patterns of ongoing invasion pathways and prioritizes quarantine resources accordingly to areas at high risk of invasion. The longhorn crazy ant, *Paratrechina longicornis*, is considered to be a significant invasive species due to its ecological impacts. Although *P. longicornis* has been reported worldwide for more than a century, its origin and invasion history remain controversial. Examining the global genetic pattern of *P. longicornis* may provide insight into the invasion history of this species. The present study aims to elaborate the evolutionary biology of *P. longicornis*, the longhorn crazy ant, focusing on the invasion history of this species and its symbionts to address questions through the lens of bioinvasion and evolutionary genetics.

The global genetic structure of *P. longicornis* has not yet been extensively studied, partially because of the limited number of genetic markers currently available. In the present study, we developed 36 polymorphic microsatellite markers for *P. longicornis* and characterized these markers by genotyping *P. longicornis* workers from 74 colonies in East and Southeast Asia. The results show that these new microsatellite markers are effective as a practical tool for reconstructing routes of invasion and assessing the population genetics of this invasive ant.

Analyses of mtDNA sequences of *P. longicornis* from 13 geographic regions reveal two highly diverged mtDNA clades that co-occur in most of the regions studied. The finding that numerous mtDNA haplotypes are shared among regions implies that inadvertent long-distance movement through human commerce is common in *P. longicornis* and has shaped the genetic structure of this invasive ant worldwide. In addition, our results indicate that the two highly diverged mtDNA clades are associated with different *Wolbachia* infection patterns, but are not congruent with patterns of nDNA (microsatellite) variation. Two *Wolbachia* strains, *w*LonA and *w*LonF, occur: *w*LonA appears to be primarily transmitted maternally, and its infection status is consistent with a relatively recent *Wolbachia*-induced selective sweep, while the history of *w*LonF infections in *P. longicornis* appears to be characterized by frequent gains and losses over time.

No. nests (percentage)	wLonA	wLonAF	wLonF	Uninfected
Clade I	42 (33%)	56 (44%)	8 (6%)	21 (17%)
Clade II	0 (0%)	0 (0%)	47 (39%)	74 (61%)
Total	42 (17%)	56 (23%)	55 (22%)	95 (38%)

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We further elucidated the sources of wLonF by surveying *Wolbachia* infection in various ant guests. Although *Wolbachia* is primarily transmitted maternally in arthropods, horizontal transmission between species has been commonly documented [1-3]. Our present results indicate that *P. longicornis* and a specialist ant cricket *Myrmecophilus americanus* share an identical *Wolbachia* strain, implying the occurrence of horizontal *Wolbachia* transmission between the ant and its specialist ant guest. We also identified two additional cases of *Wolbachia* transfer among ants and ant crickets, elucidating the role of intimate ecological associations in successful horizontal *Wolbachia* transmission.

The estimation of nDNA variation in worldwide populations reveals an extremely high level of heterozygosity in *P. longicornis*, a possible genetic consequence of its unusual reproductive mode in which workers are produced from the hybridization of a divergent queen and male clones. Our study showed that this system is widespread across our studied populations of *P. longicornis* and might act as an adaptive trait linked to the invasion success of this species given that it potentially relaxes the costs associated with inbreeding.



Figure 1. Clonal reproduction in queens and males of *P. longicornis*. This figure was adopted from Pearcy *et al.* (2011)[4]. Maternal (light) and paternal (dark) chromosomes are displayed. Contribution to the genome of the offspring is indicated by arrows (the dashed arrow represents the mother laying haploid eggs with no actual contribution to the genome).

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Study on novel rectifiers for microwave wireless power transfer system

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Ce Wang

In the 1970s, W. C. Brown demonstrated the first microwave wireless power transfer system. This system adopted horn antenna as transmitter and a rectenna array as a receiver. Since then, the microwave wireless power transfer technology attracted much academic attention. The technology can be applied widely in our life. For example, it can be applied as a power harvesting technology to drive low power or tiny power sensors in IoT system. To drive mobile electronic devices such as mobile phones, laptops, and tablets, a medium-power microwave wireless power transfer system application in life is highly anticipated. In recent years, with the popularity of electric vehicles and new electrical equipment such as drones, high power microwave wireless power transfer system has also been developed.

The microwave wireless power transfer system consists of transmitter and receiver, the DC to RF conversion efficiency in the transmitter, and the RF to DC conversion efficiency in the receiver mainly affect the total efficiency of the system. However, the single shunt rectifiers which have high conversion efficiency commonly adopted in high frequency, the output voltage of single shunt rectifiers is too low to drive a digital circuit. In this paper, we discussed the GHz-band high-frequency and high conversion efficiency rectifier at the receiver in detail.

Charge pump rectifiers with class-F filter

For Tiny or low power applications, we designed a 5.8 GHz highly efficiency rectifier circuit based charge pump circuit in chapter 2 shown as Figure 1. And we introduced a class-F load that can make it on a process the third harmonic wave. To improve the conversion efficiency of the rectifier circuit, we adjusted the applied voltage of diodes. Owing to inserting the class-F load and adjusting the applied voltage of diodes in this circuit, the conversion efficiency increase to 78% at 30 mW in the circuit in the simulation, and obtained about 71% conversion efficiency in the experiment. In addition, the output voltage is higher than 5 V at an optimum voltage 1300 Ω .



Figure 1. The photos of normal charge pump rectifier and class-F charge pump rectifier.

Charge pump rectifier array application

For medium-power applications, rectenna array often applied in a microwave wireless power transfer system. Based on chapter 2, we theoretically compare the diode losses in a charge pump and single shunt rectifier, and experimentally verify the results. Apart from this, we consider that the class-f charge pump rectifiers will be used for a rectenna array. To know the DC load change of a class-f charge pump circuits is connected as a rectenna array, we measured the conversion efficiencies of a 2 by 2 rectenna array, connected in series and parallel. The results of the experiment indicate that the optimum load of the rectifier changes to 4 times DC load when connected in series, and to 1/4 the DC load when connected in parallel.

Multistage Dickson charge pump rectifier

For medium-power applications and high voltage application, based on the class-F charge pump rectifiers, we designed multistage Dickson charge pump rectifiers shown as Figure 2. The maximum conversion efficiency of the 2-stage class-F filter Dickson charge pump rectifiers is approximately 70%,

and the maximum conversion efficiency of the 4-stage rectifiers is approximately 60%. The maximum output voltage of the 2-stage charge pump rectifiers is over 9 V, and the maximum output voltage of the 4-stage charge pump rectifiers is over 15 V. Additionally, we compared the multistage class-F filter Dickson charge pump with normal multistage Dickson charge pump rectifiers. We found that the performance of the multistage class-F filter charge pump is better than the normal charge pump rectifiers. Because the charge pumps rectifiers at each stage affect each other, the harmful effects of harmonic components are amplified in multistage Dickson charge pump rectifiers.

High conversion efficiency and high power rectifiers design

For high power application such as electric vehicle applications and drone applications, a high conversion efficiency rectifiers are required. However, a conventional silicon semiconductor hardly rectifies high power and high-frequency. In chapter 5, we applied individual GaAs Schottky diodes to design a high power high frequency rectifier. Experimental results show that the conversion efficiency of the rectifiers with GaAs Schottky diodes is 91% at optimal input power 3 W and optimal load 100 Ω . In addition, we measured the SPICE parameters and package parameters of the diodes. And we analyzed the effect



Figure 2. The photos of 2-stage and 4-stage Dickson class-F charge pump rectifier.

of the diode SPICE parameters on the rectification conversion efficiency.

Optimal load adjustment by band-stop structure for GHz-band rectifiers design

The conversion efficiency of rectifiers affects the total efficiency of microwave wireless power transfer systems; moreover, rectifiers are load-dependent. At optimal load, a rectifier has its highest conversion efficiency; as the load increases or decreases, the conversion efficiency of the rectifier decreases. In chapter 6, we propose a method in which the optimal rectifier load is controlled by changing the band-stop structure length. We design 2.45 GHz single shunt rectifiers, 2.45 GHz charge pump rectifiers, and 5.8 GHz two-stage Dickson charge pump rectifiers to verify the method. The experimental results show that for the 2.45 GHz single shunt rectifiers, when the optimal load changes from 300 Ω to 500 Ω , the conversion efficiency is approximately 72%; for the 2.45 GHz charge pump rectifiers, when the optimal load changes from 1000 Ω to 1100 Ω , the conversion efficiency is approximately 78%; and for the 5.8 GHz two-stage Dickson charge pump rectifiers, when the optimal load changes from 1000 Ω to 1900 Ω , the average conversion efficiency is approximately 65%.

Summary

In summary, microwave wireless power transmission technology has a broad application prospect. To satisfy a variety of application conditions, we proposed the GHz-band class-f filter charge pump rectifiers for tiny or low power applications. Furthermore, the load variation of the class-F filter charge pump rectifier array and multistage Dickson class-F filter charge pump rectifiers is discussed in chapter 3 and chapter 4. For high power applications, we designed high power 2.45 GHz single shunt rectifiers by GaAs Schottky diodes in chapter 5. Finally, we proposed an optimal load adjustment design method to march the load, and we verify its validity in chapter 6.

Local tracheid structure of Cryptomeria japonica and its potential for dendrochronology

(Graduate School of Agriculture, Laboratory of Biomass Morphogenesis and Information, RISH, Kyoto University)

Takeshi Nakajima

Introduction

Tree ring studies are important field of science, including dendrochronology, dendroclimatology and modeling the tree growth environmental response system¹). In general, softwood often used in these studies generates tracheids accurately aligned along the radial directions and their shape and size are known to be influenced by surrounding environment. In most cases analyses have been conducted using one parameter from one tree-ring, e.g. ring-width, density, ratio of stable isotopes, and so on. However, the information within a ring, i.e. intra-annual variety of anatomical characteristics, has been less considered as a parameter of tree ring analyses²). Therefore, intra-annual anatomical features in *Cryptomeria japonica* from Ashiu Experimental Forest were scrutinized to extract possible relationships between climate and intra-annual ring pattern using correlation analysis.

Experimental

Ashiu experimental forests located in the center of Japan was used as a research site, and 12 cores from 6 individuals of *cryptomeria japonica* were collected. Cross-sectional optical micrographs in chronological order were created from each core. Lumen radial diameter (LRD) and cell wall thickness (CWT) were measured from all tracheids in each annual ring by image analysis. Then the local tracheid structure, that is, seasonal transition of these two parameters by small window with width equal to 10% of ring-width moving by 1% of ring-width step, was calculated. Finally, relationship between the chronologies and 31-day averaged temperature and 51-day averaged precipitation in Ashiu experimental forests was analyzed in detail.

Result and discussion

Notably, strong correlation between anatomical parameters and climates at least 6 positions was detected. Especially, temperature and precipitation in June largely affected tracheid structure from middle to latewood region. Two statistical indices, RE and CE often used in dendroclimatology revealed that local tracheid structure have a possibility to be proxies of climate reconstruction.

Furthermore, a crossdating based on the most sensitive parameter, precipitation in June (Figure 1), instead of the conventional method using ring-width was examined. Interestingly, this method reliably crossdated the core which was completely failed to crossdate by a conventional method and showed that local tracheid structure can better extract simultaneity between target cores accurately. These lines of evidences suggested that the local tracheid structure has a possibility to be a new parameter for dendrochronology.



Figure 1. Time series of normalized LRD (solid) and 51-day mean precipitation sum in June (dashed).

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Identification of two anatomically similar Cupressaceae species using two-dimensional MFA mapping

(Graduate School of Agriculture, Laboratory of Biomass Morphogenesis and Information, RISH, Kyoto University)

Yusuke Kita

Introduction

Wood identification is an important task for the better understanding of Japanese cultural background. One of the representative examples is the identification of *Chamaecyparis obtusa* and *Thujopsis* spp. because their usage has strongly depended on the ranks and localities of traditional buildings in spite of their similarities. Conventionally, they have been identified by the observation of cross-field pitting in earlywood appearing junctions between tracheids and rays. However, this methodology cannot give us a clear answer in many cases. Our research aims to invent a brand-new technique for the identification of *C. obtusa* and *Thujopsis* spp. via a quantitative criterion. Hence, we introduce MFA in a two-dimensional manner and extract wood species specific features from MFA using image recognition techniques.

Methods

Present and old wood specimens of *C. obtusa* and *Thujopsis* spp. were prepared (12 specimens each in present and 2 specimens each in old). Their cross sections in 10 µm thickness were cut using a sliding microtome and sealed by gum chloral. These sections were put on a revolving stage of a polarized optical microscope equipped with a sensitive color plate and 40x objective lens. Radial and tangential walls were roughly parallel to a slow and fast axis of the plate. For quantifying interference colors, hyperspectral images targeting a part of visible right regions (461-602 nm) were acquired by a CCD camera equipped with a liquid crystal tunable filter. Hyperspectral images were converted to two-dimensional retardation and MFA images using crystal optics equations. Finally, almost 500 images were obtained (10-20 images per one specimen). For utilizing two-dimensional information of MFA, a convolutional neural network (CNN) called VGG16 [1] was applied to the two-class (*C.obtusa* and *Thujopsis* spp.) classification problem. Accuracy decay by channel-wise erasing and Grad-CAM [2] were implemented for the sake of the interpretation of results obtained by CNN.

Results and discussions

Classification accuracy reached almost 90% in the both cases, present and old wood specimens. These results strongly suggest the applicability of MFA for extracting and describing wood anatomical features. Large accuracy decays were observed selectively in small and middle MFA regions (0°-20°). In addition, Grad-CAM heatmaps highlighted only radial walls in *C. obtusa* and tangential walls in *Thujopsis* spp. as grounds for decision makings by CNN. These visualizations implied that MFA anisotropy in S₂ wall induced by pits creates wood species specific characteristics discriminating them.

Acknowledgements

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Screening and identification of aldehyde dehydrogenases in a white-rot fungus, *Ceriporiopsis subvermispora*

(Graduate school of Agriculture, Laboratory of Biomass Conversion, RISH, Kyoto University)

Junseok Lee

In these days, an interest in renewable energy becomes higher due to depletion of fossil resources and global warming by greenhouse gas. Many researchers have been focusing on utilizing carbon-neutral wood biomass for conversion to biofuels and valuable chemical products. Wood biomass is mainly composed of cellulose, hemi-cellulose and lignin. Lignin physically surrounds polysaccharides of plant cell wall; therefore, pretreatment steps should be conducted to remove lignin before ethanol production. Throughout the pretreatment process, however, phenolic byproducts—lignin-derived aromatic compounds—are released. Of these compounds, aryl-aldehydes, such as vanillin (Figure 1), are known to inhibit ethanol fermentation and growth rate of yeast. Particularly, this growth inhibition by vanillin is also reported even in various lignin-degrading white-rot fungi [1-2]. However, a selective lignin-degrading white-rot fungus, *Ceriporiopsis subvermispora*, increased growth rate, glucose consumption and lipid biosynthesis in the presence of vanillin [3]. In this study, we tried to screen and identify (aryl-)aldehyde dehydrogenases from *C. subvermispora* as part of the molecular elucidation of its vanillin metabolism.



Figure 1. Chemical structure of vanillin

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Screening and characterization of candidate genes involved in the biosynthesis of ceriporic acid B from *Ceriporiopsis subvermispora*, a selective lignin-degrading fungus

(Graduate school of Agriculture, Laboratory of Biomass Conversion, RISH, Kyoto University)

Akito Morita

A white-rot fungus, *Ceriporiopsis subvermispora*, is characterized as one of the best bio-pulping fungi because it can selectively degrade lignin without serious damage to cellulose. This fungus extracellularly produces a series of itaconic acid derivatives, ceriporic acids A-C, having a long alkyl and alkenyl side chain at the C3 position (Figure 1). In particular, ceriporic acid B suppresses iron redox reactions to inhibit the production of cellulolytic hydroxyl radicals by the Fenton reaction [1-2]. However, its biosynthetic pathway remains to be elucidated although its model pathway is proposed [3]. Therefore, it is of interest to characterize the biosynthetic genes of ceriporic acids for the elucidation of the molecular mechanism of the selective lignin degradation. So far, we previously found 10 candidate genes from the genome of *C. subvermispora* and cloned 6 genes of them. In this study, we tried to clone the remaining 4 genes, to express all candidate genes heterologously, and also measure the activities of their recombinant enzymes.



Figure 1. Suppression of the production of cellulolytic hydroxyl radicals by ceriporic acids

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Stepwise enzymatic fractionation and structural analysis of lignin-carbohydrate complex

(Graduate School of Agriculture, Laboratory of Biomass Conversion, RISH, Kyoto University)

Saho Kashima

Lignocelluloses are mainly composed of cellulose, hemicelluloses, and lignin. Lignin is a heterogeneous aromatic polymer with high potentials to utilize as resources for energy, chemicals, and materials supply. Lignin associates with hemicelluloses in plant cell walls. The amphipathic complex is called Lignin-Carbohydrate Complex (LCC). LCC contributes to the physical strength of plant cell walls, but at the same time, it hinders the separation of the plant cell wall components. Chemical structures of LCC, especially covalent linkages between lignin and carbohydrates have not been fully understood because of the low frequency of lignin-carbohydrate (LC) linkages, compared with its polymer backbones, lignin and hemicellulose moieties. Therefore, the concentration of LC linkages is essential for the entire structural analysis. Recently, ester-type LC linkages have been elucidated from pine wood [1]. In this study, we aimed at purifying the LC fragments with increased LC linkages by combining two steps enzymatic treatments degrading lignin and hemicelluloses from wood.

In the lignin degradation, we used five kinds of lignin-degrading enzymes derived from a marine microorganism, *Novosphingobium* sp. These five enzymes catalyze three-step reactions cleaving β -O-4 linkages, a major linkage in lignin. It is shown that the dimeric lignin model compounds can be degraded into aromatic phenylpropanone monomer. We proved these enzymes act on natural lignin substances and produce monomer in one pot [2]. The advantage in using these enzymes is selective cleavage of the interunit linkages between phenylpropane units in lignin without the scission of LC bonds, which facilitates purification of the LCC fragments with decreased molecular mass. We evaluated the enzyme reactivity towards LCC fractions by LC-ESI-MS and 2D-NMR.

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Production of antiviral compounds from sugarcane bagasse by microwave solvolysis

(Graduate School of Agriculture, Laboratory of Biomass Conversion, RISH, Kyoto University)

Tatsuya Miyazaki

Due to depletion of fossil resources and global warming, it has become an urgent issue to establish a biorefinery system which converts biomass to fuels, chemicals, materials and other value-added products. In particular, conversion of lignocellulosic biomass has gathered increasing interest due to its abundance and non-competitiveness with food supply. Humanities are now facing a thread of pathogenic viruses. Warming of average temperatures of the atmosphere and globalization of transportation have been causing widespread infection of pathogenic viruses harmful to humans and livestock. Therefore, development of antiviral agents suppressing spread of pathogenic viruses is rapidly becoming important.

In the present study, we studied production of antiviral compounds from sugarcane bagasse using microwave reactions by combining the needs for biorefinery and antiviral agents. Sugarcane bagasse, a major by-product of sugarcane industry is a lignocellulosic agricultural residue. Sugarcane bagasse was decomposed by microwave acidic solvolysis. The reaction solution was neutralized, and the decomposition products were extracted with ethyl acetate and methanol. The extracted products were subjected to plaque assay against influenza A virus and enterovirus 71 (EV71). Influenza A virus is an enveloped single strand RNA virus classified as the family *Orthomyxoviridae*. EV71 is a nonenveloped single strand RNA virus classified as the family *Orthomyxoviridae*. EV71 methanol that acidolysis of sugarcane bagasse produced lignin fractions having strong antiviral activity against influenza virus A virus and EV71. Thus, reactions producing antiviral compounds against enveloped and nonenveloped viruses from sugarcane bagasse were found.

Characterization of an *O*-methyltransferase involved in antitumor lignan biosynthesis in *Thujopsis dolabrata*

(Graduate School of Agriculture, Laboratory of Metabolic Sciences of Forest Plants and Microorganisms, RISH, Kyoto University)

Yu Matsuura

Lignans are phenylpropanoid dimers that are linked at C8 positions of their propyl side chains and known for having various physiological activities. For examples, podophyllotoxin, aryltetralin lignan, is known as an antitumor lignan and used as a precursor for the chemical synthesis of the anticancer drugs etoposide, teniposide and etopophos [1]. *Podophyllum hexandrum* and *Anthriscus sylvestris* are known as plants that produce antitumor lignans including podophyllotoxin. Biosynthetic pathway of antitumor lignans has been studied using several plants, and some pathways were proposed. Sakakibara et al. [2] carried out feeding experiments using several stable isotope-labeled precursors to *A. sylvestris*, and proposed the pathway from matairesinol to yatein via 4,5-*O*,*O*-dimethylthujaplicatin. Recently, Lau and Sattely [3] were successful in isolation many genes involved in lignan biosynthesis of *P. hexandrum* based on RNA-seq gene expression analysis, and demonstrated that each of isolated genes can catalyze each reaction step from matairesinol to deoxypodophyllotoxin via bursehernin by agroinfiltration into tabacco leaves and biochemical characterization using recombinant protein.

Recently, author's laboratory cloned putative lignan OMT genes (TdOMT1, 3, 4, 5, and 6) from *Thujopsis dolabrata*, a closely related to *T. occidentalis*, as a collaboration with Shizuoka University [4]. Recombinant protein of TdOMT6 showed OMT activities for 4-*O*-demethylyatein and 5-*O*-methylthujaplicatin, while the functions of other genes (TdOMT1, 3, 4, and 5) were also unknown. On the other hand, although *T. dolabrata* produces deoxypodophyllotoxin [5], the biosynthetic pathway leading to the compound has not been elucidated. In this study, the author performed biochemical characterization of TdOMTs and estimated the biosynthetic pathway.

In this study, several lignans including matairesinol and deoxypodophyllotoxin were found in leaves of *T. dolabrata*. Furthermore, recombinant TdOMT6 catalyzed *O*-methylation of hydroxy group at C4 position of 4-*O*-demethylyatein, 5-*O*-methylthujaplicatin, and pluviatolide. The OMT activity for 4-*O*-demethylyatein was much greater than those for 5-*O*-methylthujaplicatin and pluviatolide, when the mixture of equal amounts of these three lignans was used as a substrate for enzyme assay. These results strongly suggest that TdOMT6 functions as 4-*O*-demethylyatein OMT, and biosynthetic pathway from matairesinol to deoxypodophyllotoxin via 4-*O*-demethylyatein in *T. dolabrata* exists.

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Analysis of shikonins in the rhizosphere of Lithospermum erythrorhizon

(Graduate School of Agriculture, Laboratory of Plant Gene Expression, RISH, Kyoto University)

Ko Sato

The rhizosphere is a small region around root defined as "region affected by plant roots", and plant metabolites are one of the major effects from plants to the rhizosphere soil. To define the rhizosphere area in soil, the dynamics of these plant metabolites are important, but it is difficult to analyze the dynamics of these plant metabolites in soil. Thus, defining the spatiotemporal distribution of plant metabolites in the rhizosphere is a crucial step to understand the rhizosphere. In order to analyze the metabolites. Visualization is a good indicator for the detailed information on the dynamics of metabolites. Visualization is expected to lead to the elucidation of the mechanisms of various rhizosphere interactions in soil. So far, methods to visualize the molecules have been established to clarify the localization of minerals and metabolites in the rhizosphere using gels containing adsorbents, radioisotopes, fluorescent proteins, and computer tomography scans. On the other hand, there remain difficult for the application of these methods to analyze the dynamics of the metabolites in long period, mostly due to the limitation of observation time, soil condition, and scale of observation system. In this study, we analyzed the dynamics of plant metabolites in the rhizosphere using shikonins, which are colored purple. Shikonin is produced in *Lithospermum erythrorhizon*, and have high biological activities and are involved in interactions between organisms in the rhizosphere.

In order to observe the secretion of shikonins in *L. erythrorhizon*, plants grown in a pot were transferred to the root box containing Toyoura sand. These plants were cultivated in a dark room to prevent the inhibition of the shikonin biosynthesis, and the root growth was observed by time-lapse imaging. In order to analyze the distribution of shikonins in the soil, roots and soils in pots were fractionated into root, root surface soil, rhizosphere soil, and root zone soil by the water fractionation method using phosphate buffer, following the established protocol. The amount of shikonins in these samples was analyzed using high pressure liquid chromatography. In order to analyze the transfer of shikonins to the water phase in the soil fraction, the shikonins transferred to the aquatic fraction when the rhizosphere soil was fractionated using phosphate buffer, and an average of 2.6% was transferred to the root surface soil when the rhizosphere soil was fractionated. It was shown that the phosphate buffer fractionation method is applicable to soil fraction in the rhizosphere of *L. erythrorhizon*. It was shown that most of the shikonins in pots were localized in the root surface.

Establishment of virus-induced gene silencing (VIGS) system by use of domestic plant viruses in *Lithospermum erythrorhizon*

(Graduate School of Agriculture, Laboratory of Plant Gene Expression, RISH, Kyoto University)

Yuki Izuishi

Lithospermum erythrorhizon is a boraginaceaeous medicinal plant that produces a unique red naphtoquinone called shikonin as its ester derivatives. These pigments are accumulated in the roots of this herbal plant. The dried roots have been used as a crude drug in Asian countries, and shikonin derivatives are major active pharmaceutical components of this herbal medicine. Many divergent biological activities have been reported in shikonin and its stereo-isomer alkannin; i.e., conventionally, antibacterial, anti-inflammatory, anti-oxidant, antitumor, granulation enhancer, and more recently anti-angiogenic [1], anti-topoisomerase [2], increment of glucose uptake [3], and decrease in adiposity [4].

Biosynthetic pathway of shikonin were actively studied by biochemical methods using enzymatic analysis, and also by feeding experiments with radio-labeled compounds from 1970s, as summarized in a review [1]. Currently, synthetic biology approaches are actively studied to synthesize high value plant products in unicellular organisms, while the shikonion biosynthetic pathway is not completely clarified. There are many candidate genes paving the gap in the latter shikonin biosynthetic pathway, while an effective evaluation system for the gene functions is missing. RNAi-mediated suppression is only one feasible method for this, but it needs long period to evaluate the gene function after generating hairy roots, while virus-induced gene silencing (VIGS) system provides more rapid and efficient evaluation system as it provides an easy knockdown system by simple infection.

In this study, we tried several plant viruses as vectors to induce gene fragments, by which the functions of candidate genes are knocked down. As a model gene, we selected phytoene desaturase (*PDS*) gene to knock down in this plant, because the phenotype of VIGS is easily recognized by visual evaluations.

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Secretion of tomatine from tomato roots and analysis of tomatine in the field

(Graduate School of Agriculture, Laboratory of Plant Gene Expression, RISH, Kyoto University)

Kohei Ohno

It is known that plants secrete various secondary metabolites from roots, and these metabolites are involved in the interaction between plants and microorganisms in the rhizosphere. Rhizosphere microbiota play an important role in plant growth and health. To archive sustainable agriculture with reduced fertilizers and pesticides, it is important to deepen our understanding of the interactions between plants and microorganisms and control of rhizosphere environment through root exudates. Tomato is one of the most cultivated vegetable in the world. Tomato biosynthesizes the glycoalkaloid α -tomatine and accumulates it in each organ of the plant. Tomatine is toxic to a wide range of organisms including insects and fungi, and has a function to protect plants from various biological stresses. Although tomatine is recently shown to be secreted into the rhizosphere, the function of tomatine in the tomato rhizosphere and the mechanism of secretion from the roots are still unknown. The purpose of this study was to clarify the secretion of tomatine using hydroponic and field-grown tomatoes in order to elucidate the function of tomatine in the rhizosphere.

Tomato (*Solanum lycopersicum*) was grown in hydroponic culture, and tomatine contents in leaves, roots, and root exudates was quantified using LC-MS at 2 week intervals from 3 weeks to 13 weeks after sowing. Tomato was also grown in the field, and tomatine in plant and rhizosphere soil was quantified, and rhizosphere microbiota was analyzed.

In hydroponically grown tomatoes, the amount of tomatine was highest at 7 weeks, which is the flowering stage, and was hardly secreted after fruit setting. Tomatine content was higher in the leaves than in the roots throughout the growing periods. The roots of young plants accumulate a large amount of tomatine, which may protect roots from potential pathogens. Field-cultivated tomatoes also secreted tomatine throughout the growth period. The content of tomatine in roots was higher in the field cultivation than in hydroponic cultivation. In the field, it was considered that tomatine biosynthesis may be activated in the roots for the protection from pathogenic bacteria and fungi. To analyze the expression of genes involved in tomatine biosynthesis in leaves and roots, RNA was extracted and reverse-transcription quantitative PCR was performed. We also analyzed the tomato rhizosphere microbiota in order to investigate the effects of tomatine secretion on rhizosphere microorganisms.

A transport engineering approach to synthetic biology for artepillin C production in yeast

(Graduate School of Agriculture, Laboratory of Plant Gene Expression, RISH, Kyoto University)

Koki Yanagihara

Propolis is a resinous substance that honeybees prepare by collecting wax-rich part from buds and other aerial parts of different plant species in order to seal physical damages (e.g., cracks and holes) of beehives, to prevent invasion of their enemies [1]. This honeybee product has been sold as natural medicines and food supplements worldwide because of its broad pharmaceutical and health-promoting activities attributed to its complex chemistry of over 500 constituents [2,3]. The chemical composition of propolis is highly diversified depending on its botanical sources, geological locations and bee species, which gives unique spectrum of bioactivities for each type of propolis [2]. Brazilian green propolis is one of the most globally spread types for commercial purposes. It is characterized by the presence of bioactive prenylated derivatives of *p*-coumaric acid, such as drupanin and artepillin C, the latter of which is a major constituent exceeds 10% at highest levels in the Brazilian propolis [3]. It is to be noted that this main compound exhibits its activities by oral administration as reported, for instance, suppression of colon and pulmonary carcinogenesis chemically induced in mice [4,5]

Despite the high value of artepillin C, this compound is mainly accumulated only in Brazilian plants belonging to *Baccharis* species, such as *B. dracunculifolia*, a bush distributed in South America. Because of the Nagoya protocol, applied sciences with the Brazilian plant contain potential risks of benefit share. We have thus searched domestic plants that have productivity of artepillin C, namely diprenyltransferase for *p*-coumaric acid. As the results, we identified AcPT-1 from *Artemisia capillaris*, which was then subsequently applied to produce artepillin C in yeast [6].

In the production procedure, we have realized that the produced artepillin C remains at the cellular level, while non-prenylated substrate, *p*-coumaric acid, is almost exclusively secreted to the medium leading to the inefficient usage as the enzyme substrate. This observation encouraged us to identify transporter molecules responsible for the secretion of artepillin C in the intact plant. Then, we have thoroughly listed candidate transporter genes that may be involved in the excretion of artepillin C in the native plant.

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In silico search of candidate genes involved in shikonin production in Lithospermum erythrorhizon

(Graduate School of Agriculture, Laboratory of Plant Gene Expression, RISH, Kyoto University)

Hao Li

Shikonin is a red naphthoquinone pigment produced by some boraginaceaeous plants. *Lithpospermum* erythrorhizon is a representative of those plants, and other limited species belonging to Arnebia, Anchusa, Alkanna, Echium, and Onosma also produce the red pigment. Shikonin derivatives exist as ester derivatives with low molecular weight fatty acids in the root bark of those plants. The dried roots of *L. erythrorhizon* and other shikonin-containing plants are widely used as crude drugs in the area from Europe to Asian countries, taking the advantage that these naphthoquinone derivatives exhibit a variety of biological activities, such as anti-inflammatory, anti-bacterial, hemostatic activities [1]. Recently, an inhibitory effect on main protease of COVID-19 causing serious pandemic is also reported in shikonin [2]. Shikonin derivatives have also been used as a natural dye to stain cloths for over 2 thousand years to have bright purple color with aluminum-containing mordants. *L. erythrorhizon* is, however, recognized as an endangered species despite the high market needs in the last decades. By those backgrounds, cell suspension culture systems were established, which were applied for the industrial production of shikonin derivatives in 1980s. This success brought an outbreak of plant cell/tissue culture studies in scientific communities, and also in bio-industry sectors at the time.

Biosynthetic rout of shikonin were actively studied by biochemical methods using enzymatic analysis, and also by feeding experiments with radio-labeled compounds from 1970s, as summarized in a review article [1]. The biosynthetic pathway of shikonin has been, however, remained to be clarified yet for over 50 years, in particular the latter half of the pathway, in particular the naphthalene ring formation reaction.

The genome sequence read of *L. erythrorhizon* is deposited in public databases without assembling in 2018. Thus, a draft genome sequence was constructed by use of public softwares, which enabled us to search genes of interest at the genome level. By analyzing the data, it was figured out that some secondary metabolic genes exist in the genome as single exon genes. Taking the shikonin biosynthesis as a model, it was shown that, at least some transferase families, several genes are present in the genome as single exon genes, which are potentially involved in the biosynthesis of shikonin [3].

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Development of a low noise RASS using a parametric array

(Graduate School of Informatics, Laboratory of Atmospheric Sensing and Diagnosis, RISH, Kyoto University)

Koki Muguruma

The frequency of unpredictable local heavy rain is increasing in the world. Various studies have been made to predict local and short-term weather phenomena. One of them is to improve the temporal and spatial resolutions of meteorological observation data. If meteorological observation data can be obtained with high resolution, understandings of meteorological phenomena will progress, and short-term weather forecasts will become more accurate, which will contribute to the safe operation of aircraft.

Radio acoustic sounding system (RASS) is a ground-based remote-sensing technique to obtain temperature profiles in the troposphere with high temporal and height resolutions by combining an atmospheric radar (or wind profiling radar) and a high-power acoustic transmitter shown in Figure 1. However, the RASS requires strong output sounds in the audible frequency, so observation sites are restricted due to a noise problem.

The purpose of this study is to develop a low noise and all-weather RASS system using a parametric array having a sharp directivity. Considering the application of parametric speakers to RASS, securing waterproofness is an important issue because of outdoor use of RASS speaker. We ensured waterproof property of a speaker by making it turn to downward and reflective type shown in Figure 2.

We carried out an observation experiment by using an LQ7 wind profiling radar and the reflective speaker. When the wind was calm, it was confirmed that RASS echoes could be obtained up to 1 km above the ground as shown in Figure 3. However, it was difficult to obtain RASS echoes when the wind was strong. In order to improve the RASS observability in the presence of wind, we considered speaker layout as *Uncapacitated Facility Location Problem* based on wind data obtained with a Doppler sodar, and we examined the optimal speaker layout.

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Figure 1. The principle of RASS



parametric speaker



Development of low-latitude ionosphere observation technique with dual-band beacon experiment from COSMIC-2 and TBEx satellites

(Graduate School of Informatics, Laboratory of Radar Atmospheric Science, RISH, Kyoto University)

Nobuhiro Ujihara

In the low-latitude ionosphere, it is known that plasma bubbles, depletion of electron density, are generated. There are social needs for the studies to reveal mechanisms of plasma bubble generation, which can lead to the prediction of plasma bubble occurrences. The ionospheric Total Electron Content (TEC) measurement by satellite beacon is useful for studying the plasma bubble. In this study, we developed the TEC observation method using two different frequency beacon signals from COSMIC-2 and TBEx satellites which were launched on June 25, 2019. We conducted TBEx orbital simulation, developed signal analysis software, developed and set new beacon receiver, examined method estimating TEC by COSMIC-2 satellites. 400MHz/965MHz signals are used in beacon observation by COSMIC-2. As our old model the GRBR used 150MHz/400MHz signals, the signal processing was largely depending on integer frequency ratio of 3:8. We then developed a new signal processing for GRBR2 that is suitable to the COSMIC-2 beacon. There are two bias estimation method, single point method and multipoint method. Comparing the results, we found that the latter is better than the former in the point of estimation accuracy. We deployed three GRBR2 at three locations in Southeast Asia. We succeeded to find longitudinal TEC distribution like Figure 1 automatically by the above-mentioned observation scheme. Figure 2 is Average TEC distribution in October near Bangkok and Ho Chi Minh. We also showed TEC distribution at the solar eclipse event on December 26, 2019. TEC observation by TBEx satellites have not been done yet. However, observations using conventional satellites that use 150MHz/400MHz signals were successful without any problems.



Figure 1. Longitudinal TEC distribution



As described above, in this study, we developed GRBR2 for TEC observation using dual frequency beacon signal using COSMIC-2 and TBEx satellites, and established a basic data analysis flow. We could not detect LSWS because of low solar activity. It is expected that LSWS is detected by TEC observation method developed in this study.

Artificial lignification improves mechanical property of cellulose microfibril gel

(Graduate School of Agriculture, Laboratory of Active Bio-based Materials, RISH, Kyoto University)

Tsubasa Yonekawa

Introduction

Plants accomplished to advance into the land from the sea about 475 million years ago, and especially tall woody plants had been flourishing by acquiring lignin since 425 million years ago. Therefore, it is considered that the mechanical property of wood was improved by depositing lignin into the cell wall. However, how the deposition of lignin, so called lignification, contributes to the mechanical property of the cell wall still remains unknown. In this research, to clarify the effects of the lignification on the mechanical property of the cell wall, artificial wood cell wall model was prepared by using cellulose microfibril hydrogel extracted from wood and artificial lignin model compound. The mechanical property of this cell wall model was studied in detail.

Experiment

By removing lignin and hemicellulose from the wood powder of hinoki (*Chamaecyparis obtusa*), the pulp water suspension was obtained. This suspension was mechanically fibrillated by the grinder to get viscous cellulose microfibril suspension. This suspension was then diluted and was filtrated to obtain wet cellulose microfibril sheet. By alkaline treatment and following neutralization, cellulose microfibril hydrogel was obtained. Then the lignin model compound was sequentially deposited inside the gel. The resulting cell wall model was subjected to observation by scanning electron microscopy and tensile test.

Results and discussion

By repeating the deposition of lignin, the surface of the cellulose microfibrils has been gradually covered by paste-like lignin, and the number of the pore was decreased (Fig. 1). The ratio of Klason lignin to cellulose content was gradually increased, and the value was 28.1% in 10 cycle. The thickness of the hydrogel was decreased by repeating the deposition of lignin, which means lignin deposition caused excess removal of water. The Young's modulus of the cell wall model was gradually increased by depositing lignin. Generally, the decrease of water content leads to higher Young' modulus. However, even taking into account the effect of the decrease in water content, lignin deposited samples showed higher Young's modulus than blank ones (Fig. 2). This is because the movement of the cellulose network was restricted by the surrounding lignin network. When the lignin deposited hydrogel was treated with 1,4-dioxane, almost all the lignin deposited cellulose network by physical bonding. Therefore, it is suggested that there exists interaction between cellulose and lignin in the real cell wall. Also, it is suggested that the mechanical property of the plant cell wall was improved by lignification even in never-dry state, by the formation of lignin network as well as the excess removal of water. Thus, it is considered that lignification enabled plants to stand alone against the gravity and to become taller.



Fig. 1. Cross-sectional views of cellulose microfibril hydrogels. *left*: blank, *right*: 10 cycle of lignin deposition.



Fig. 2. Young's modulus *vs* water content of various hydrogels.

Development of cellulose nanofiber/collagen sponge for 3D cell culture device

(Graduate School of Agriculture, Laboratory of Active Bio-based Materials, RISH, Kyoto University)

Tomoaki Shimpo

Introduction

Recently, the field of regenerative medical science have been getting attention. Especially in cell research, "Three-dimensional culture (3D cell culture)" for research has been attracted because it is required to mimic the in-vivo situation in cell culture. At present, collagen sponge with high biocompatibility has been used as a scaffold of cell for therapeutic area, however, using collagen sponge for cell research or cell experiment as cell culture device have not progressed. That is because collagen sponges have low physical strength, and there is a problem that the space where cells can survive is getting narrow by the deformation of the sponge during cell culture. Then, we tried to reinforce collagen sponge with cellulose nanofiber which has high elasticity, and aimed at development of Cellulose nanofiber/Collagen sponge for 3D cell culture device which can be cultured for a long time.

Experiment

A suspension of pH3 TEMPO-oxidized cellulose nanofiber (TOCN) (about 3 nm in width) was mixed with a collagen (COL) solution at 4 °C, and then the mix solution was immediately frozen at -20 °C after pH-induced. Next, these are dried with a freeze dryer to produce a sponge. After that, this sponge was subjected to dehydrothermally cross-linked treatment by a vacuum oven, sterilized by EOG, and then subjected to scanning electron microscope observation, physical property test, and cell culture test.

Results and discussion

In a 7-day shaking cell culture, the COL sponge broke in the medium on 4 day, while the TOCN/COL sponge retained its shape. Furthermore, the cell mitochondrial activity was significantly increased as the TOCN amount was increased in the MTT test for measuring the cell mitochondrial activity (Figure 1). And also, after 4 day of culture, cell cryopreservation was performed, and cell mitochondrial activity after cryopreservation was compared. As a result, cell mitochondrial activity of TOCN/COL sponge was significantly higher than that of COL sponge (Figure 2).

These results suggest that reinforcement by TOCN is effective on maintaining the space where cells can survive during culture and also after cryopreservation, and the TOCN/COL sponge is useful for 3D cell culture.



Figure 1. Cell proliferation on 7-day cell culture by MTT assay. (p*<0.05, p**<0.01)



Figure 2. Cell proliferation on cryo-preservation of cells with the sponge by MTT assay. ($p^{*}<0.05$, $p^{**}<0.01$)

Effect of kraft pulping on cellulose nanofibrillation

(Graduate School of Agriculture, Laboratory of Active Bio-based Materials, RISH, Kyoto University)

Ting-Hsuan Ku

Introduction

Cellulose nanofiber (CNF) owns the characteristics of high strength, high Young's modulus, low coefficient of thermal expansion to be applied to various industries, however, the high production cost causes it difficult to be used by the industries. Currently, kraft pulping is the dominant pulping process to produce commercial kraft pulp, which is utilized as a starting material to make CNF in many areas. The mechanism that affects the kraft pulping process on nanofibrillation has remained unclear until today. The present study aims to explore the effects affecting the kraft pulping process on nanofibrillation as a foundation for modifying the low efficiency in CNF production.

Materials and methods

The wood powder of 60-100 mesh from Todomatsu (*Abies sachalinensis*) was used. The kraft pulp sample was prepared by cooking the wood powder with NaOH/Na2S solution (kraft pulping) at 160°C for 5 hours and further purified with sodium chlorite under acidic condition and potassium hydroxide solution. The non-kraft pulp was prepared the same as the kraft pulp but without the kraft pulping process. Furthermore, non-kraft pulp was treated at 160°C and 1 hour with water and alkaline respectively. Samples were prepared into 0.7% suspension and treated with a high-speed blender with different time to observe the degree of fibrillation. CNF was prepared from non-kraft pulp after passing two times of stone grinder. 250 mL of 1% CNF was prepared and cooked in the portable reactor at 160°C for 1 hour to simulate the pulping process.

Results and discussion

Kraft suspension and non-kraft suspension has a different degree of fibrillation shown in Figure 1(a) and 1(b). Also, the non-kraft treated suspension has a higher specific surface area and water retention value comparing with kraft treated suspension. The non-kraft suspension is more easily to fibrillate then the kraft suspension. The non-kraft suspension cooked at 160°C with water and alkaline solution shown in Figure 1(c) and 1(d) have a different degree of fibrillation with the non-kraft suspension shown in Figure 1(b). The non-kraft pulp treated with 160°C for 1 hour with water and alkaline in Figure 1(c) and 1(d) showed a similar suspension state with the non-kraft suspension in Figure 1(a). CNF suspension shown in Figure 2 cooked at 160°C with water and alkaline solution aggregated after cooking. Kraft pulping process will have an unfavorable effect on the nanofibrillation of pulp. The reason for the unfavorable effect may due to microstructural change during the high temperature and high-pressure cooking.







Figure 2. CNF suspension cooked at 160°C for 1 hour with water and alkaline respectively

The structural performance of reinforced concrete flames with CLT infills

(Graduate School of Agriculture, Laboratory of Structural Function, RISH, Kyoto University)

Yutaka Taki

Introduction

Recently, from the viewpoint that the wood use is promoted, using wooden structural materials to middle or high-rise buildings is expected in Japanese architectural field. But, it's difficult to design those buildings due to fire resistance. Therefore, the composite structure with wooden structural materials and steel or RC (Reinforced Concrete) structural materials is gathering attention. For example, floors and walls of steel or reinforced concrete structures are composed of wooden structural materials. And CLT (Cross Laminated Timber) can play a role as walls and floors because CLT is panel, has high rigidity and strength. So, in this research, to confirm the structural performance and reinforcement of composite structure with CLT wing walls and RC flames as shown in Figure 1 is aimed.

Method

In this research, steel plate insertion type drift pin joint and slip stopper shown in Figure 2 are selected as the joints between CLT infills and RC flames. After that, flame test specimens shown in Figure 1 are designed. Flame test specimens are prepared 3 types. The parameters are presence of CLT wing walls and joint type. Type A specimen has CLT wing, and joint type is steel plate insertion type drift pin joint. Type B specimen has CLT wing walls, and joint type is slip stopper. Type C specimen has only RC flames. And flame test and material test are conducted, the examination about structural performance, reinforcement and the stress state of structural members is conducted.

Result and discussion

The skeleton curve of load-deformation angle is shown in Figure 3. Comparing between each specimen, maximum load of A specimen is 1.8 times higher than C specimen, and B specimen is 1.6 times higher than C specimen. In addition, Initial rigidity of A specimen is 1.8 times higher than C specimen, B specimen is 1.5 times higher than C specimen. From the above, reinforcement of RC flames is detected by filling CLT wing walls in RC flames.







Figure 2. The specification of joint



Figure 3. The skeleton curve of each

Full-scale shaking table test of high-strength CLT structure with framework and verification of performance by analysis

(Graduate School of Agriculture, Laboratory of Structural Function, RISH, Kyoto University)

Akira Takise

Introduction

The 2016 notification of the General Design Law for Buildings Using CLT is expected to increase opportunities to use CLT in buildings for a variety of uses, including non-residential buildings. I focused on a construction method that enables a rational design by reducing the amount of CLT walls by using stronger hardware than the existing joining method, and by compensating for the vertical load with axial members such as columns and beams instead. However, it is not clear how they will be joined together or how they will perform as a building in terms of earthquake resistance1). Therefore, this study verifies the performance of the CLT with framework structure by analyzing the seismic behavior and load bearing elements.

Methods

In this study, a shaking table experiment is conducted, and then the behavior is tracked using a numerical analysis program. The perspective view of the test specimen used in the experiment is shown in Figure 1. The test specimen is a two-story full-scale structure that resists seismic forces by a centrally located CLT wall. In order to effectively demonstrate the performance of the CLT, original high-yielding hardware was used for the joint, and three types of specifications were set depending on the type of joint between the test piece and the foundation. The seismic waves used for excitation were unidirectional waves in the longitudinal direction and were input at several magnifications. There are two types of ground



relationship of shear force under 135% excitation of BSL wave (1st layer).

motions: the seismic motion observed during the south part of Hyogo prefecture Earthquake in 1995 (JMA Kobe wave) and the artificial seismic motion (BSL wave), which was created to correspond to a major earthquake under the Building Standards Law.

Results and conclusions

Figure 2 shows the relationship between layer shear force and interlayer deformation under 135% excitation of BSL waves for each specification. In the double-bolts specification (two bolts installed at the bottom of the first floor CLT quake-resistant wall) and the single-bolt specification, the deformation angle between the layers was less than 1/30 rad, which is the safety limit for wooden construction, and there was no major damage to the test specimens. In the un-bolted specification, the interlaminar deformation exceeded the safety limit, and the maximum damage was about 1/17 rad. The damage to the CLT included compression cracking at the wall legs and shear failure at the drift pin joint, but none of the damage was minor enough to collapse.

It is considered that this test specimen can withstand large earthquake inputs with fewer seismic elements than the existing CLT structure and has high seismic performance. In order to make use of this information in the actual design, it is necessary to study in detail the mechanism of force manifestation.

The development of optimal CLT floor with two-by-four

(Graduate School of Agriculture, Laboratory of Structural Function, RISH, Kyoto University)

Soichi Abe

Introduction

Cross-Laminated Timber (CLT), illustrated as Fig.1, is used for the wall or floor of buildings and supports the structure. CLT panels consist of some timbers, which is called laminae. Usually in Japan, CLT laminae is cut from logs and CLT consists of laminae having equal thickness. Then there are few examples that the laminae which is now generally on sale is used for CLT laminae. We can save time and cost on the manufacturing process if CLT constructed by laminae now generally on sale are made happen in realty. It is also beneficial that we can sell these laminae as original use. Two-by-four and stud are focused on in this research as laminae on sale. Two-by-four is the timber used for platform flame construction. Stud is the

timber put between posts and nonstructural member of the building.

More stress occurs in outer layers than inner layers when CLT panels are used for floors. The transversal layers don't contribute to CLT bending strength. Therefore, the transversal layers have the role in fixing the effect of swelling and shrinkage of cross laminae.



Figure 1. Typical CLT in Japan

The purpose of my research is to develop CLT floor panels using 204 and stud, and to reduce CLT volume and cost.

Method

Laminae bending test and CLT out-of-plane bending test were conducted. The cross section of two-by-four is $38\text{mm} \times 89\text{mm}$ and stud is $27\text{mm} \times 105\text{mm}$. Thirty CLT specimens were divided into three groups, which were H (height) 190mm, H179 and H211. H190 has 5 layers 5 ply cross section, and all layers constructed with two-by-four. H179 has 3 layers 5 ply, and outer layers constructed with two-by-four and inner layer constructed with stud. H211 has 5 layers 7 ply cross section, and only outermost layers constructed with two-by-four and the others' layers with stud. Then each type of laminae has about 150 specimens. And all was conducted by bending tests.

Optimal arrangement of the laminae for various types regarding length and design loads, were decided after calculated values with experimental results of two types of laminae.

Results and discussion

Each experimental date was put into Table.1, which include bending Young's modulus and 5% lower limit value of bending strength ($F_{b_5\%}$). It was found that all $F_{b_5\%}$ of two-by-four was over strength criteria, being set on two-by-four, and that has higher bending strength. Therefore, it is suggested that optimal arrangement for CLT need two-by-four outermost layers and stud transversal layers. The higher the strength of CLT is on strong axis in general, the less ratio of transversal layers for cross section. However, transversal layers have an important role to keep dimensional stability. In fact, a layer of CLT can have two plies at most. It is suggested that we can manufacture more improved CLT on bending performance if CLT with less ratio of transversal layers for cross section stability.

Table 1: CLT out-of-plane bending

	Tuble 1.011 but of plane beliang							
		Young's	Fh 5%					
		modulus	(N/mm^2)					
		(kN/mm ²)	(1 (11111)					
;	H190	5.46	17.6					
;	H179	6.81	23.4					
	H211	6.47	20.3					

Tabl	le 2 : lai	mnae	bend	lıng

n	204	6.90	26.8
al	Stud	6.93	16.8
Distribution, morphology, and phylogenetic analysis of the genus *Coptotermes* Wasmann (Rhinotermitidae) in Sumatra and West Java, Indonesia

(Graduate School of Agriculture, Laboratory of Innovative Humano-habitability, RISH, Kyoto University)

Bramantyo Wikantyoso

Introduction

The distribution of *Coptotermes* spp. has received little attention in Indonesia [1], and it is common to find difficulties in their identification if only soldiers' body measurements are taken into consideration [2]. The purpose of the present study was to map the distribution of *Coptotermes* spp. by identifying specimens collected from the Sundaland region. Multivariate and phylogenetic analyses were used to understand the potential of the morphological characteristics of *Coptotermes* spp.

Soldier head morphology: The most difficult characteristic in Coptotermes spp. discrimination

Four *Coptotermes* species were identified, among which *C. curvignathus* was most closely associated with forested areas and *C. gestroi* with urban areas. The postmentum waist and labrum seta characteristics were consistent across the identified species, while head width, pronotum seta, head capsule seta, and postmentum median length were found to be the most useful characteristics for species discrimination. However, multicollinearity in the data blurs overlapped variables and potentially informative characteristics.

High intraspecies morphological variation was observed in *C. getsroi*, which may be a contributing factor to the difficulty of identifying this species. All *C. gestroi* specimens with head capsule variations were categorized into the same clade based on a phylogenetic analysis of their 12S and 16S gene fractions. *Coptotermes curvignathus, C. sepangensis,* and *C. kalshoveni* possess a set character of low waist



Figure 1. Two species, *C. curvignathus* (A) and *C. gestroi* (B and C) look different at first glance.

However, looking solely at their head morphology would not be sufficient to distinguish them in field practice.

postmentum type and a pair of paraterminal seta, while *C. gestroi* was characterized by middle waist postmentum and less visible paraterminal seta. Finally, it was suggested that postmentum waist and labrum seta may shed light on the grouping of *Coptotermes* spp. in Indonesia.

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Microplastics in a forest soil environment: interactions with termites

(Graduate School of Agriculture, Laboratory of Innovative Humano-habitability, RISH, Kyoto University)

Hiroki Yabumoto

Introduction

Even though plastics have enormous benefits to society, they cause a serious environmental problem. In particular, plastics of <5 mm in size, called microplastics (MPs), have been intensively studied for their impacts on aquatic environments. However, there is little information about microplastics in terrestrial environments. Thus, it is essential to conduct research on the problem of soil pollution by plastics. The purpose of this research was to verify the effect of the activities of soil organisms on the movement of MPs in terrestrial environments, using termites as representatives of soil organisms. It is well known that termites play an important role in terrestrial environments, especially in the tropics and subtropics.

Materials and methods

Soil samples 5 cm in diameter and 30 cm in depth were obtained in Japan and Indonesia from sites showing a variety of termite activities and at different distances from the shore. MPs from the samples were extracted with a $ZnCl_2$ solution (density: 1.6) and analyzed with a Fourier-transform infrared (FTIR) spectrometer.

The pot experiment was employed as a model system for interaction between MPs and termites. The pots were 5.8 cm in diameter and 15 cm in height. Two subterranean pest termites, the Formosan Subterranean Termite (FST: *Coptotermes formosanus*) in Japan (obtained from a laboratory colony maintained at the Deterioration Organisms Laboratory, RISH, Kyoto University) and the Asian Subterranean Termite (AST: *C. gestroi*) in Indonesia (obtained from a laboratory colony maintained at the Research Centre for Biomaterials, Indonesian Institute of Sciences [LIPI]) were employed in combination with polyethylene (PE) and polypropylene (PP) MPs in three different sizes (2-5 mm, 1-2 mm, 0.5-1 mm), and polystyrene (PS) blocks ($2 \times 2 \times 1$ cm). The pot was filled with soil to a depth of 15 cm (moisture content = 20%), and 200 mg MPs and 220 termites (200 workers and 20 soldiers) were placed on the soil surface. The assembled pot was kept in the termite culturing room at $28 \pm 2^{\circ}$ C and > 60% relative humidity in the dark. After 3 weeks, the soils from each 3 cm of depth (a total of 5 samples) and the sample piled over the original soil surface were collected and quantitatively analyzed for MPs.

Results and discussion

Although the soil analyses are still ongoing, MPs were obtained from the soil samples collected from a pine forest 500 m inland from the beach in Kagoshima Prefecture and from a pine forest just behind the beach in Wakayama Prefecture.

In the pot experiments in Japan and Indonesia, it was observed that MPs were not only buried in the soil piled over the original soil surface, but had also been transported to depths of >3 cm. In the cases of PE and PP, smaller particles tended to be transported deeper, and particles of <0.5 mm in size were also detected. In the case of PS, the termites heavily attacked PS blocks and broke them into many smaller particles. MPs were seen in layers deeper than those where PE and PP were found. ASTs transported the MPs much more and deeper than FSTs.

Our study shows that MPs exist in the forest soil environment near the coast, that termites are associated with the movement of MPs from the soil surface into the soil, and that termites break plastics into smaller particles. These effects depend on the size and type of plastics. When termites break plastics into smaller pieces, and those pieces are then retained in soil where the environment is relatively stable, it is possible that there would be long-term negative impacts on soil biota, soil organisms, and plants. Further research is needed on the effects and fate of MPs in soil environments.

Relativistic acceleration of protons by EMIC waves in Jovian magnetosphere

(Graduate School of Engineering, Laboratory of Computer Simulation for Humanospheric Sciences, RISH, Kyoto University)

Tomohiro Sekine

We perform test particle simulations of interactions between protons and a coherent electromagnetic ion cyclotron (EMIC) wave around Jupiter. Simulation results indicate that non-relativistic protons are trapped and accelerated by the EMIC wave. We also simulate the motion of relativistic protons interacting with the EMIC wave. The results show that the relativistic protons are accelerated by the EMIC wave with very high efficiency compared with the case of non-relativistic protons. We find two characteristics from the simulation results. First, the gyrophases of the protons with low equatorial pitch angles are bunched together and almost all of the protons are trapped. We reveal that the cause of phase bunching is the Lorentz force generated by the wave magnetic field and protons. Our simulations show that the effect of phase bunching becomes stronger when the amplitude of the EMIC wave is larger or the pitch angles of protons are low. Second, the direction of the trapped relativistic protons reverses, causing a long-time acceleration process. In the process, since the Lorentz factor increases, the resonance condition changes. Therefore, parallel motion of resonant protons changes from opposite direction to the same direction of wave propagation, resulting in longer trapping. We confirm that this process is the same as the Relativistic Turning Acceleration (RTA) process of electrons interacting with whistler mode waves. We find that the further the turning point is from the equator, the more efficient the RTA process is. Moreover, the acceleration efficiency of the RTA process increases when the amplitude of the EMIC wave is large.

A method for estimation of cold plasma density from whistler mode waves observed by Magnetospheric Multiscale mission spacecraft

(Graduate School of Engineering, Laboratory of Computer Simulation for Humanospheric Sciences, RISH, Kyoto University)

Keita Takahashi

We developed a method to obtain the cold plasma density by using a phase difference of whistler mode waves observed by two of the Magnetospheric Multiscale Mission. We chose large amplitude events with coherent packet structures such as chorus emissions. We applied a band-pass filter to the magnetic and electric wave-form data to extract whistler mode waves. We calculated time variation of phase differences of the waves between the two spacecraft. If the phase differences are nearly constant, we can assume that the waves detected by two spacecraft consist of the same waveforms. When the distance between the two MMS spacecraft is less than one wavelength, we can easily determine the wave number vector from the phase difference. Combining with the background magnetic field vector, the wave vector and instantaneous wave frequency, we can obtain the local plasma frequency from the oblique whistler mode dispersion relation with the quasi-longitudinal approximation. The plasma frequency gives the background plasma density, which is in good agreement with that from upper hybrid resonance (UHR) emission observed by the electric field double probes. This result suggests that it is possible to observe fluctuations in the actual cold plasma density through analysis of coherent whistler-mode wave waveform data recorded by the MMS spacecraft at high time resolution. We calculated frequency sweep rate by the estimated plasma density obtained from this method. We found that the frequency sweeps rate are in good agreement with the sub-packet structure of the instantaneous frequency.

Study on efficiency improvement of microwave power transfer system for stratospheric platform

(Graduate School of Engineering, Laboratory of Applied Radio Engineering for Humanosphere, RISH, Kyoto University)

Yuta Nakamoto

A stratospheric platform (SPF) is attracting attention as a new communication platform for communication area expansion and disaster communication. In SPF, unmanned airplanes stay at the stratosphere and are used as communication relay stations. Therefore, a light and stable power supply is needed for long-term operation. Microwave power transfer (MPT) is proposed as a solution (Fig.1). We investigate the feasibility of MPT on SPF by numerical simulations. The MPT system on SPF is restricted by the shape of receiving array antenna, the size of the transmitting array antenna, the flight altitude and the coverage of communication service. In the present research, the MPT system with 2kW DC output under those requirements is proposed, allowing the uninterrupted operation of SPF. We calculate electric field intensity at the receiver by taking superposition of transmitted electric field from each antenna element. The maximum beam efficiency in free space is 23.4% with the operation frequency, the size of the receiving antenna, the size of transmitting antenna are 24GHz, 2m×80m and 40m×2.5m, respectively(Fig.2). The atmospheric attenuation of receiving power is negligible, whereas the efficiency is significantly decreased due to the rain attenuation. The total beam efficiency over the flight route is investigated. The SPF is positioned in the3km radius from the transmitting antenna at an altitude of 20km. We find that the beam efficiency for D-shaped route is 12.0%, which is the highest between the potential four route; a circle, the figure-8 and the oval route. By using the rectifier model in the previous research, the net efficiency including RF-DC conversion is 6.7% (Fig.3). With the 30kW power from the transmitting antenna, 2kW DC output is realized.



Fig.1. Microwave power transfer system for SPF

Fig.2. Calculated beam efficiency



Fig.3. (a) Proposed antenna and flight root for maximum efficiency (b) Estimated efficiency by flight root

Study on microwave power transmission systems in multipath environments

(Graduate School of Engineering, Laboratory of Applied Radio Engineering for Humanosphere, RISH, Kyoto University)

Taichi Sasaki

This paper describes that retrodirective systems improve the efficiency and safety of microwave power transmission (MPT) system in multipath environments. The retrodirective array antenna sends back the phase conjugate signal toward the pilot signal transmitted from the receiver (Fig.1). In indoor environments, multipath waves are generated by reflection from objects and propagate as well as the direct wave between the transmitter and the receiver. Using array pattern synthesis calculation, we confirmed that the beam pattern of the retrodirective signal reproduces the multipath of the pilot signal and completely conforms with the optimal beam pattern calculated by the rotating element electric field vector (REV) method. Next, electromagneticfieldsimulationverifiedthattheretrodirectivesignalformedmultibeams, concentrated at the receiver and improved the MPT efficiency. Even if there is an obstacle between the transmitter and the receiver, the retrodirective signal could track the receiver, avoiding direct exposure of the obstacle from the beam (Fig.2). Furthermore, it was found that the MPT efficiency of the retrodirective was further improved by using not only the phase information but also the amplitude information of the pilot signal. When there were two receivers, the retrodirective signal could track each target simultaneously with separate multibeams. By adjusting the phase difference between the two pilot signals, the retrodirective signal that maximizes the received power could be estimated. Finally, we experimentally demonstrated the retrodirective MPT system by fabricating and combining antennas and phase conjugate circuits (Fig.3). By using two orthogonal polarization, we tried to prevent interference between the pilot and retrodirective signals. Measured efficiency agreed with the simulation results (Fig.4), and it was confirmed that the system operates correctly in actual environments.



Fig.1. Principle of hardware retrodirective Fig.2. Power signal distribution of retrodirective using amplitude information



Fig.3. Experimental setup of multi-pass retrodirective



Study on high efficiency wireless power transfer method for the In-Line Inspection robot

(Graduate School of Engineering, Laboratory of Applied Radio Engineering for Humanosphere, RISH, Kyoto University)

Isami Sato

Gas pipelines are important infrastructures, and it is necessary to conduct inspection of the pipes. In-Line Inspection (ILI) robots had been invented for this inspection. These robots are currently driven with batteries, and thus the robots cannot move more than 1km. This limitation can be removed with wireless power transfer system using microwave (Fig.1). Since gas pipes are made of steel, pipes can be regarded as circular waveguide, and microwave can propagate through the pipe with less attenuation. At first, we conducted power transmission experiments in a gas pipe using a microstrip antenna as a transmitting antenna and a dipole antenna as a receiving antenna (Fig.2). Our attempt to calculate attenuation rate from decrease of receiving power failed because of mixed mode. Therefore, we designed a transmitting antenna which only generates TE₀₁ mode. Receiving antenna with high receiving efficiency for all propagation mode including TE_{01} mode was also designed. Using these antennas, we achieved 55 % transmission efficiency in experiments (Fig.3). From the experiment, attenuation rate of TE_{01} mode was calculated as 11.9dB/km. Furthermore, we estimated conductivity of the pipe from the attenuation rate. We assumed the conductivity as 1.0×10^7 S/m. However, the actual conductivity is 1.9×10^6 S/m, which is lower than we assumed, and this is due to rust and scratches on the pipe. Next we designed a receiving antenna which fits the ILI robot. When designing this antenna, we considered degradation of antenna characteristics when placed in a gas pipe. Transmission efficiency with this antenna was 48%. Decrease in the efficiency was due to the antenna being tilted. With attenuation rate and receiving efficiency that we obtained from our experiments, we can calculate transmission efficiency at any distance. For further research, we have to design rectifier circuit to convert microwave power to DC power.



Fig.1. Microwave power transfer system for robot in gas pipe



Fig.2. Experimental setup of MPT in gas pipe Fig.3. Measured data of microwave propagation in gas pipe

Development and integration of the high-speed current detection circuits in particle sensors dedicated to Wave-Particle Interaction Analyzer

(Graduate School of Engineering, Laboratory of Space Electromagnetic Environment Exploration, RISH, Kyoto University)

Motoyuki Kikukawa

Charged particles in space plasma do not exchange their kinetic energies through their collisions but through plasma waves. This is so-called "wave-particle interaction." It is indispensable for understanding space electromagnetic environments. Wave-Particle Interaction Analyzer (WPIA) is a new method of observing wave particle interaction. The size of WPIA system is large, because the WPIA consists of plasma wave receivers, particle detectors and the interface between them. It is difficult to mount the WPIA on a small satellite. Our attempt is to miniaturize the WPIA system using the Application Specific Integrated Circuit (ASIC) technology. In this research, we developed and integrated the new high-speed current detection circuits dedicated to particle sensors. The circuits feed particle detection pulses of particle detectors into an arithmetic logic unit on the WPIA.

The circuit implemented inside the ASIC consists of three stages. The first stage is the current voltage conversion circuit. It picks up current pulses of corresponding particles detected by particle sensors and converts into voltage signals with large enough amplitudes to drive the second stage. The second stage contains a comparator and a peak-hold circuit. They ensure picking up real signals by setting a threshold level.

We found that the circuits we developed output detection signals within 30 ns after particle detectors emit current pulses and the output signals reset within 12 ns after reset signals. The developed chip showed the capability to detect particles with a count rate of 106 /s and the enough accuracy of the arrival time of particles. The size of one channel in the developed circuits is 210 μ m × 570 μ m.

We can connect 16 of these circuits in parallel on one chip of 5mm square. Since we realize the very high-speed and small circuits, we can expect to mount them on a micro satellite.



Figure 1. Layout of the designed chip for one channel of particle detectors.

A study on data processing for the Wave-Particle Interaction Analyzer onboard the Arase satellite

(Graduate School of Engineering, Laboratory of Space Electromagnetic Environment Exploration, RISH, Kyoto University)

Jumpei Miki

The Arase satellite was launched in December 2016 for the purpose of understanding wave-particle interaction processes in the inner magnetosphere. Software-type Wave-Particle Interaction Analyzer (S-WPIA) is installed in the Arase satellite. In the S-WPIA, it is possible to identify a relative phase angle of an instantaneous plasma wave electric and magnetic field vectors and a velocity vector of each detected electron. Since energy exchanges between plasma waves and particles are represented by the inner product of electric field vectors and particle velocity vectors, the relative phase angle is essential in wave-particle interaction processes. The objective of the present thesis is to evaluate a method for calibrating electric and magnetic fields in the view point of the WPIA and data of the chorus emissions obtained by the S-WPIA onboard the Arase satellite.

We established the calibration processes of the amplitude and phase of electric and magnetic fields affected by sensors, filters and amplifiers of the receiver. We evaluated the precision of the calibrated data by comparing the observed values with the theoretical values of the refractive index and the amplitude ratios and phase differences between each component of electric and magnetic fields. Our analysis showed that the electric fields data was not accurate enough to perform the WPIA analysis due to the lack of the spin-axis electric field sensor. On the other hand, we succeeded in showing that the calibration of the magnetic fields data is precise.

Since the magnetic field observations are accurate, we evaluated the wave-particle interaction from the relative phase angle between the instantaneous magnetic field vector and the particle velocity vector. The result showed that it is necessary to correct the observability bias of the relative phase angle when the target waves propagate obliquely relative to the ambient magnetic field. We established the compensation method of this observation bias considering the configurations and specifications of the particle sensors.



Figure 1. Frequency-spectrum diagram of chorus emissions observed by the Arase satellite. Chorus emissions is the main target of the S-WPIA.

Study of Electron Cyclotron Harmonic waves in the Earth's inner magnetosphere

(Graduate School of Engineering, Laboratory of Space Electromagnetic Environment Exploration, RISH, Kyoto University)

Airi Shinjo

The present study focuses on Electron Cyclotron Harmonic (ECH) waves observed by the Arase satellite. ECH waves are commonly observed in the low latitude region just outside the terrestrial plasmapause. They are purely electrostatic and their frequency spectra show harmonic structures. The Arase satellite is equipped with the high-performance plasma wave receiver called PWE (Plasma Wave Experiments). They can observe the electric field waveforms with high time resolutions for a longer time period than that by former satellites. Utilizing this advantage, we examined new spectral features of the ECH. The most striking point in the newly found spectral features is that each harmonic spectrum shows further fine spectral structures. The time variation of each fine spectrum frequency is completely independent.

We applyed a new method to estimating phase velocities of ECH waves using phase differences of waveforms observed by two monopole electric field antennas. This method is so-called "interferometry method." It is effective when the wavelength is in the same order of the length of the electric field antenna. The electric field data of the Arase satellite is two-dimensional. We estimated the direction of phase change by using the satellite spin dependence. Using this spin dependence, we calculated the phase velocity projected on the spin plane and converted it to the absolute value of the phase velocity. In addition, we attempted to estimate the temperature of low-energy electrons from the obtained phase velocities consulting linear dispersion relations of the ECH waves.

In conclusion, we succeeded in calculating the phase velocities and low energy temperatures by applying these methods to the ECH observed by the Arase.



Figure 1. Frequency-time spectrogram of the ECH waves observed by the Arase satellite.

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