九州大学農学部遺伝子資源開発研究センター年報　第18号　二〇一四年度

九州大学農学部
遺伝子資源開発研究センター

年　報　　　　第18号

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Faculty of Agriculture
Kyushu University

Number 18

九州大学大学院　農学研究院
遺伝子資源開発研究センター

年報　第 18 号

平成 26 年

九州大学大学院　農学研究院附属遺伝子資源開発研究センター

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I. センターの概要

1. 目的

本センターは、遺伝子資源の収集、保存、開発から評価、利用に至る研究教育を遂行する。特に、高度な技法で遺伝情報の解析を行い、遺伝子レベルでの農業遺伝子資源に関する応用展開研究と戦略的プロジェクト研究の推進並びに遺伝子資源のDNA・細胞レポジトリー機能の充実を図る。

2. 沿革

昭和62年5月　本学附属家蚕遺伝子実験施設を振替え、附属遺伝子資源研究センターが10年の時限施設として設置され、教授、助教授、助手各2名が配置された。

平成元年4月　教授、助教授各1が追加配置された。本学大学院農学研究科に設置された独立専攻遺伝子資源工学専攻の協力講座として、昆虫遺伝子資源学、遺伝子開発管理学の2講座に参加した。

平成3年4月　遺伝子資源工学専攻に微生物遺伝子工学講座が新設され、これに参加した。

平成9年4月　附属遺伝子資源研究センターは時限により廃止され、新たに、教授3名、助教授3名、助手2名の振替えによって附属遺伝子資源開発研究センターが設置された。

平成12年4月　大学院重点化に伴い、大学院　農学研究院附属遺伝子資源開発研究センターに改組された。また、大学院教育として生物資源環境科学府　遺伝子資源工学専攻　遺伝子資源開発学講座（昆虫遺伝子資源学分野、植物遺伝子資源学分野、微生物遺伝子工学分野）に改組された。

平成22年4月　農学研究院・学府組織改組に伴い、昆虫遺伝子資源学分野、植物遺伝子資源学分野は、生命機能科学部門　システム生物学講座に、微生物遺伝子工学分野は分子微生物学・バイオマス資源科学講座に所属し、教育に参画した。

3. 組織・教職員

センター長　日下部宜宏

家蚕遺伝子開発分野

准教授	伴野　豊	技術職員	西川　和弘
助教	山本　幸治	技術職員	田村　圭
学術研究員	藤井　告	技術職員	山本　和典
学術研究員	福森　善寿	技術補佐員	江口　誠一

植物遺伝子開発分野

准教授	熊丸　敏博	特任助教	福田　真子
		学術研究員	松坂　弘明
		研究支援推進員	仲島　久美子
		テクニカルスタッフ	藤田　喜久男
			原田　良子
			有隅　久美子
			須山　奈緒美
		技術補佐員	村田　亜紀
			米田　未和子

微生物遺伝子開発分野

講師	土居　克実	テクニカルスタッフ	中嶋　ひとみ

4. 研究と事業内容

家蚕遺伝子開発分野

- カイコ遺伝子資源の収集、開発、評価、保存、活用並びに遺伝子機能の発現機構の解明
- 文部科学省ナショナルバイオリソースプロジェクトNBRP（カイコ）の中核機関として我国のバイオリソース事業への貢献

植物遺伝子開発分野

- イネ種子貯蔵タンパク質の生合成･集積を制御する遺伝的機構の解明
- ナショナルバイオリソースプロジェクト(NBRP)におけるイネ突然変異系統の整備
- 在来イネ遺伝子資源の保存と特性評価に関する研究

微生物遺伝子開発分野

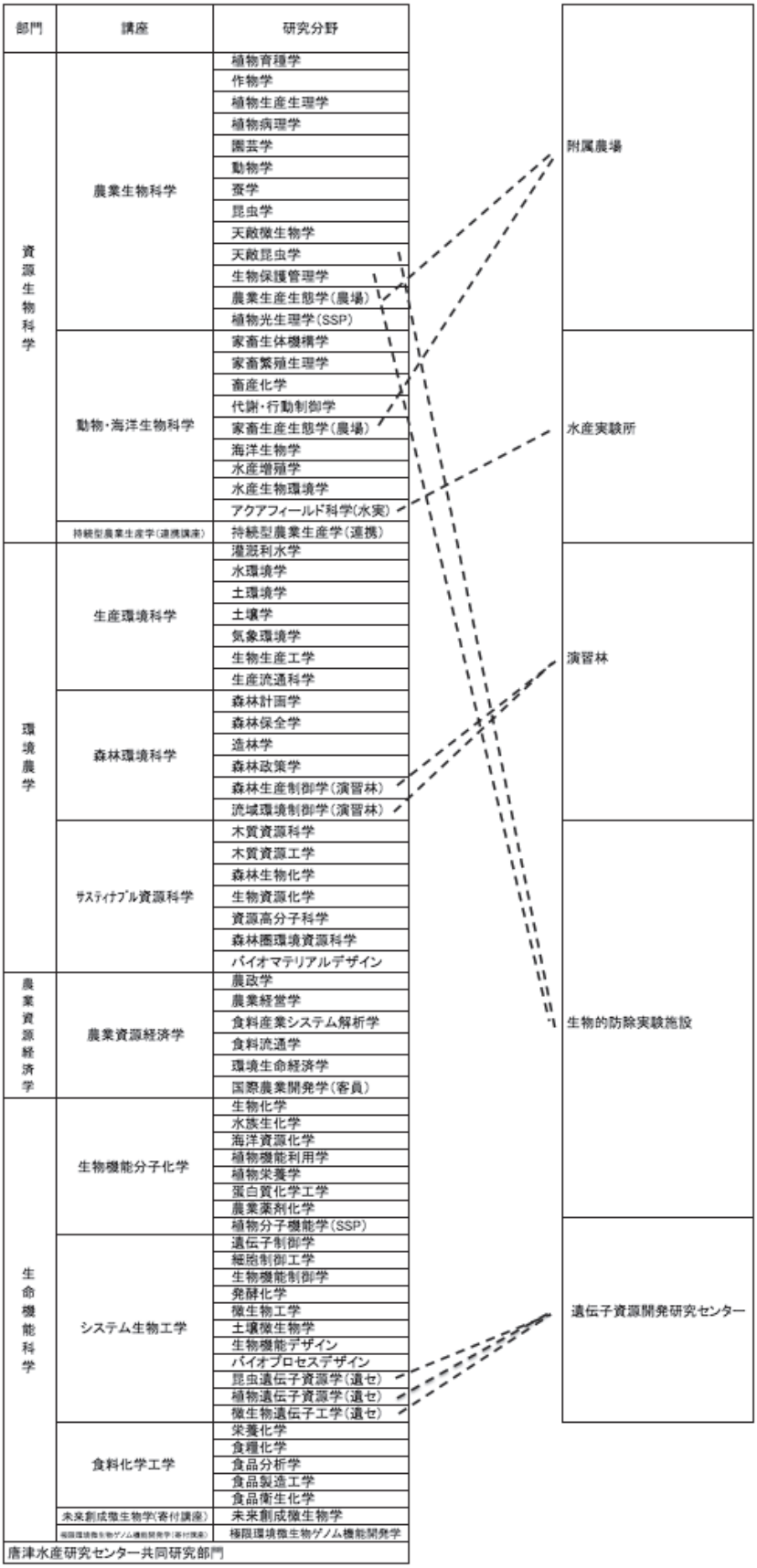
微生物遺伝子資源の探索と評価、保存と利用開発、並びに有用遺伝子の高度機能化と応用展開に関する研究

各分野とも、所定の許可を得た本学部内外の学生や研究者等に対し、研究の場や遺伝子資源材料を提供し、さらに研究指導と教育を行っている。

5. 遺伝子資源開発研究センター運営委員会委員（平成26年3月31日現在）

委員長	日下部　宜宏	（資源生物科学部門）
准教授	熊丸　敏博	（遺伝子資源開発研究センター）
准教授	伴野　豊	（遺伝子資源開発研究センター）
講師	土居　克実	（遺伝子資源開発研究センター）
教授	久原　哲	（生命機能科学部門）
准教授	片倉　喜範	（生命機能科学部門）
教授	吉村　淳	（資源生物科学部門）
准教授	小名　俊博	（環境農学部門）
教授	南石　晃明	（農業資源経済学部門）
教授	望月　俊宏	（附属農場）
教授	大賀　祥治	（附属演習林）
教授	高木　正見	（生物的防除研究施設）

6. 組織図


部門
講座
研究分野
資源生物科学
農業生物科学
植物育種学
作物学
植物生産生理学
植物病理学
園芸学
動物学
蚕学
昆虫学
天敵微生物学
天敵昆虫学
生物保護管理学
農業生産生態学（農場）
植物光生理学（SSP）
動物・海洋生物科学
家畜生体機構学
家畜繁殖生理学
畜産化学
代謝・行動制御学
家畜生産生態学（農場）
海洋生物学
水産増殖学
水産生物環境学
アクアフィールド科学（水実）
持続型農業生産学（連携講座）
持続型農業生産学（連携）
環境農学
生産環境科学
灌漑利水学
水環境学
土環境学
土壌学
気象環境学
生物生産工学
生産流通科学
森林環境科学
森林計画学
森林保全学
造林学
森林政策学
森林生産制御学（演習林）
流域環境制御学（演習林）
サスティナブル資源科学
木質資源科学
木質資源工学
森林生物化学
生物資源化学
資源高分子科学
森林圏環境資源科学
バイオマテリアルデザイン
農業資源経済学
農業資源経済学
農政学
農業経営学
食料産業システム解析学
食料流通学
環境生命経済学
国際農業開発学（客員）
生命機能科学
生物機能分子化学
生物化学
水族生化学
海洋資源化学
植物機能利用学
植物栄養学
蛋白質化学工学
農業薬剤化学
植物分子機能学（SSP）
システム生物工学
遺伝子制御学
細胞制御工学
生物機能制御学
発酵化学
微生物工学
土壌微生物学
生物機能デザイン
バイオプロセスデザイン
昆虫遺伝子資源学（遺セ）
植物遺伝子資源学（遺セ）
微生物遺伝子工学（遺セ）
食料化学工学
栄養化学
食糧化学
食品分析学
食品製造工学
食品衛生化学
未来創成微生物学（寄付講座）
未来創成微生物学
極限環境微生物ゲノム機能開発学（寄付講座）
極限環境微生物ゲノム機能開発学
唐津水産研究センター共同研究部門
附属農場
水産実験所
演習林
生物的防除実験施設
遺伝子資源開発研究センター

II. 研究成果

1. 研究業績・出版物リスト

家蚕遺伝子開発分野

A. 原著論文

1) Fukumori H, Teshiba S, Shigeoka Y, Yamamoto K, Banno Y, Aso Y, Purification and characterization of cocoonase from the silkworm, *Bombyx mori*, *Bioscience Biotechnology and Biochemistry*, 78, 202-211 (2014)
2) Hossain MDT, Yamada N, Yamamoto K, Glutathione-binding site of a *Bombyx mori* theta-class glutathione transferase, *PLoS One*, 9 (5), e97740 (2014)
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4) Hossain MDT, Nagaoka S, Yamamoto K, Identification of residues essential for catalytic activity of a *Bombyx mori* arginase, *Journal of Insect Biotechnology & Sericology*, 83, 47-51 (2014)
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7) Yoda S, Yamaguchi J, Mita K, Yamamoto K, Banno Y, Ando T, Daimon T, Fujiwara H, The transcription factor Apontic-like controls diverse colouration pattern in caterpillars, *Nat Commun.*, 5 4936 (2014)
8) Xu J, Kusakabe T, Yamamoto K, Suetsugu Y, Mon H, Li Z, Zhu L, Iiyama K, Banno Y, Yoshimura K, Lee JM, A novel third chromosomal locus controls susceptibility to Autographa californica multiple nucleopolyhedrovirus in the silkworm, *Bombyx mori*, *Appl Microbiol Biotechnol*., 98(7) 3049-58 (2014)

B. 著書・総説

1) 山本幸治, 特集「昆虫由来酵素・タンパク質の機能解析とその応用」にあたって，蚕糸・昆虫バイオテック，83 (1)，3 (2014)
2) 山本幸治, 昆虫グルタチオン転移酵素群の機能解析と応用，蚕糸・昆虫バイオテック，83 (1)，5-10 (2014)

3) Yamamoto K, Higashiura A, Nakagawa A, Suzuki M, Crystal structure of Omega-class glutathione transferase of the silkworm, *Bombyx mori*. *Photon factory activity report 2011*, 29 (2014)

C. 学会発表

1) 香月太地, 山田直隆, 山本幸治, 平島明法, カイコ glutathione S-transferase に対する農薬の影響, 農芸化学会西日本支部大会, 2014 年 9 月 18 日, 佐賀.
2) 山本幸治, 東浦彰史, 山田直隆, 中川敦史, トビイロウンカ由来 Delta-class グルタチオン転移酵素の X 線立体構造解析, 日本農芸化学会西日本支部講演会, 2014 年 9 月 18 日, 佐賀.
3) Yamamoto K, Structural basis of catalytic mechanism of *Bombyx mori* prostaglandin E synthase, 9th International Workshop on the Molecular Biology and Genetics of the Lepidoptera, 2014 年 8 月 22 日, Greece.
4) 広瀬義躬, 横山岳, 松尾和典, 伴野豊, チョウ目昆虫の卵寄生蜂のカイコ卵への寄生には卵の硬さとサイズが影響する, 第 59 回日本応用動物昆虫学会大会, 2015 年 03 月 27 日, 山形.
5) 佐原健, 鈴木一生, 安河内祐二, 伴野豊, 染色体異系統を用いてカイコ突然変異の原因遺伝子にせまる, 第59回日本応用動物昆虫学会大会, 2015年03月28日, 山形.

植物遺伝子開発分野

A. 原著論文

1) Hoai TTT, Suu TD, Satoh H, Kumamaru T., Diversity of glutelin acidic subunit polypeptides in rice cultivars collected from northern Vietnam, *Plant Breeding*, 133 (3), 341-347 (2014)
2) Hoai TTT, Matsusaka H, Toyosawa Y, Suu TD, Satoh H, Kumamaru T., Influence of single-nucleotide polymorphisms in the gene encoding granule-bound starch synthase I on amylose content in Vietnamese rice cultivars, *Breeding Science*, 64, 142-148 (2014)
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4) Tanaka W., Y. Ohmori, T. Ushijima, H. Matsusaka, T. Matsushita, T. Kumamaru, S. Kawano, H. Hirano., Axillary meristem formation in rice requires the *WUSCHEL* ortholog *TILLERS ABSENT1, Plant Cell*, 27, 1173-1184 (2015)
5) Wen L., M. Fukuda, M. Sunada, S. Ishino, Y. Ishino, T. W. Okita, M. Ogawa, T. Ueda, T. Kumamaru., Guanine nucleotide exchange factor 2 for Rab5 proteins coordinated

with GLUP6/GEF regulates the intracellular transport of the proglutelin from the Golgi apparatus to the protein storage vacuole in rice endosperm, *J. Exp. Bot*., (Accepted) (2015)

B. 学会発表

1) 永田 俊文, 長谷川 陽一, 濱田 茂樹, 熊丸敏博, 松坂 弘明, 鈴木 保宏, 米の主要なトリアシルグリセロールリパーゼの同定と酵素化学的特性の解明, 日本育種学会, 2015 年 3 月 22 日, 玉川大学.
2) 久保文香, 安居佑季子, 佐藤豊, 熊丸敏博, 平野博之, イネ葉脈パターンの解析と細葉遺伝子 ALM1 の単離, 日本育種学会, 2015 年 3 月 22 日, 玉川大学.
3) ELakhdar Ammar, Ahmed Abd ELSattar, Khairy Amer, T. Kumamaru. Genetic diversity in barley (Hordeum vulgare L.) during salinity stress based on simple sequence repeats SSR-markers, 日本育種学会, 2015 年 3 月 22 日, 玉川大学.
4) Satoh, R., S. Shiraishi, T. W. Okita, M. Maeshima, T. Kumamaru., Physiological role of plastid membrane transporters involved in transport of intermediates of starch biosynthesis in rice, Plant Bioligy Europe FESPB/EPSO, 2014. 6. 23., Dublin, UK.
5) 熊丸敏博, 安井秀, 吉村淳, 土井一行, 久保貴彦, 野々村賢一, 倉田のり, イネ NBRP -イネ属の多様性を生かすリソース基盤の構築-, 日本育種学会第 9 回九州育種談話会, 2014 年 9 月 4 日, 九州大学.
6) 中村哲洋, 熊丸敏博, 植物性グリコーゲンを種子に高蓄積するイネ突然変異体の選抜及び解析, 日本育種学会第 9 回九州育種談話会, 2014 年 9 月 4 日, 九州大学.
7) 松坂弘明, 橋本博之, 佐藤匡央, 熊丸敏博, TILLING 法を用いたイネ Oleosin 変異体の選抜, 日本育種学会第 9 回九州育種談話会, 2014 年 9 月 4 日, 九州大学.
8) 福田真子, 村上孝裕, 熊丸敏博, イネグルテリン前駆体を多量に蓄積する glup2 変異遺伝子の連鎖地図構築及び同変異体種子の組織学的解析, 日本育種学会第 9 回九州育種談話会, 2014 年 9 月 4 日, 九州大学.
9) Ammar El-Akhdar, T. Kumamaru, Gene action for yield and its components in barley, 日本育種学会第 9 回九州育種談話会, 2014 年 9 月 4 日, 九州大学.
10) 長谷川陽一, 濱田茂樹, 熊丸敏博, 松坂弘明, 鈴木 宏, 米の主要なトリアシルグリセロールリパーゼ候補遺伝子の推定と TILLING 法による変異系統候補の選抜, 日本育種学会, 2014 年 9 月 26 日, 南九州大学.

C. 特許出願

1) 鈴木保宏、長谷川陽一、永田俊文、濱田茂樹、秋田祐介、熊丸敏博、松坂弘明. トリアシルグリセロールリパーゼ変異植物. 特願2014-058474

D. データベース等

突然変異系統データベース　（Oryzabase上）
http://www.shigen.nig.ac.jp/rice/oryzabaseV4/
TILLINGオープンラボ (Oryzabase上)
http://www.shigen.nig.ac.jp/rice/oryzabaseV4/tilling/openLab
イネ保存品種データベース
http://w3.grt.kyushu-u.ac.jp/Rice_Kyushu/rice-kyushu/htdocs/main.html

微生物遺伝子開発分野

A. 原著論文

1) R. Ogura, T. Wakamatsu, Y. Mutaguchi, K. Doi and T. Ohshima, Biochemical characterization of an L-tryptophan dehydrogenase from the photoautotrophic cyanobacterium *Nostoc punctiforme*, *Enzyme Microb. Tech*., 60, 40-46 (2014)

2) H. Akita, Y. Imaizumi, H. Suzuki, K. Doi and T. Ohshima, Spectrophotometric assay of D-isoleucine using an artificially created D-amino acid dehydrogenase, *Biotechnol. Lett.*, **36**, 2245-2248 (2014)

3) H. Akita, H. Suzuki, K. Doi and T. Ohshima, Efficient synthesis of D-branched-chain amino acids and their labeled compounds with stable isotopes using D-amino acid dehydrogenase. *Appl. Microbiol. Biotechnol.*, 98: 1135-1143 (2014)

4) J. Kobayashi, J. Yukimoto, Y. Shimizu, T. Ohmori, H. Suzuki, K. Doi, and T. Ohshima, Characterization of *Lactobacillus salivarius* alanine racemase: short-chain carboxylate-activation and the role of A131, *SpringerPlus*, in press,（2015）

B. 学会発表

1) 田上諒, 藤野泰寛, 土居克実, シリカ誘導性プロモーターを利用した異種タンパク質発現系の開発, 第 51 回化学関連支部合同九州大会, 2014 年 6 月 28 日, 北九州国際会議場.
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C. 特許出願

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Glutathione-Binding Site of a *Bombyx mori* Theta-Class Glutathione Transferase

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Abstract

The glutathione transferase (GST) superfamily plays key roles in the detoxification of various xenobiotics. Here, we report the isolation and characterization of a silkworm protein belonging to a previously reported theta-class GST family. The enzyme (bmGSTT) catalyzes the reaction of glutathione with 1-chloro-2,4-dinitrobenzene, 1,2-epoxy-3-(4-nitrophenoxy)-propane, and 4-nitrophenethyl bromide. Mutagenesis of highly conserved residues in the catalytic site revealed that Glu66 and Ser67 are important for enzymatic function. These results provide insights into the catalysis of glutathione conjugation in silkworm by bmGSTT and into the metabolism of exogenous chemical agents.

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Introduction

Glutathione (GSH) conjugation is essential for the detoxification of xenobiotics [1,2]. Several studies have also implicated conjugation reactions with endogenous compounds, such as α,β-unsaturated aldehydes and prostaglandin [2–4], resulting in the excretion of at least one water-soluble compound. GSH transferases (GSTs, EC 2.5.1.18) are responsible for catalysis of this conjugation and are distributed ubiquitously among aerobic organisms [5]. GSTs are cytosolic enzymes, widely distributed across both prokaryotic and eukaryotic kingdoms [6]. In mammals, there are seven GST classes (alpha, mu, pi, omega, sigma, theta, and zeta) that can be distinguished based on their primary amino acid sequence; identity is approximately 50% within a class and less that 30% between different classes [7,8]. Six GST classes (delta, epsilon, omega, sigma, theta, and zeta) have been identified in dipteran insects, such as *Anopheles gambiae* [9] and *Drosophila melanogaster* [10,11]. Insect GSTs can determine sensitivity to insecticides [9,12], and since the Lepidoptera are the principal insect pests in agriculture, knowledge of lepidopteran GSTs is of great importance. We have previously characterized several GSTs in the silkworm, *Bombyx mori*, a lepidopteran model insect [13–19], and a sigma-class GST in the fall webworm, *Hyphantria cunea*, one of the most serious lepidopteran pests of broad-leaved trees [16]. However, there have been no reports to date on the characterization of theta-class GSTs from silkworms.

Here, we report the identification and classification of a theta-class GST isolated from *B. mori*, which we named bmGSTT. While bmGSTT shares some common substrates with human theta-class GSTs (hGSTT), it has a distinct substrate profile when compared to other *B. mori* GSTs studied to date. Furthermore, bmGSTT does not participate in the response to agents that generate oxidative stress, in contrast to previously identified *B. mori* GSTs. The activity profile of bmGSTT sheds further light on the way in which insects deal with xenobiotic agents and contributes to a more detailed understanding of the GST system in general.

Materials and Methods

Insects and tissue dissection

Larvae of the silkworm, *B. mori*, were reared on mulberry leaves in the Institute of Genetic Resources, Kyushu University Graduate School (Fukuoka, Japan). At day -1 fifth instar larvae, fat bodies were dissected from the larvae on ice and stored at −80°C until use. Total RNA was extracted rapidly from the dissected fat bodies with the RNeasy Plus Mini Kit (Qiagen Inc., Valencia, CA), in accordance with the manufacturer's instructions, and the resultant RNAs were subjected to RT-PCR.

Cloning and sequencing of cDNA encoding bmGSTT

Total RNA was processed using RT-PCR. First-strand cDNA was produced using SuperScript II Reverse Transcriptase (Life Technologies, Carlsbad, CA) and an oligo-dT primer. The resulting cDNA was used as a PCR template with the oligonucleotide primers 5′-TATACCATGGTTTTAAAACTATATTATGAT-3′ (sense) and 5′-CCGGATCCTTAAAGTTTAGAATTAGCCGCA-3′ (antisense), based on a sequence obtained from the SilkBase EST database [20]. Underlined and double-underlined regions in the primer sequences represent *Nco*I and *Bam*HI restriction enzyme sites, respectively, which were used to insert the PCR product into an expression plasmid. PCR was performed with 1 cycle at 94°C for 2 min; then 35 cycles at 94°C for 1 min, 50°C for 1 min, and 72°C for 2 min; followed by 1 cycle at 72°C for 10 min. The resulting bmGSTT cDNA (*bmgstt*) was ligated into the pGEM-T Easy Vector (Promega, Madison, WI), which was then used to transform *E. coli* DH5α cells. Genetyx software (ver. 14.0.12, Genetyx Corp., Tokyo, Japan) was used to

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Identification of Residues Essential for Catalytic Activity of a *Bombyx mori* Arginase

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Arginase is an oligometric protein that catalyzes the conversion of arginine to ornithine and urea in the presence of metal. Arginase in the silkworm, *Bombyx mori*, has been shown to participate in sperm maturation. In this study, we examine the enzymatic properties of *B. mori* arginase identified previously. The recombinant enzyme (bmArg-r) was purified to near homogeneity by ammonium sulfate fractionation, anion-exchange chromatography and gel-filtration chromatography. The isolated bmArg-r exhibits activity toward arginine in the presence of manganese ions, whereas it was unable to catalyze lysine hydrolysis. Copper and magnesium ions did not accelerate the activity toward arginine. Mutagenesis of His^{107} in the metal-binding site of bmArg-r revealed that this residue is important for enzymatic function. We found that the mutation of His^{132}, another histidine residue present in the metal-binding site, reduced the solubility of recombinant bmArg-r. It was considered that these histidine residues located in the metal-binding site of bmArg-r play a crucial role in catalytic activity.

Key words: arginine, arginase, *Bombyx mori*, Lepidoptera, site-directed mutagenesis

INTRODUCTION

Arginase (EC 3.5.3.1) is capable of yielding L-ornithine and urea from L-arginine as a substrate (Christianson and Cox, 1999). Manganese, which is contained in the arginase, is implicated in this reaction. The enzyme is found across both prokaryotic and eukaryotic organisms (Jenkinson *et al.*, 1996). In mammals, there are two types of isoforms: arginase I and arginase II. Although the occurrence of these arginases is approximately 50% each, they both contain a binuclear metal cluster (Mn^{2+}-Mn^{2+}) for catalysis (Cama *et al.*, 2003). L-Arginine is the only substrate of arginase *in vivo*. Arginase plays important roles in urea genesis and L-arginine homeostasis, and in the formation of polyamines and protein synthesis (Christianson and Cox, 1999).

Previously, two arginase cDNAs were cloned in the silkworm, *Bombyx mori;* named *bmarg-f* and *bmarg-r* (Nagaoka *et al.*, 2011). The *bmarg-f* mRNA was expressed in non-reproductive organs including the fat, body and muscle of larval and adult silkworms, whereas the *bmarg-r* mRNA was expressed in reproductive organs of the male such as the glandula lacteola and seminal vesicle (Nagaoka *et al.*, 2011).*B. mori* arginase-r (bmArg-r) appears to provide energy to sperm activity, thus playing an essential role in sperm maturation (Nagaoka *et al.*, 2011). In this context, it is necessary to study the catalytic function of the latter arginase and identify the active site.

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It has been shown that bmArg-r has His^{107} and His^{132} residues (Nagaoka, *et al.*, 2011). These residues may correspond to His^{101} and His^{126} located in active sites cleft of human arginase I, and also to His^{120} and His^{145} of human arginase II, which were described as being among the ligands for Mn^{2+}(Cama *et al.*, 2003). The histidine residues are highly maintained in all arginases and intrinsic in binding the metal ions to form metal-binding sites (Colleluori *et al.*, 2005). The structural basis of rat arginase I shows that the enzyme includes an Mn^{2+}- Mn^{2+} cluster (Cox *et al.*, 2001; Kanyo *et al.*, 1996). Manganese ions are believed to be involved in the activation of water molecules to produce a hydroxide that binds to metal and attacks the guanidino carbon of the substrate in a nucleophilic manner (Kanyo *et al.*, 1996). To ascertain the importance of His^{107} and His^{132} of bmArg-r in their interaction with substrate and manganese ions, these residues were mutated to alanine by site-directed mutagenesis to generate H107A and H132A.

The present article reports the results of experiments along this line for the wild-type (WT) bmAgr-r and its mutant produced by overexpression in *Escherichia coli*.

MATERIALS AND METHODS

Overexpression and purification of recombinant protein

A cDNA encoding arginase (*bmarg-r*; Genebank no. AB479104) was linked to a pET-11b vector (Novagen, EMD Biosciences, Inc., Darmstadt, Germany) and used to transform into competent *E. coli* cells (Rosetta2 (DE3) pLysS , Novagen) (Nagaoka, *et al.*, 2011). The cells were

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Purification and characterization of cocoonase from the silkworm *Bombyx mori*

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Cocoonase (CCN) which facilitates the degradation of a cocoon is recognized as a trypsin-like serine protease. In this study, CCN from the silkworm *Bombyx mori* was purified and comprehensively characterized. Its activity was maximal at about pH 9.8. It was stable above pH 3.4 at 4 °C and below 50 °C at pH 7.5. $CuSO_4$, $FeSO_4$, and $ZnSO_4$ showed inhibitory effects on CCN, but other salts improved activity. Typical trypsin inhibitors inhibited CCN, but the relative inhibitory activities were much lower than those against bovine trypsin. An extract of cocoon shells inhibited trypsin, but it was only slightly inhibitory against CCN. There were significant differences in catalytic efficiencies and substrate specificities as between CCN and bovine trypsin.

Key words: silkworm; *Bombyx mori*; protease; cocoonase; trypsin

Sericulture rearing of silkworm larvae and spinning of cocoons into thread is an industry having a history of nearly 5000 years. Hence, knowledge of the silkworm, *Bombyx mori* has long been accumulated over a wide range including genetics, physiology, and biochemistry, and the silkworm is recognized to be one of the suitable model animals for bioscience research. It is a holometabolous insect having four life-stages clearly differentiated from each other: egg, larva, pupa, and adult. Morphologically striking changes from larva to adult occur in the pupa by a finely regulated metabolism, which includes the degradation, remodeling, and neogenesis of tissues.

A cocoon is the living space of an immobile silkworm pupa that protects it from a variety of unfavorable situations such as desiccation and predation,[1–3] but an adult moth must escape from its cocoon after the completion of metamorphosis. The cocoon of a silkworm is composed principally of two silk proteins: fibroin and sericin. Fibroin is a fibrous protein secreted from the posterior silk gland. Sericin is a globular protein secreted from the middle silk gland, and it plays a role as the glue binding fibers made of fibroin together.[4,5] Cocoonase (CCN) catalyzes the proteolysis of sericin which weakens the mechanical strength of the cocoon and facilitates the emergence of the adult silkmoth.[6] Studies of CCNs have been done mostly on the ones from the Polyphemus moth (*Antheraea polyphemus*),[7–10] the Indian tasar silkworm (*A. mylitta*),[11] the Chinese Tussah silkmoth (*A. pernyi*),[12,13] and *B. mori*.[14–16] Nearly 40 years since extensive studies of CCN in the 1970s,[7–14] few results have been reported except for those focused on *B. mori* CCN (bmCCN).[15–18] Recently the recombinant protein of bmCCN was overproduced by means of yeast cells in a technological effort to use it in a more efficient silk-degumming procedure to remove sericin from raw silk.[19] The structural and functional features of CCN have been reported to be similar to those of a bovine pancreatic trypsin,[13,20] but to date we do not have sufficient information about the protein chemistry of bmCCN. In the present study, bmCCN was purified from the cephalic parts of silkmoth adults and characterized. We found that there were several considerable differences in the properties of bovine trypsin and bmCCN.

Materials and methods

Reagents. The following peptidyl 4-metylcoumaryl-7-amide (MCA) compounds were purchased from the Peptide Institute (Osaka, Japan): Bz-Arg-MCA (R), Z-Arg-Arg-MCA (RR), Z-Phe-Arg-MCA (FR), Boc-Val-Pro-Arg-MCA (VPR), Boc-Asp(OBzl)-Pro-Arg-MCA (DPR), Boc-Ala-Gly-Pro-Arg-MCA (AGPR), Boc-Phe-Ser-Arg-MCA (FSR), Boc-Leu-Arg-Arg-MCA

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Abbreviations: AGPR, Boc-Ala-Gly-Pro-Arg-MCA; AMC, 7-amino-4-methylcoumarin; apCCN, *Antheraea pernyi* CCN; BAEE, benzoyl-L-arginine ethyl ester; bmCCN, *Bombyx mori* CCN; Boc, *tert*-butoxycarbonyl; BPTI, bovine pancreatic trypsin inhibitor; BSA, bovine serum albumin; CCN, cocoonase; CHES, 2-(cyclohexylamino)ethanesulfonic acid; CSE, cocoon shell extract; CST1, cocoon shell-associated trypsin inhibitor; DPR, Boc-Asp(OBzl)-Pro-Arg-MCA; EGR, Boc-Glu(OBzl)-Gly-Arg-MCA; FR, Z-Phe-Arg-MCA; FSR, Boc-Phe-Ser-Arg-MCA; HIC, hydrophobic interaction chromatography; K_I, inhibition constant; LRR, Boc-Leu-Arg-Arg-MCA; MCA, 4-metylcoumaryl-7-amide; QRR, Boc-Gln-Arg-Arg-MCA; R, Bz-Arg-MCA: RR, Z-Arg-Arg-MCA; SBTI, soybean trypsin inhibitor; SDS-PAGE, SDS-polyacrylamide gel electrophoresis; SE, standard error; SEC, size-exclusion chromatography; TCA, trichloroacetic acid; TLCK, *N*-α-p-tosyl-L-lysine chloromethyl ketone; VPR, Boc-Val-Pro-Arg-MCA; Z, benzoyl-carbonyl.

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Structural characterization of the catalytic site of a *Nilaparvata lugens* delta-class glutathione transferase

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ABSTRACT

Glutathione transferases (GSTs) are a major class of detoxification enzymes that play a central role in the defense against environmental toxicants and oxidative stress. Here, we studied the crystal structure of a delta-class glutathione transferase from *Nilaparvata lugens*, nlGSTD, to gain insights into its catalytic mechanism. The structure of nlGSTD in complex with glutathione, determined at a resolution of 1.7 Å, revealed that it exists as a dimer and its secondary and tertiary structures are similar to those of other delta-class GSTs. Analysis of a complex between nlGSTD and glutathione showed that the bound glutathione was localized to the glutathione-binding site. Site-directed mutagenesis of nlGSTD mutants indicated that amino acid residues Ser11, His52, Glu66, and Phe119 contribute to catalytic activity.

Introduction

Glutathione transferases (GSTs,[1] EC 2.5.1.18) are a class of ubiquitously expressed enzymes that are responsible for the intracellular detoxification of diverse xenobiotics and endogenous substances. GSTs function by conjugating the toxicant to reduced glutathione (GSH) [1,2]. Multiple classes of mammalian GSTs, including the alpha, mu, pi, omega, sigma, theta, and zeta classes, have been defined on the basis of differences in their amino acid sequences [3]. The delta, epsilon, omega, sigma, theta, and zeta classes are present in dipteran insects such as *Anopheles gambiae* and *Drosophila melanogaster* [4].

The brown planthopper *Nilaparvata lugens* is a notorious rice-crop pest in Asian countries. *N. lugens* causes severe damage to plants through direct crop consumption and by transmission of viruses such as rice ragged stunt virus [5] and rice grassy stunt virus [6], resulting in high economic losses. Currently, the only effective method for pest-control is the application of chemical insecticides. Insect GSTs are of particular importance because they play an essential role in mediating the detoxification of xenobiotics such as insecticides. Other GST classes that are induced in response to insecticide application also play an important role in adapted resistance against the insecticide by increasing insecticide metabolism. Studying the structural and functional properties of GST from brown planthopper will help elucidate the mechanism that underlies the detoxifying ability of the brown planthopper, and aid in the identification of vital GSTs of other hemipteran insects.

In this study, we sequenced an mRNA encoding a delta-class GST from *N. lugens* (nlGSTD). To understand the molecular basis for substrate recognition and catalysis, we determined the three-dimensional crystal structure of recombinant nlGSTD and elucidated the structure–function relationship involved in its catalytic action. Comprehensive research of *N. lugens* GSTs might aid the development of novel pesticides that may be used to regulate agricultural pests.

Materials and methods

Insects and RNA extraction

N. lugens (strain:Izumo) was reared and maintained by the National Institute of Agrobiological Sciences. Total RNA was isolated from adult insects using an SV Total RNA Purification kit (Promega, Madison, WI) following the manufacturer's instructions.

Cloning and sequencing of the cDNA encoding nlGSTD

We used an *N. lugens* cDNA expressed sequence tag (EST) database [7] to identify candidate GST genes. Primer designs for reverse

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[1] *Abbreviations used:* GSH, glutathione; GST, glutathione transferase; GSTD, delta-class GST; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

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Note

Structural insight into the active site of a *Bombyx mori* unclassified glutathione transferase

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Glutathione transferases (GSTs) are major detoxification enzymes that play central roles in the defense against various environmental toxicants as well as oxidative stress. Here, we identify amino acid residues of an unclassified GST from *Bombyx mori*, bmGSTu-interacting glutathione (GSH). Site-directed mutagenesis of bmGSTu mutants indicated that amino acid residues Asp103, Ser162, and Ser166 contribute to catalytic activity.

Key words: glutathione; glutathione transferase; *Lepidoptera*; site-directed mutagenesis

Glutathione transferases (GSTs, EC 2.5.1.18) are ubiquitously expressed and are responsible for the intracellular detoxification of diverse xenobiotics and endogenous substances by conjugation to reduced glutathione (GSH).[1,2] Insect GSTs are particularly interesting because of their role in insecticide metabolism. In Lepidoptera, delta, omega, sigma, and zeta classes of GSTs, as well as unclassified GSTs, have been characterized for the silkworm *Bombyx mori*.[3–8] Recently, the three-dimensional structures of *B. mori* delta-class (bmGSTD), sigma-class (bmGSTS), omega-class (bmGSTO), and unclassified (bmGSTu) GSTs were determined.[9–12]

We found that the mRNA encoding a bmGSTu is induced in a silkworm strain resistant to diazinon after exposure to the insecticide, suggesting that bmGSTu may play a role in insecticide resistance in *B. mori*. In a previous study, we determined the X-ray crystal structure of bmGSTu (PDB ID: 3AY8).[9] Thus, in the current study, in order to improve our understanding of the molecular basis for catalysis by the enzyme, we examined the structure and catalytic function of the enzyme. Because the silkworm provides a model for studying lepidopterans,[13,14] comprehensive research on silkworm, GSTs should provide insights into combatting the species considered agricultural pests.

Recombinant bmGSTu was overexpressed and purified according to the published methods.[8] Amino acid-substitution mutants of bmGSTu were constructed using a plasmid containing the coding sequences of wild-type bmGSTu and a QuikChange Site-Directed Mutagenesis Kit (Agilent Technologies, Santa Clara, CA, USA), according to the manufacturer's recommendations. The nucleotide sequence of the full-length mutant cDNA was determined by DNA sequencing.

The hydrogen-bonding network may be essential for the GSH ionization step of the catalytic mechanism.[15] The active sites of insect GSTs, including the network, have been well characterized in *Anopheles dirus* GST D3-3 (adGSTD3-3).[16] The configuration of the glutamyl α-carboxylate group of GSH, together with the G-site residues Ser65, Arg66, Asp100, Thr158, and Thr162 of adGSTD3-3, facilitate the formation of a hydrogen-bonding network for distribution of the charge, which can be in the form of either a proton or an electron. Electrostatic interactions between the GSH glutamyl and carboxylic Glu64, as well as with Arg66 and Asp100, were found to extend to the hydrogen-bonding motif identified previously. This network appears to be a functionally conserved motif and it can be divided into types I and II.[15] The type I hydrogen-bonding networks, exemplified by delta, theta, omega, and tau classes of GSTs, contain an acidic amino acid residue at position 64, whereas the type II networks (alpha, mu, pi, and sigma classes of GSTs) have a polar amino acid residue (glutamine) capable of interacting with the γ-glutamyl portion of GSH. A DALI search (http://ekhidna.biocenter.helsinki.fi/dali_server/) was performed to obtain the root mean square (RMS) deviation of 1.6 Å between structures of adGSTD3-3 (PDB ID: 1JLV) and bmGSTu (PDB ID: 3AY8). The equivalent residue (Glu67) is conserved in the sequence of bmGSTu, which resembles a member of the type I network. A hydrogen-bonding network for a type I GST (adGSTD3-3) has been previously described.[15] The network type I contains Glu64, Ser65, Arg66, Asp100, Thr158, and Thr162 in adGSTD3-3 (PDB ID: 1JLV[16]); these residues were superposed with Glu67, Ser68, Arg69, Asp103, Ser162, and Ser166 in bmGSTu (Fig. 1).

Among the five residues, we characterized Glu67, Ser68, and Arg69 by site-directed mutagenesis in our

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Abbreviations: GSH, glutathione; GST, glutathione transferase; GSTu, unclassified GST; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

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ORIGINAL ARTICLE

Silkworm strains that can be clearly destined towards either embryonic diapause or direct development by adjusting a single ambient parameter during the preceding generation

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Abstract

In the silkworm, *Bombyx mori* (Lepidoptera: Bombycidae), embryonic diapause is under maternal control and the decision between diapause and direct development in bivoltine strains depends on environmental factors such as temperature and photoperiod experienced by the preceding generation. We reared ten bivoltine silkworm strains (c10, g32, k06, n25, p21, p22, p24, p44, p50 and p63) under various thermal and light conditions and examined the incidence of embryonic diapause in the next generation to identify strains in which the incidence of diapause can be controlled within the range of 0 to 100% by adjusting a single ambient parameter. Some strains were clearly destined towards either diapause or direct development. The diapause incidence in the c10, p22 and p50 strains was controlled by temperature during the egg stage (0% at 18°C and 100% at 25°C), that in the p50 strain was also determined to be dependent on illumination during the egg stage (0% under continuous darkness and 100% under continuous illumination), and photoperiod during the larval stage regulated diapause in p44 and p50 (0% and 100% under long-day and short-day photoperiod, respectively), when all other external parameters remained constant under each experimental condition. These diapause-controllable silkworm strains might serve as model systems for studies of insect diapause, especially for the differential screening of related factors.

Key words: bivoltine, Bombycidae, diapause-controllable, Lepidoptera, maternal control.

INTRODUCTION

Diapause is the hormonally mediated arrest of development and it is one of the most important adaptations that allow relatively small ectothermic insects to survive predictable adverse conditions such as cold winters and hot summers (Tauber *et al*. 1986; Denlinger *et al*. 2011). Some insects decide between diapause and direct development depending on external cues including temperature, photoperiod and diet (Nylin 2013). However, the molecular mechanisms that integrate environmental stimuli and consequently trigger diapause remain largely unknown.

Diapause is found at different ontogenetic stages of insect species. In the silkworm, *Bombyx mori* (L.), embryonic diapause is under maternal control and the decision as to whether or not to enter diapause in bivoltine strains depends on environmental factors such as the temperature and photoperiod experienced by the preceding generation (Watanabe 1924; Kogure 1933). Silkworms have been used in studies on diapause for several decades. These insects are easily reared to produce a genetically uniform population and the large body facilitates surgical procedures. In addition, diapause is easily discriminated from non-diapause based on egg color.

The aim of this study is to identify currently available silkworm strains in which the incidence of diapause can be controlled within the range of 0 to 100% by

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NATURE COMMUNICATIONS | ARTICLE

The transcription factor Apontic-like controls diverse colouration pattern in caterpillars

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Abstract

Abstract · References · Author information · Supplementary information

Genetic polymorphisms underlie the convergent and divergent evolution of various phenotypes. Diverse colour patterns on caterpillars, which are ecologically important, are good models for understanding the molecular backgrounds of phenotypic diversity. Here we show that a single evolutionarily conserved gene *apontic-like* (*apt-like*) encoding for a putative transcription factor accounts for the silkworm *p* locus, which causes at least 15 different larval markings involved in branch-like markings and eye-spot formation. The expression of *apt-like* and melanin synthesis genes are upregulated in association with pigmented areas of marking mutants *Striped* (p^S) and *normal* ($+^P$) but not in the non-marking allele *plain* (*p*). Functional analyses, ectopic expression, RNAi and TALEN, demonstrate that *apt-like* causes melanin pigmentation in a cell-autonomous manner. These results suggest that variation in *p* alleles is caused by the differential expression of the gene *apt-like* which induces targeted elevation of gene expressions in the melanin synthesis pathway.

Subject terms: Biological sciences · Evolution · Genetics

At a glance

Figures | Compounds | Genes and Proteins

Figure 1 Figure 2 Figure 3 Figure 4 Figure 5

First | 1-5 of 8 | Last

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A novel third chromosomal locus controls susceptibility to *Autographa californica* multiple nucleopolyhedrovirus in the silkworm, *Bombyx mori*

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Abstract Baculovirus demonstrates specific infection spectrums and thus one certain host exhibits particular response to single baculovirus isolate. *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) is considered to be not an innate pathogen to *Bombyx mori*, but some silkworm strains have been identified to be permissive to AcMNPV, indicating the positive or negative involvement of certain host factors in baculovirus replications in vivo. To provide a fundamental knowledge of this process, we performed large-scale screening to investigate the responses of 448 silkworm strains against recombinant AcMNPV inoculation. By genetic analysis between permissive and resistant strains identified, we further confirmed that a potential corresponding locus on chromosome 3 regulates host responses to AcMNPV in silkworm. Additionally, we found that it is available for AcMNPV–silkworm baculovirus expression vector system to produce proteins of interest.

Keywords Silkworm · Baculovirus · AcMNPV · Replication · Genetic analysis

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Introduction

Members of the family Baculoviridae are large rod-shaped enveloped viruses with double-stranded, circular, supercoiled genomes that vary in size from 80 to 180 kb (Herniou et al. 2012). Each baculovirus isolate demonstrates a narrow host range (Goulson et al. 2003), while one exception is that *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) and several closely related viruses, such as *Rachiplusia ou* MNPV (RoMNPV) which displays relatively wider host range and replicates in hosts derived from several families of Lepidoptera (Gröner et al. 1986; Vail et al. 1993; Harrison and Bonning 1999). Nucleopolyhedroviruses cause infection that is typically fatal to the insect. Due to the nature of the virus particles, it is difficult to prevent the NPV infections and thus these cause severe annual economic losses in the silkworm sericulture industry (Jiang et al. 2013). To date, in-depth studies on the specificity of NPVs are very limited and little is known about the mechanisms of baculovirus DNA replication in host cells. Until recently, the evidence from emerging researches is beginning to identify several virus genes involved in the baculovirus host range determination

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Diversity of glutelin acidic subunit polypeptides in rice cultivars collected from Northern Vietnam

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Abstract

The diversity of glutelin acidic polypeptides in rice cultivars collected from Northern Vietnam was characterized via sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) and isoelectric focusing (IEF) electrophoresis. Glutelin acidic subunits were separated into four bands by molecular mass, as α-1 (39 kDa), α-2 (38 kDa), α-3 (37.5 or 37 kDa) and α-4 (34 or 33 kDa). One hundred and eighty-five Vietnamese rice cultivars were divided into three types, based on differences in staining intensity and the molecular size of the α-3 and α-4 polypeptides derived from SDS-PAGE analysis. Wide variation was also observed in the isoelectric point (pI) staining intensity, in addition to the absence/presence of pI bands detected via IEF analysis. A total of 16 pI bands, ranging from pI 6.30 to pI 7.52, were identified in the various local rice cultivars. The maximum and minimum of IEF bands detected were 14 and 10, respectively. The genetic variability index (H′) ranged from 0.280 to 0.820, which confirms that local rice cultivars from Northern Vietnam have diverse glutelin seed storage units.

Key words: electrophoresis — seed storage glutelin — diversity — rice germplasm

Cereals are an important source of dietary protein for humans. Rice is unquestionably a superior source of energy among existing cereal crops. Rice is the most important food crop worldwide, providing over 21% of the calorific needs of the world's population in South-East Asia. (Fitzgerald et al. 2008).

Seed storage proteins do not contain enzymes and have the sole purpose of providing the proteins (nitrogen and sulphur source) required for germination and the establishment of a new plant (Mandal and Mandal 2000). Seed storage proteins are classified based on their solubility in water (albumins), dilute saline (globulin), aqueous alcohol (prolamin) and dilute acid or alkali (glutelin) (Osborn 1924). Prolamin and glutelin are the major seed storage proteins found in rice. Prolamin is deposited in protein body I, which is derived from the endoplasmic reticulum (ER). In comparison, glutelin is deposited in protein body II, which is derived from the protein storage vacuole (PSV) (Tanaka et al. 1980, Ogawa et al. 1987). Glutelin, which accounts for 80% of total rice storage proteins, is easily digested. In contrast, it is difficult to digest prolamin, which accounts for about 20% of total rice storage proteins.

There has been extensive effort to improve the protein content of rice grains. Several seed storage protein mutants have been artificially generated through the use of *N*-methyl-*N*-nitrosourea (MNU) mutagenesis (Satoh and Omura 1981, Kumamaru et al. 1988). It is important to analyse these mutants to understand the synthesis, processing and deposition of seed storage proteins in rice. In addition to artificial mutants, spontaneous seed storage protein mutants have also been found in the local rice cultivars of some countries (Jahan et al. 2001, Aung et al. 2003). Characterization of seed storage protein in rice germplasm therefore plays a critical role in developing the novel genetic resources for rice quality improvement.

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) is a valuable tool for characterizing variation in the storage proteins of different rice cultivars. Proteins may be separated by size under denaturing conditions; therefore, this method also facilitates the estimation of the relative molecular mass of individual polypeptides (Hilu and Essen 1988). While SDS-PAGE separates proteins based on their molecular masses, isoelectric focusing (IEF) separates proteins based on the isoelectric point. Moreover, each subunit fractioned by SDS-PAGE is composed of at least two polypeptides, which are isolated through IEF electrophoresis (Wen and Luthe 1985, Uemura et al. 1996, Qu et al. 2002). Many studies have evaluated the genetic diversity of seed storage proteins from local crop cultivars by using SDS-PAGE. Examples include *Brassica* (Rahman and Hirata 2004), triticale (Igrejas et al. 1999), foxtail millet (Kumarn and Parameswaran 1998), cereal (Damania et al. 1983, Gorinstein et al. 1999, Alvarez et al. 2006, Lerner et al. 2009), *Arachis* (Bertozo and Valls 2001) and alfalfa bean (Zivkovi'c et al. 2012). Some studies have demonstrated that the glutelin profile is highly varied in local rice cultivars sampled from Madagascar, Myanmar, Bangladesh and Pakistan (Satoh et al. 1990, Aung et al. 2003, Siddiqui et al. 2003, 2010, Jahan et al. 2005). Studies focusing on variation in the glutelin profile might provide valuable information for collecting and conserving genetic resources, in addition to broadening the genetic material available for seed quality enhancement programmes.

Previous studies have reported high genetic diversity in *Oryza sativa,* cultivars from China, Thailand, Laos, Bangladesh, Myanmar and Vietnam (Oka 1988, Jahan et al. 2001, Aung et al. 2003, Fukuoka et al. 2003, 2006). As reported by Chang (1976), the belt of primary genetic diversity extends from the Ganges plains below the eastern foothills of the Himalayas, through upper Burma, Northern Thailand, Laos and Northern Vietnam, to the south-western and southern parts of China. This phenomenon is supported by the existence of high genetic diversity of larger numbers of local rice cultivars in the Northern Vietnam (Trinh et al. 1993, Okuno et al. 1996, Fukuoka et al. 2003, 2006).

In this study, we examined the diversity of glutelin in local rice cultivars from Northern Vietnam using SDS-PAGE and IEF

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Influence of single-nucleotide polymorphisms in the gene encoding granule-bound starch synthase I on amylose content in Vietnamese rice cultivars

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Amylose content is one of the most important factors influencing the physical and chemical properties of starch in rice. Analysis of 352 Vietnamese rice cultivars revealed a wide range of variation in apparent amylose content and the expression level of granule-bound starch synthase. On the basis of single-nucleotide polymorphisms (SNP) at the splicing donor site of the first intron and in the coding region of the granule-bound starch synthase I gene, *Waxy* gene, alleles can be classified into seven groups that reflect differences in apparent amylose content. The very low and low apparent amylose content levels were tightly associated with a G to T in the first intron whereas intermediate and high amylose was associated with a T genotype at SNP in exon 10. The correlation between the combination of T genotype at SNP in the first intron, C in exon 6, or C in exon 10 was predominant among low amylose rice varieties. Our analysis confirmed the existence of Wx^{op} allele in Vietnamese rice germplasm. The results of this study suggest that the low amylose properties of Vietnamese local rice germplasm are attributable to spontaneous mutations at exons, and not at the splicing donor site.

Key Words: amylose, granule-bound starch synthase I, rice germplasm, single-nucleotide polymorphism, *Waxy* gene.

Introduction

Starch consists of two kinds of glucan polymers, amylose and amylopectin. Amylose is predominantly a linear molecule of α-1,4-linked D-glucose, although some of the molecules are slightly branched by α-1,6-glucosidic linkages (Takeda and Hizukuri 1987). Amylopectin is composed of highly branched α-1,4-polyglucans, which are short, linear α-1,4-glucan chains that are regularly branched by α-1,6-glucosidic linkages (Takeda *et al.* 1987). The physicochemical properties of rice starch are affected by the ratio of amylose to amylopectin and their molecular structures (Nakamura *et al.* 2006).

Amylose is synthesized by the granule-bound starch synthase (GBSS), whereas amylopectin is synthesized by the concerted action of four classes of enzymes: ADP-glucose pyrophosphorylase, starch synthase, branching enzyme, and debranching enzyme (Denyer *et al.* 2001, Hannah and James 2008). Granule-bound starch synthase I (GBSSI) in rice is encoded by the *Waxy* (*Wx*) gene (Hirano and Sano 1991, Hirose and Terao 2004, Okagaki 1992). In rice cultivars, there are three functional alleles at the *Waxy* locus: Wx^a, Wx^b, and Wx^{op}. Wx^a and Wx^b are found mainly in Indica and Japonica rice, respectively. The expression level of Wx^a and Wx^b are associated with amylose content (Sano 1984, Sano *et al.* 1985, 1986). Expression level of mRNA and accumulation of waxy protein in Wx^a cultivars is 10-fold higher than that of Wx^b cultivars (Isshiki *et al.* 1998). The *Waxy opaque* (Wx^{op}) allele is the modified form of the Wx^a gene and endosperms with the Wx^{op} allele have 10% amylose content (Mikami *et al.* 1999, 2008).

The association between amylose content (AC) and single-nucleotide polymorphisms (SNPs) in the rice *Wx* gene has been described at the splicing donor sites of the first intron (Isshiki *et al.* 1998, Sano *et al.* 1985), exon 4 (Larkin and Park 1999, Mikami *et al.* 1999, 2008), exon 6 (Cai *et al.* 1998, Larkin and Park 2003, Mikami *et al.* 2008, Wang *et al.* 1995), and exon 10 (Cai *et al.* 1998, Hirano *et al.* 1996, Mikami *et al.* 2008, Wang *et al.* 1995). The cytosine and thymidine (CT_n) dinucleotide repeats in the 5'-untranslated region (UTR) of the *Wx* gene were reported to be a factor associated with AC (Ayres *et al.* 1997, Bergman *et al.* 2001, Bligh *et al.* 1995). However, the relationship between these polymorphisms and amylose contents is not clear.

Subsequent studies demonstrated that the SNP at the splicing donor site of the first intron reduces the efficiency of GBSS prior to processing of mRNA and causes the low levels of the mature *Waxy* transcript, GBSS, and apparent amylose content (AAC) (Cai *et al.* 1998, Hirano *et al.* 1996, 1998, Larkin and Park 1999, 2003, Wang *et al.* 1995). Moreover, recent reports show a tight correlation between SNP in the first intron, coding regions, and AAC (Chen *et*

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Characterization of RNA binding protein RBP-P reveals a possible role in rice glutelin gene expression and RNA localization

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Abstract RNA binding proteins (RBPs) play an important role in mRNA metabolism including synthesis, maturation, transport, localization, and stability. In developing rice seeds, RNAs that code for the major storage proteins are transported to specific domains of the cortical endoplasmic reticulum (ER) by a regulated mechanism requiring RNA cis-localization elements, or zipcodes. Putative trans-acting RBPs that recognize prolamine RNA zipcodes required for restricted localization to protein body-ER have previously been identified. Here, we describe the identification of RBP-P using a Northwestern blot approach as an RBP that recognizes and binds to glutelin zipcode RNA, which is required for proper RNA localization to cisternal-ER. RBP-P protein expression coincides with that of glutelin during seed maturation and is localized to both the nucleus and cytosol. RNA-immunoprecipitation and subsequent RT-PCR analysis further demonstrated that RBP-P interacts with glutelin RNAs. In vitro RNA–protein UV-crosslinking assays showed that recombinant RBP-P binds strongly to glutelin mRNA, and in particular, 3′ UTR and zipcode RNA. RBP-P also exhibited strong binding activity to a glutelin intron sequence, suggesting that RBP-P might participate in mRNA splicing. Overall, these results support a multifunctional role for RBP-P in glutelin mRNA metabolism, perhaps in nuclear pre-mRNA splicing and cytosolic localization to the cisternal-ER.

Keywords Rice · RNA binding protein · RNA localization · Zipcode · Storage protein · Glutelin

Kelly A. Doroshenk and Li Tian have contributed equally to this work.

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Introduction

The fate of cytosolic mRNAs is first established in the nucleus, where events regulated by cis- and trans-acting factors determine stability, localization, and translation (Giorgi and Moore 2007). Trans-acting factors include RNA binding proteins (RBPs) that, together with their associated mRNAs and other interacting factors, form a ribonucleoprotein complex that is eventually exported to the cytosol (Glisovic et al. 2008). Although this complex is dynamic, some RBP components recruited in the nucleus remain with the mRNA well after export and can be found at the site of translation (Dreyfuss et al. 2002; Glisovic et al. 2008). This is true of some heterogeneous nuclear ribonucleoproteins (hnRNPs), RBPs that are involved in one or more aspects of nuclear and cytosolic RNA processing including RNA splicing, turnover, localization, and translation (Krecic and Swanson 1999; Dreyfuss et al. 2002). In fact, many RBPs have been reported to perform multiple duties within the cell and this functional diversity is due in part to the presence of one or more RNA binding domains,

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Axillary Meristem Formation in Rice Requires the *WUSCHEL* Ortholog *TILLERS ABSENT1*[OPEN]

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Axillary shoot formation is a key determinant of plant architecture. Formation of the axillary shoot is regulated by initiation of the axillary meristem or outgrowth of the axillary bud. Here, we show that rice (*Oryza sativa*) *TILLERS ABSENT1* (*TAB1*; also known as Os *WUS*), an ortholog of *Arabidopsis thaliana WUS*, is required to initiate axillary meristem development. We found that formation of the axillary meristem in rice proceeds via a transient state, which we term the premeristem, characterized by the expression of *OSH1*, a marker of indeterminate cells in the shoot apical meristem. In the *tab1-1* (*wus-1*) mutant, however, formation of the axillary meristem is arrested at various stages of the premeristem zone, and *OSH1* expression is highly reduced. *TAB1/WUS* is expressed in the premeristem zone, where it shows a partially overlapping pattern with *OSH1*. It is likely, therefore, that *TAB1* plays an important role in maintaining the premeristem zone and in promoting the formation of the axillary meristem by promoting *OSH1* expression. Temporal expression patterns of *WUSCHEL-RELATED HOMEOBOX4* (*WOX4*) indicate that *WOX4* is likely to regulate meristem maintenance instead of *TAB1* after establishment of the axillary meristem. Lastly, we show that the prophyll, the first leaf in the secondary axis, is formed from the premeristem zone and not from the axillary meristem.

INTRODUCTION

Plant architecture such as shoots and inflorescences are greatly influenced by the branching pattern (reviewed in Wang and Li, 2008; Domagalska and Leyser, 2011). Shoot branches grow from axillary buds, which are derived from the axillary meristems formed at the axil of leaf primordia. Branch formation is regulated at two developmental stages: initiation of the axillary meristem and outgrowth of the axillary bud. The latter event, which is induced by derepression of bud dormancy, is regulated by a coordinated action of phytohormones such as auxin, strigolactone, and cytokinin (reviewed in Domagalska and Leyser, 2011). Whereas there has been rapid progress in our understanding of bud outgrowth, the mechanism of axillary meristem initiation is insufficiently understood at present.

Genetic studies have revealed that initiation of the axillary meristem is regulated by genes such as *MONOCULM1* (*MOC1*), *LAX PANICLE1* (*LAX1*), and *LAX2* in rice (*Oryza sativa*) (Komatsu et al., 2003; Li et al., 2003; Oikawa and Kyozuka, 2009; Tabuchi et al., 2011); *barren stalk1* in maize (*Zea mays*) (Gallavotti et al., 2004); *LATERAL SUPPRESSOR* (*LAS*), *REGULATOR OF AXILLARY MERISTEMS*, and *REGULATOR OF AXILLARY MERISTEM FORMATION* (*ROX*) in *Arabidopsis thaliana* (Greb et al., 2003; Keller et al., 2006; Müller et al., 2006; Yang et al., 2012); and *Lateral suppressor* (*Ls*) and *Blind* in tomato (*Solanum lycopersicum*) (Schumacher et al., 1999; Schmitz et al., 2002). Most of these genes encode transcription factors belonging to the GRAS, MYB, and basic/helix-loop-helix families and are required for the early steps of axillary meristem initiation. These genes are expressed in distinct patterns in the axil of leaf primordia, for example, in the region where the axillary meristem initiates or the boundary region that discriminates the developing axillary meristem from other tissues. Recently, it has been demonstrated that auxin depletion and cytokinin signaling in the leaf axil are required for axillary meristem formation in Arabidopsis (Wang et al., 2014a, 2014b).

The shoot branch in rice is called a tiller, and rice propagates vegetatively by tillering (reviewed in Wang and Li, 2008; Pautler et al., 2013). Therefore, understanding the mechanism of tiller formation in rice is important not only for basic biological knowledge but also for agricultural improvement. A tiller is formed from an axillary bud (tiller bud). Unlike in Arabidopsis and tomato, where the axillary meristem forms on the adaxial side of the leaf, rice generates the axillary bud on the culm (stem) in the leaf axil (Li et al., 2003; Oikawa and Kyozuka, 2009). In rice *moc1*, *lax1*, and *lax2* mutants, the tiller is absent or highly reduced, and the branching of the inflorescence (panicle) is compromised (Komatsu et al., 2003; Li et al., 2003; Oikawa and Kyozuka, 2009; Tabuchi et al., 2011). The genes responsible for these mutations play crucial roles in initiating the axillary meristem. *OSH1*, which is a marker of meristematic cells and a homolog of Arabidopsis

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RESEARCH PAPER

Guanine nucleotide exchange factor 2 for Rab5 proteins coordinated with GLUP6/GEF regulates the intracellular transport of the proglutelin from the Golgi apparatus to the protein storage vacuole in rice endosperm

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Abstract

Rice glutelin polypeptides are initially synthesized on the endoplasmic reticulum (ER) membrane as a proglutelin, which are then transported to the protein storage vacuole (PSV) via the Golgi apparatus. Rab5 and its cognate activator guanine nucleotide exchange factor (GEF) are essential for the intracellular transport of proglutelin from the Golgi apparatus to the PSV. Results from previous studies showed that the double recessive type of *glup4/rab5a* and *glup6/gef* mutant accumulated much higher amounts of proglutelin than either parent line. The present study demonstrates that the double recessive type of *glup4/rab5a* and *glup6/gef* mutant showed not only elevated proglutelin levels and much larger paramural bodies but also reduced the number and size of PSVs, indicating a synergistic mutation effect. These observations led us to the hypothesis that other isoforms of Rab5 and GEF also participate in the intracellular transport of rice glutelin. A database search identified a novel guanine nucleotide exchange factor, Rab5-GEF2. Like GLUP6/GEF, Rab5-GEF2 was capable of activating Rab5a and two other Rab5 isoforms in *in vitro* GTP/GDP exchange assays. GEF proteins consist of the helical bundle (HB) domain at the N-terminus, Vps9 domain, and a C-terminal region. By the deletion analysis of GEFs, the HB domain was found essential for the activation of Rab5 proteins.

Key words: Guanine nucleotide exchange factor, helical bundle domain, intracellular transport, *in vitro* GEF assay, Rab5, storage protein.

Abbreviations: COP, coat protein; CTAB, cetyltrimethylammonium bromide; DDBJ, DNA data bank of Japan; esp, endosperm storage protein; ER, endoplasmic reticulum; GEF, guanine nucleotide exchange factor; glup, glutelin precursor; GMP-PNP, 5′-guanylyl imidodiphosphate; HB, helical bundle; KOME, knowledge-based *Oryza* molecular biological encyclopedia; MNU, *N*-methyl-*N*-nitrosourea; PB, protein body; PDIL, protein disulfide isomerase-like; PMBs, paramural bodies; PSV, protein storage vacuole; RAP-DB, rice annotation project database; PVC, prevacuolar compartment; RiceXRro, rice expression profile database; RT, reverse transcript; SALADA, alignment diagram and the association dendrogram; SPR, surface plasmon resonance; WAF, week after flowering.

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Biochemical characterization of an L-tryptophan dehydrogenase from the photoautotrophic cyanobacterium *Nostoc punctiforme*

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ABSTRACT

An NAD^+-dependent L-tryptophan dehydrogenase from *Nostoc punctiforme* NIES-2108 (NpTrpDH) was cloned and overexpressed in *Escherichia coli*. The recombinant NpTrpDH with a C-terminal His_6-tag was purified to homogeneity using a Ni-NTA agarose column, and was found to be a homodimer with a molecular mass of 76.1 kDa. The enzyme required NAD^+ and NADH as cofactors for oxidative deamination and reductive amination, respectively, but not $NADP^+$ or NADPH. L-Trp was the preferred substrate for deamination, though L-Phe was deaminated at a much lower rate. The enzyme exclusively aminated 3-indolepyruvate; phenylpyruvate was inert. The pH optima for the deamination of L-Trp and amination of 3-indolpyruvate were 11.0 and 7.5, respectively. For deamination of L-Trp, maximum enzymatic activity was observed at 45 °C. NpTrpDH retained more than 80% of its activity after incubation for 30 min at pHs ranging from 5.0 to 11.5 or incubation for 10 min at temperatures up to 40 °C. Unlike L-Trp dehydrogenases from higher plants, NpTrpDH activity was not activated by metal ions. Typical Michaelis–Menten kinetics were observed for NAD^+ and L-Trp for oxidative deamination, but with reductive amination there was marked substrate inhibition by 3-indolepyruvate. NMR analysis of the hydrogen transfer from the C4 position of the nicotinamide moiety of NADH showed that NpTrpDH has a pro-*S* (B-type) stereospecificity similar to the Glu/Leu/Phe/Val dehydrogenase family.

1. Introduction

Amino acid dehydrogenases (EC 1.4.1.X) catalyze the reversible $NAD(P)^+$-dependent oxidative deamination of amino acids to their corresponding 2-oxoacids and ammonia [1–3]. More than fifteen kinds of amino acid dehydrogenases, including those acting on L-Glu, L-Ala, L-Ser, L-Val, L-Leu, L-Gly, L-Lys, L-Phe and L-Asp, have been identified in various organisms [1,4,5], and detailed analyses of the structures and functions of L-Glu dehydrogenase [5], L-Leu dehydrogenase [6,7] and L-Phe dehydrogenase [8] have been reported. In addition, several amino acid dehydrogenases have been applied for use in biosensors for L-amino acid, 2-oxoacid and ammonia assays [1,9], disease diagnosis [10,11], and amino acids synthesis [9,12].

As compared to other amino acid dehydrogenases, L-Trp dehydrogenase (EC 1.4.1.19, TrpDH), which catalyzes the reversible oxidative deamination of L-Trp to 3-indolepyruvate in the presence of $NAD(P)^+$, has not been extensively investigated from the viewpoint of it biochemical or biotechnological potential due in large part to its extremely limited distribution. The enzyme was first identified in several higher plants (e.g., *Pisum sativum*, *Spinacia oleracea* and *Zea mays*, etc.) in the mid 1980s [13], and was partially characterized at that time [14–17]. There was then no further investigation of the enzyme until the product of the Npun_R1275 gene (*npun_r1275*) from the cyanobacterium *Nostoc punctiforme* ATCC 29133 was found to exhibit NAD^+-dependent oxidative deamination activity toward L-Trp [18]. This was the first microbial TrpDH known, and its gene (*npun_r1275*) was located

Abbreviations: TrpDH, L-Trp dehydrogenase; NpTrpDH, NAD^+-dependent L-tryptophan dehydrogenase from *Nostoc punctiforme* NIES-2108.

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ORIGINAL RESEARCH PAPER

Spectrophotometric assay of D-isoleucine using an artificially created D-amino acid dehydrogenase

Hironaga Akita · Yoshifumi Imaizumi · Hirokazu Suzuki · Katsumi Doi · Toshihisa Ohshima

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Abstract D-isoleucine (D-Ile) can be assayed using chiral chromatography but the availability of that method is limited by the necessity for special expertise and expensive equipment. We therefore developed a simple and specific colorimetric assay system for D-Ile determination using an artificially created $NADP^+$-dependent D-amino acid dehydrogenase (DAADH). The system consists of two reaction steps: the first is the quantitative conversion of D-Ile to (3*R*)-2-oxo-3-methyl valerate by DAADH in which $NADP^+$ is converted to NADPH, while the second is chemical conversion of NADPH to reduced water-soluble Tetrazolium-3 via a redox mediator. D-Ile was determined from 1 to 50 μM, and the assay was unaffected by the presence of any of three other isomers (100 μM), alcohol and organic acids.

Keywords D-Amino acid · D-Amino acid dehydrogenase · Endpoint assay · D-Isoleucine · Spectrophotometric assay · Water-soluble Tetrazolium-3

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BIOTECHNOLOGICALLY RELEVANT ENZYMES AND PROTEINS

Efficient synthesis of D-branched-chain amino acids and their labeled compounds with stable isotopes using D-amino acid dehydrogenase

Hironaga Akita · Hirokazu Suzuki · Katsumi Doi · Toshihisa Ohshima

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Abstract D-Branched-chain amino acids (D-BCAAs) such as D-leucine, D-isoleucine, and D-valine are known to be peptide antibiotic intermediates and to exhibit a variety of bioactivities. Consequently, much effort is going into achieving simple stereospecific synthesis of D-BCAAs, especially analogs labeled with stable isotopes. Up to now, however, no effective method has been reported. Here, we report the establishment of an efficient system for enantioselective synthesis of D-BCAAs and production of D-BCAAs labeled with stable isotopes. This system is based on two thermostable enzymes: D-amino acid dehydrogenase, catalyzing NADPH-dependent enantioselective amination of 2-oxo acids to produce the corresponding D-amino acids, and glucose dehydrogenase, catalyzing NADPH regeneration from $NADP^+$ and D-glucose. After incubation with the enzymes for 2 h at 65°C and pH 10.5, 2-oxo-4-methylvaleric acid was converted to D-leucine with an excellent yield (>99 %) and optical purity (>99 %). Using this system, we produced five different D-BCAAs labeled with stable isotopes: D-[1-^{13}C,^{15}N]leucine, D-[1-^{13}C]leucine, D-[^{15}N]leucine, D-[^{15}N]isoleucine, and D-[^{15}N]valine. The structure of each labeled D-amino acid was confirmed using time-of-flight mass spectrometry and nuclear magnetic resonance analysis. These analyses confirmed that the developed system was highly useful for production of D-BCAAs labeled with stable isotopes, making this the first reported enzymatic production of D-BCAAs labeled with stable isotopes. Our findings facilitate tracer studies investigating D-BCAAs and their derivatives.

Keywords D-Branched-chain amino acid synthesis · D-Amino acid dehydrogenase · Stable isotope · D-[^{13}C,^{15}N]amino acid · NADPH regeneration

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Introduction

It is now known that many organisms contain D-amino acids in either free or conjugated forms (Friedman 2010; Friedman and Levin 2012; Martínez-Rodríguez et al. 2010). And although their physiological functions are still largely unknown, their involvements in several specific bioactivities and cell functions have been reported in recent years. For example, it was recently reported that accumulation of D-serine is involved in mammalian brain function (Nishikawa 2011), and that incorporation of D-amino acids into proteins is associated with some human diseases (Katane and Homma 2011). In addition, notable bioactivity has been reported for D-branched-chain amino acids (D-BCAAs). For example, D-isoleucine appears to be involved in promoting growth of the matsutake mushroom (*Tricholoma matsutake*) (Kawagishi et al. 2004), while D-leucine triggers biofilm disassembly in *Bacillus subtilis*

III. センターの活動状況

1. 教育活動

【家蚕遺伝子開発分野】

大学院生物資源環境科学研究科
システム生物学教育コースとして大学院教育に参画

【植物遺伝子開発分野】

大学院生物資源環境科学府（博士課程）

中村哲洋　　Study on rice sugary2 mutation altering amylopectin structure in both storage and photosynthetic tissues

【微生物遺伝子開発分野】

農学部生物資源環境科学科（学士課程）

中村　彩乃　*Thermus*属繊維状ファージの宿主認識およびアセンブリ機構の解明

李　華強　　超好熱アーキアの糖代謝機構の解明

大学院生物資源環境科学府（修士課程）

田上　諒　　好熱性細菌の過飽和シリカへの応答機構と大腸菌への応用

ﾏﾙﾄﾉ　ﾋﾝﾄﾞﾗ　　溶原性ファージφOH2由来耐熱性溶菌酵素の特性解析

和田　圭介　　細胞内分子進化を利用した新規耐熱性酵素遺伝子群の創出

黒木　未知瑠　　好熱性ファージφOH2由来溶菌酵素holinによるガン細胞のアポトーシス誘導

児玉　恵子　　好熱性ファージφOH16の複製機構の解明

酒井　紀利人	好熱好酸性アーキアに感染する新規ウィルスの探索と性状解析
東　大輔	地熱環境におけるシリカ沈殿形成メカニズムの研究
前田　純平	*L. otakiensis*由来D-分岐鎖アミノ酸ラセマーゼの性質とその役割
Jimmy Kayumba	アフリカ原産乳酸発酵物より分離した乳酸菌の多様性解析
Kim MinA	好熱性ファージ由来耐熱性溶菌酵素を用いたファージセラピー技術の開発

大学院生物資源環境科学府（博士課程）

永吉　佑子	極限環境ウイルスの構造・機能解析と分子進化の解明

2. 科学研究費・共同、受託研究等

【家蚕遺伝子開発分野】

基盤研究（C）研究代表　山本 幸治
「昆虫不妊化剤開発を指向したプロスタグランジン合成酵素の機能解析」

ナショナルバイオリソースプロジェクト中核的拠点整備プログラム
課題管理者　伴野　豊
「カイコバイオリソースの収集・高品質化と効率的保存・供給体制の整備」

ナショナルバイオリソースプロジェクト基盤技術整備プログラム
課題管理者　伴野 豊
「カイコの凍結保存技術の開発」

挑戦的萌芽研究　研究代表　伴野 豊
「CAS冷却を用いたカイコの永久保存システムの構築に関する基盤研究」

【植物遺伝子開発分野】

ナショナルバイオリソース中核的拠点整備プログラム　機関代表　熊丸 敏博
「イネ属の多様性を生かすリソース基盤の構築（多様な高品質イネ実験系統の整備）」

基盤研究 (A)　研究分担　熊丸 敏博
「外国産ジャポニカ米の官能食味試験および国産米競争力分析に関する学際研究」

農林水産業・食品産業科学技術研究推進事業　研究分担　熊丸 敏博
「難消化性澱粉構造と高水分吸収性を有する変異体米を用いた低カロリー食品の開発」

ワシントン州立大学. Thomas W. Okita
「イネ種子成分の集積を制御する遺伝子に関する研究」

【微生物遺伝子開発分野】

平成 26～29 年度　科学研究費補助金　基盤研究(B)（代表）
「地熱環境における好熱性微生物によるシリカバイオミネラリゼーション形成の統合解析」

平成 26～28 年度　科学研究費補助金　基盤研究(B)（海外）（分担）
「ネパール野生キノコのライブラリーと健康機能を含むデータベースの構築」

平成 26～27 年度　小林国際奨学財団研究助成（代表）
「乳酸発酵過程における D-アミノ酸代謝機構の網羅的解析と効率的 D-アミノ酸生産法の開発」

平成 26 年度　NEDO エネルギー・環境新技術先導プログラム（分担）
「地熱発電量を 10 倍化する酸性熱水利用および還元井減衰防止技術の開発」

平成26年度　科学技術振興機構A-STEP（研究成果最適展開支援プログラム）（代表）
「耐熱性溶菌酵素を用いたファージセラピー基盤の開発」

3. 講演会・セミナー・講習会

【家蚕遺伝子開発分野】
伴野 豊

九州大学　夏休み公開講座　2014 年 7 月
九州大学　福岡県箱崎中学校職場体験学習協力　2014 年 8 月
九州大学　カイコの形質評価と利用に関する講習　2014 年 5 月
九州大学　博多織デベロップメントカレッジ講義 2014年5月
九州大学　博多織デベロップメントカレッジ講義　2014 年 7
九州大学　カイコの糸とり教室　2014 年 8 月
海の道むなかた館　蚕とクワコに関する講演と実習　2014 年 12 月

【植物遺伝子開発分野】
熊丸 敏博
福岡県立明善高等学校 大学セミナー　2014年7月

4. 海外渡航

【家蚕遺伝子開発分野】
山本 幸治
Greece，Crete，9th International Workshop on the Molecular Biology and Genetics of the Lepidoptera，2014年8月

【植物遺伝子開発分野】
熊丸 敏博
アメリカ合衆国，FRC，サクラメント, 2014年6月

【微生物遺伝子開発分野】

土居 克実
アメリカ合衆国，Madison, 2014 Molecular Genetics of Bacteria and Phages Meeting, University of Wisconsin, Madison, 2014年8月

ラオス人民民主共和国，Vientiane, JDS留学生（九州大学受入分）現地面接及びラオス国教育研究機構視察、2015年1月

5. 訪問研究員等

【植物遺伝子開発分野】

Ammar Abd El-Azeim El-Akhdar，訪問研究員, Field Crops Research Institute,

Agricultural Research Center, 2014年6月～2015年3月, エジプト

【微生物遺伝子開発分野】

Onanong Pringsulaka, 訪問研究員, Srinakharinwirot University,2015年3月, タイ王国
「日本学術振興会 Core-to-Core Programによる共同研究実施」

IV. 遺伝子資源の保存、収集の状況

【家蚕遺伝子開発分野】

本センター保存の家蚕（カイコ）系統は、アカデミックリソースとしては、世界最大のコレクションであり、カイコ研究の拠り所として国の内外の研究者から利用されている。2002 年 7 月からスタートした文部科学省ナショナルバイオリソース（NBRP）のカイコの中核拠点として本分野は指定され、本センターの果たすべき役割は益々高まっている。保存系統はまずその主要目的形質によってアルファベットで分類し、それに2位数を附し系統番号としている（同一起源の分枝系は3位数）。分類記号の内容及びおよび、記号別保有数は以下の如くである。それらは、下記の系統約 500 系統がコアとなっている。コア系統の遺伝子情報の詳細はナショナルバイオリソースプロジェクトのホームページに掲載されている。
http://www.shigen.nig.ac.jp/silkwormbase/index.jsp

コア系統以外に TG（ゲノム改変カイコ）系統 138 系統、クワコへカイコを連続戻し交配して作成した染色体置換系統 53 系統、ケミカルミュータジェネシス(ENU)25 系統、他機関から寄託された系統等が維持されている。

p （地域型品種）	２３	a （胚子，幼虫期致死）	２０
b （繭形・繭質）	１７	c （繭色）	２５
d （卵形・卵殻色）	３５	e （卵色）	２８
f （幼虫肢・斑紋）	３８	g （幼虫斑紋）	１７
i （幼虫眼紋・頭尾斑）	１３	k （幼虫体色）	２４
l （幼虫体色）	２８	m （モザイク・畸形）	１７
n （幼虫体形）	２８	o （油蚕）	４０
r （染色体異常・交叉率）	１６	t （発育・眠性）	２５
u （蛹・成虫）	２１	w （連関分析用合成系）	２７
x （分析未了の新突然変異）	１４		

（提供：系統の分譲件数）

本分野の過去５年間のカイコ系統の分譲件数を示す。分譲依頼者は、研究、教育

関係が大半である。農学部、理学部系から依頼が多いが近年は薬学関係からの依頼も増えている。哺乳類等の代替の実験生物としての利用が創薬分野で広がっている。

		2010	2011	2012	2013	2014
生物体での分譲	国内	920件	733件	910件	973件	1295件
	国外	71件	94件	117件	178件	75件
DNAでの分譲	国内	44件	0件	76件	470件	20件
	国外	0件	0件	0件	0件	0件

（カイコバイオリソースに関する専門知識・情報の提供）

リソース分譲の増加と共にカイコに関する生物学的知識、利活用に関する専門知識、技術相談、研修依頼、また来訪者への対応が増大している。その主な項目を下記に列挙する。括弧内は主な対象者。

- カイコ突然変異体を中心とした形質特性、起源に関する情報提供（研究者、院生）
- 研究に適した系統の選出依頼や、研究計画に対する助言依頼（研究者、院生）
- カイコバイオリソースに関する遺伝を中心とした文献や知識の提供（研究者、院生）
- カイコの系統維持に関する専門知識の提供（研究者、教育関係者）
- カイコ系統の凍結保存に関する技術移転に関する相談（研究者）
- 桑の分譲、栽培に関する専門知識の提供（研究者、一般）
- カイコ全般に関する知識提供（教員、一般）
- 養蚕に関する知識、技術の提供（農業関係者、一般）
- カイコの教材としての活用方法に関する相談（教員、教育関係者）
- 報道、出版機関からのカイコ、養蚕に関する問い合わせや専門用語の解説依頼や知識の提供、監修依頼（報道、出版関係者）
- カイコを用いたイベント開催に関するアドバイスや講演依頼（教員、自治体関係者、一般）

（系統の受入）

トランスジェニック系統 20 系統
（農業生物資源研究所瀬筒秀樹研究ユニット長から）

（カイコ系統の保存事業）

文部科学省研究振興局ライフサイエンス課研究開発施設共用等促進費補助金（ナショナルバイオリソースプロジェクト）の支援を受け実施

1）カイコバイオリソースの収集と管理

・ 独立行政法人農業生物資源研究所からトランスジェニック系統を 20 系統収集した。
・ ENU 誘発新規突然変異系統を 5 系統収集した。
・ １本の常染色体のみをｸﾜｺの染色体で置換することを最終目的としたｺﾝｿﾐｯｸ系統を 2 系統収集した
・ 既存の保有系統の遺伝的特性の調査を行い、新規変異体候補を 3 系統見出した。
・ DNA リソースの収集は 30 個体（カイコ 24 個体、クワコ 6 個体）であった。
・ カイコバイオリソースの安全かつ効率的な保存体制の構築
・ 750 系統のカイコ系統を飼育し、卵、幼虫、蛹時期に形質の評価を行い、平成 27 年度へと継代する保存業務を行った。
・ 凍結保存の実用化に取組み、新規に 111 系統の凍結保存を行った。累計の凍結保存数は 603 系統となった。
・ 新たに３種類の致死遺伝子について、効率的に系統維持を行うために分子マーカーを設計した。
・ DNA リソース 1610 個体を保存した。
・ カイコで確立された卵巣移植方法を、分担機関（東京大学）で保存しているイチジクカサンに応用することを試みた。移植した卵巣がﾎｽﾄの産卵管と結合し、受精卵を得ることができた。今後は、凍結・解凍した卵巣の移植を行い、イチジクカサンの卵巣も凍結保存できるのか検討する。
・ バックアップ体制の整備として以下のことを実施した。九州大学保存のコアリソース 495 系統については保管の委託契約先である長野県松本市の風穴で、信州大学の野蚕系統は九州大学でバックアップ保存する為に、リソースの移動、管理を行なった。

3）高品質なカイコバイオリソースの保存と提供

・ 日常の飼育室管理、栄養状態の良い桑葉を確保するための桑園管理（福岡市 3 ヶ所、指宿市 1 ヶ所）を行った。
・ 提供に備えるため、第 1 期の飼育（5 月～6 月）において、浸酸種、冷浸種、人工越年種を、オリジナル系統を中心に約 500 系統で採卵を行った。その卵を基本に、年間計５回の桑葉飼育を行い、幼虫、蛹、成虫を準備し、提供事業を行った。
・ リソースの分譲件数は予定数 1,390 件（国内 1315 件、海外 75 件）であった。

4）桑園管理

カイコ飼育には餌となる桑の確保が必要で、本分野の業務は桑園管理から行われている。現在、箱崎地区、原町農場、香椎浜、指宿試験地の４カ所に約 3.5ha の桑園を保有している。桑園管理は、施肥、除草、病害虫、選定、収穫など幅広い分野

に関する知識と経験が必要な業務であり、農場職員の協力のもと行っている。大学移転が進行中であるが、桑園に関しては一部を早期に移転する計画となっているため、既に H25 年より伊都第３工区において造成工事が進んでいる。早期移転の理由は香椎浜（6000 ㎡）と原町農場の道路改良事業による売却予定（1100 ㎡）が農学部移転に先行して行われる可能性があるためである。7100 ㎡が対象面積であるが、現地の地形の都合で 9300 ㎡が早期移転面積となっている。この対応が通常の桑園管理と進行しているため、非常に多忙となっている。

昨年までに造成された土地に桑の植付けを行ったが、排水に問題があり、大半を抜き、再度植え直しを行うことになった。施設部、工事業者、コンサルタント業者、農学研究院長、同圃場計画専門委員会を始めとした関係各位と頻繁に対応を協議して改善をはかった。その結果、伊都地区に記念すべき新規桑園が誕生した。現地には写真に示したような看板も設置された。２年後には桑収穫を開始する予定である。

伊都地区新桑園の造成と平行して、本年は原町農場の跡地処分のための遺跡調査が始まった。跡地を売却する際に、調査を行う為である。その結果、桑園部分からは、紀元 700 年前後の地方政庁の跡とみられる遺跡が見つかった。粕屋町教委によると、「糟屋評（かすやのこおり）」と呼ばれる政庁跡の可能性が高いという。桑園内の試掘に伴い、一部の桑株を抜く協力を行った。

指宿試験地では沖縄グワ（沖桑：おきそう）と呼ばれる冬期間でも摘桑が可能な桑を中心に栽培している。近年 NBRP 事業の拡大で冬期間の桑の需要が高まっている。このため、指宿での栽培強化をはかった。具体的には萎縮病被害があり、休耕していた圃場に挿し木で増殖した桑を植え付けた。

◎H26年度の桑園移転に伴う管理業務の主なものは以下の通りである。

４月

群馬の桑苗業者（大竹桑園）から購入した桑苗約4000本を伊都キャンパスに造成された桑園に植付け。当初5680本（7100㎡分）発注していたが、業者の圃場で水害が発生し、依頼した本数が揃わなかった。

６月

植付けた苗の８割程が成育不良。桑園内には冠水箇所が現れ、関係各位と対策を協議。

７月から９月

現地の土壌調査を土壌学和田教授の指導のもと行う。還元状態となった土壌が広く確認された。

10月

還元土壌を改良する目的で作土と心土を含めた深さ1mまでを大型機械でミキシングした。工事対象となった部分に植え付けた約3200本の苗は成育不良であるためそのままミキシングすることとし、新たに苗を購入し、植え直すことにした。

平成27年1月

排水のために周囲に暗渠を設置。

２月

来春、納入予定の桑苗（8000本、新規発注は6000本であるが、不足している桑苗2000本分を加算）の穂木を群馬大竹桑園へ送付。

３月

群馬大竹桑園から昨年発注していた2065本の新たな桑苗が届く（当初は、4360本届く予定であった現地で根腐れがあり減少。来年納品予定)。10月の工事で失った桑株の補充として植付けを行った。

5月：4月中旬に植付けた苗から芽吹きが始まる。新規桑園は伊都キャンパスでは最も高台にあり、眺望が良好。

6月：梅雨となり、冠水箇所が出現。水が引かない状態が続き、桑の生育が悪くなり、枯死する株も出始める。

7月下旬：土壌学和田先生と現地調査を行う。表面から、20から30センチ程掘ると青灰し還元状態となった土壌が現れた。

10月：大型機械によって1mの深さまで土を撹拌。赤丸で示したバケットの内部には撹拌装置が付随し、土をミキシングできる。

3月：再植付けを行った。本写真はその後5月に写したもので、良好に成育している。明渠も設置し、排水を行っている。

桑園に設置された看板:伊都地区最初の記念すべき桑園の完成。2年後から使用を予定している。

【植物遺伝子開発分野】

現在保存している品種系統の分類基準とその数を以下に示す。

HO系統	国内外の品種系統	1, 398 系統
LO系統	1962−1965年収集したわが国在来品種	1, 341 系統
IBP系統	FAO 国際共同研究供試品種	276 系統
UP系統	国内外の陸稲品種	342 系統
CM系統	化学変異源処理突然変異系統	5, 715 系統
EM系統	胚乳形質に関する突然変異系統	1, 764 系統
	計	10, 836 系統

これらの系統の一部をデータベースとして公開している。
http://w3.grt.kyushu-u.ac.jp/Rice_Kyushu/rice-kyushu/htdocs/main.html
http://www.shigen.nig.ac.jp/rice/oryzabase/

NBRPにおいて開設したTILLINGオープンラボに国内外の研究者を受け入れている。
http://www.shigen.nig.ac.jp/rice/oryzabaseV4/tilling/openLab

遺伝子資源系統の導入と分譲

年次	開発系統	導入(件数-系統数)		分譲(件数-系統数)	
		国内	国外	国内	国外
2014	681			11-2898	1-49

【微生物遺伝子開発分野】

微生物遺伝子開発分野における菌株の収集と保存は、発酵学講座、微生物工学講座など応用微生物関連講座での有用微生物の探索とその研究過程で得られた分離株及び変異株の収集・保存に始まる。これら菌株の多くはアルコール、有機酸、アミノ酸、核酸、抗生物質、酵素等の発酵、食品、医薬、化学工業にまたがる広範囲の各種有用物質の生産に利用されている。また、産業廃棄物の処理と資源化、炭酸ガス処理を含む地球環境の改善に係わる環境科学の基礎的・応用的研究にも大きく貢献している。
現在、以下のような菌株を保存している。

I. 細菌

（A）基準株　*Bacillus*属、*Cellulomonas*属、*Lactobacillus*属、*Lactococcus*属、*Pseudomonus*属、*Thermus*属および大腸菌　65種140株
（B）分離株　*Bacillus*属、*Geobacillus*属、*Ureibacillus*属、*Lactobacillus*属、*Lactococcus*属、*Pediococcus*属、*Pseudomonus*属、*Enterococcus*属及び*Thermus*属　43種1104株
（C）変異株　*Bacillus*属、*Geobacillus*属、*Lactobacillus*属および*Thermus*属　23種185株

II. 放線菌

（A）基準株　*Micromonospora*属、*Nocardia*属、*Rodococcus*属、*Streptomyces*属および*Streptoverticillium*属　155種171株
（B）分離株　*Streptomyces*属　5種5株
（C）変異株　*Streptomyces*属　10種311株

III. プラスミド

（A）導入プラスミドベクター　大腸菌、枯草菌（含む納豆菌）、乳酸菌、放線菌および酵母系統　165種類
（B）分離プラスミド　枯草菌（含む納豆菌）、乳酸菌および放線菌系統　127種類
（C）変異・構築プラスミド　4100種類以上

IV. ファージ

（A）導入ファージ・ファージベクター　大腸菌、乳酸菌、放線菌系統　35種類
（B）分離ファージ　乳酸菌および放線菌、アーキア系統　205種類
（C）変異・構築ファージ　大腸菌、乳酸菌および放線菌系統　85種類

V. 糸状菌

（A）基準株　*Aspergillus*属、*Mucor*属および*Penicillium*属　3種25株

VI.　酵母

（A）基準株　*Saccharomyces*属および*Candida*属　3種3株

VII.　昆虫培養細胞

*Bombyx*属、*Spodoptera*属及び*Trichoplolsia*属　7種11株

VII.　昆虫ウイルス及び組換え体

（A）昆虫ウイルス　5種類

（B）組換え体ウイルス　6種類

上記以外の有用微生物資源については、現在、発酵学教室及び微生物工学教室においてそれぞれ保存・管理されている。

（導入）

理化学研究所　微生物系統保存施設

Lactobacillus otakiensis JCM 15040

Lactobacillus buchneri JCM1115

製品評価技術基盤機構　バイオテクノロジーセンター

Sulfolobus acidocaldarius NBRC 15157

Sulfolobus shibatae NBRC 15437

Sulfolobus solfataricus NBRC 15331

Sulfolobus tokodaii NBRC 100140

Thermoplasma acidophilum NBRC 15155

Thermoplasma volcanium NBRC 15438

Thermus thermophilus phage ϕYS40

Thermus thermophilus phage ϕTMA

（分譲）

Dr. Dimitris G. Hatzinikolaou（Assistant Professor,National and Kapodistrian University of Athens, ギリシャ）

プラスミドベクター　pGAM48

Dr. Fangjie Zhao（Professor, Nanjing Agricultural University, 中国）

プラスミドベクター　pGAM48
プラスミドベクター　pUCG18T
Geobacillus kaustophilus MK244
Escherichia coli BR408

Dr. Fred Antson (Professor, University of York, 英国)

*Thermus*ファージφOH2　ゲノム

遺伝子制御学講座

Kitasatospora griseola
Streptomyces endus
Streptomyces griseus
Streptomyces lividans
Streptomyces coelicolor
Streptomyces azureus
Streptomyces laurentii

V. センター規程

九州大学農学研究院附属遺伝子資源開発研究センター規程

（趣旨）
第一条　この規程は、九州大学農学部附属遺伝子資源研究センター（以下「センター」という。）の組織及び運営に関し必要な事項を定める。
（センターの目的）
第二条　センターは、遺伝子の保存、開発及び利用に関する研究を行うことを目的とする。
（分野）
第三条　センターに、次の分野を置く。
一　家蚕遺伝子開発分野
二　植物遺伝子開発分野
三　微生物遺伝子開発分野
（センターの長）
第四条　センターに長を置き、農学部の責任及び兼任の教授のうちから教授会の議を経て選定する。
2　センターの長は、センターの管理及び運営を総括する。
3　センターの長の任期は、二年とする。
4　センター長は、再任されることができる。
（運営委員会）
第五条　センターの管理運営に関する重要な事項を審議するため、遺伝子資源開発センター運営委員会（以下「運営委員会」という。）を置く。
第六条　運営委員会は、委員長及び次の各号に掲げる委員をもって組織する。
一　センターの専任の教官のうちから選ばれた者三人
二　農学科、農芸化学科、林学科及び食糧化学工学科の専任の教授及び助教授のうちから選ばれた者各一人
三　前二号に掲げる者以外の農学科の専任の教授、助教授及び講師のうちから選ばれた者一人
四　農学部附属農場及び演習林の専任の教授及び助教授のうちから選ばれた者各一人
五　農学研究科遺伝子資源工学専攻の専任の教授及び助教授のうちから選ばれた者一人
2　委員の任期は、二年とする。ただし、委員に欠員が生じた場合の後任者の任期は、前任者の残任期間とする。
3　委員は、再任されることができる。
4　委員は、農学部長が委嘱する。

第七条　委員長は、センターの長をもって充てる。
2　委員長は、運営委員会を召集し、その議長となる。
3　委員長に事故等があるときは、あらかじめ委員長の指名する委員がその職務を代行する。
第八条　運営委員会は、委員の過半数の出席がなければ、議事を開き、議決をすることができない。
2　運営委員会の議事は、出席した委員の過半数をもって決し、可否同数のときは、議長の決するところによる。
（雑則）
第九条　この規程に定めるもののほか、センターの管理運営に関し必要な事項は、運営委員会の議を経て、センターの長が定める。
附則
1　この規程は、平成九年四月一日から施行し、平成九年四月十六日から適用する。
2　九州大学農学部附属遺伝子資源研究センター規程（昭和六十二年五月二十九日施行）は、廃止する。

VI. 英文摘要

INSTITUTE OF GENETIC RESOURCES

The institute of Genetic Resources had been established in May, 1987, and was then reorganized in April, 1997, within the Faculty of Agriculture, Kyushu University. The Institute is devoted to basic and applied studies on genetics with special interest in the stock maintenance of agriculturally important organisms. Silkworm, rice and fermentative microorganisms are chosen as the main materials from the viewpoint that their scientific researches have been carried out and developed chiefly in Japan. Emphasis has also been placed on studies at molecular level to contribute to the development of biotechnology and to establish gene libraries of these biological resources.

Silkworm Genetics Division

BANNO, Yutaka	Ph.D.	Associate Professor
YAMAMOTO, Koji	Ph.D.	Assistant Professor

a) Linkage analysis of silkworm
b) Mutagenesis and teratogenesis in silkworm
c) Analysis of gene expression
d) Maintenance of the mutant stocks
e) Construction of a genetic linkage map of silkworm genome
f) Cytological studies of the deficient and translocated chromosomes

Plant Genetic Division

KUMAMARU, Toshihiro Ph. D. Associate Professor

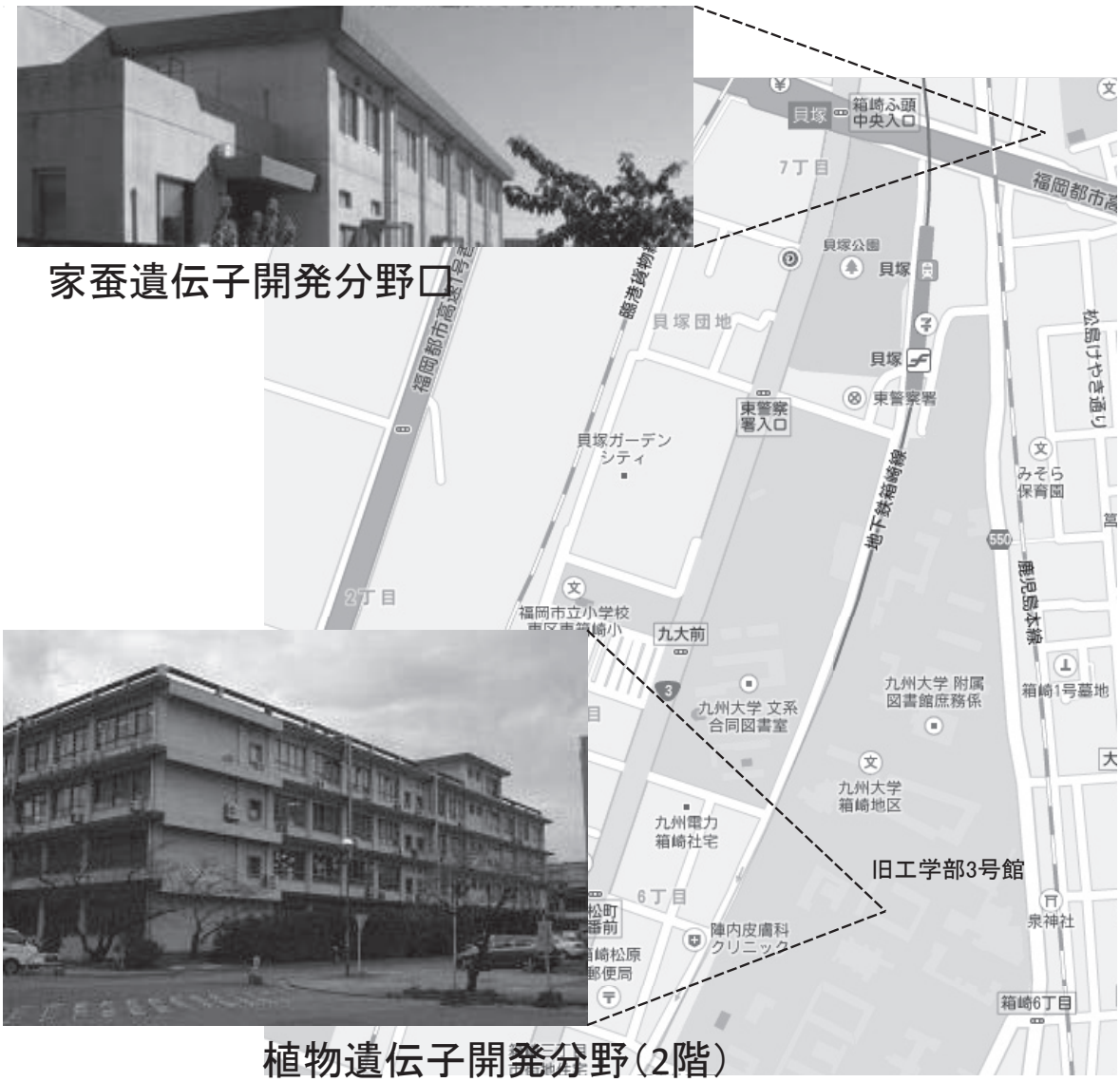
a) Resolution of the mechanism controlling the transport and the accumulation of the seed storage proteins in rice.
b) Construction of the rice mutation pool.
c) Conservation and evaluation of rice genetic resources.

Microbial Genetics Division

DOI, Katsumi Ph.D. Associate Professor

a) Survey, development and preservation of microbial genetic resources
b) Genetics and breeding of industrial bacteria: *Streptomyces*, *Lactobacillus*, *Bacillus*, *Thermus*, etc
c) Functional analysis and application of novel and useful genes found in industrial bacteria
d) Isolation and characterization of bacterial and archeal viruses
e) Investigation of biomineralization in geothermal environment

VII. センター研究棟配置図



家蚕遺伝子開発分野
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Fax. 092-641-1011, 092-624-1011

植物遺伝子開発分野
Tel.＆ Fax.　092-642-3056, 3057, 3058

微生物遺伝子開発分野
Tel.＆ Fax.　092-642-3053, 3054, 3059

編集後記

九州大学農学研究院遺伝子資源開発研究センター年報第17号を発刊致しました。平成25年度は、遺伝子資源開発研究センター長に麻生陽一先生の後任として日下部宜宏先生が就任され、センターの運営にご尽力いただいております。

平成26年4月現在、本センターの教員数は4名ですが、全員の力を合わせ、更なるセンターの発展に邁進する所存であります。今後とも、本センターの活動にご支援とご教示いただきますよう、お願いいたします。

編集者

熊丸　敏博
土居　克実
山本　幸治

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