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「Analysis of microRNA Toward Exploring a New Functional Component in Matcha Green Tea」

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資源生物科学専攻　　代謝・行動制御学分野
「Studies on Epigenetic Mechanisms Underlying Long-Term Effects of Developmental Metabolic Programming on Grass-Fed Beef Productivity in Japanese Black Cattle（黒毛和牛のグラスフェッドビーフ生産性に影響する発達期代謝プログラミングとそのエピジェネティック制御機構に関する研究）」

・WARDAK MOHAMMAD HAMAYOON（博士後期課程）
環境農学専攻　　農産食料流通工学分野
「Sustainable Preservation Solution for Fruit and Vegetables: Edible Films and Partial Coating Technologies（持続可能な青果物保存技術としての可食フィルムと部分コーティング）」

・TRAN THI VAN（博士後期課程）
環境農学専攻　　農産食料流通工学分野
「Impact of Nano-Biomaterials and Essential Oils on Carboxymethyl Cellulose-Based Edible Films and Emulsions （ナノバイオ材料および精油がカルボキシメチルセルロース基材の可食フィルムおよびエマルションに与える影響)」

・MOHAMED SAMIR MOHAMED ABO ELFETOH EL TELBANY　（博士後期課程）
生命機能科学専攻　　食品衛生化学分野
「Study on isolation, characterization of bacteriophages against multidrug-resistant enterococci, and their synergy with bacteriocins and endolysins（多剤耐性腸球菌に対するバクテリオファージの単離、特性評価、ならびにバクテリオシンおよびエンドリシンとの相乗作用に関する研究)」

Analysis of microRNA Toward Exploring a New Functional Component in Matcha Green Tea

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Matcha green tea, a traditional Japanese green tea, has various health-promoting effects; however, studies on its bioactive components remain limited except for main components such as catechins, theanine, and caffeine. As a candidate of functional factor, dietary microRNA (miRNA), a class of functional RNA with approximate 22 nucleotides in length, has been gaining considerable attentions in biomedical and food functionality research field. Here we aim to investigate the presence and basic properties of miRNAs in Matcha and to evaluate the health-promoting effects of Matcha-derived miRNAs.

We revealed for the first time the presence of miRNAs in Matcha, and their levels were affected by cultivars and harvesting seasons. Comparing with 27 commercially available plant-based dried powders including vegetable and species, Matcha exhibited the highest RNA yields and levels of representative miRNAs, indicating that Matcha is a rich miRNA source. To support future research of miRNAs, we demonstrated that miRNA yields were enhanced by increasing the hot water temperature accompanied with soaking process. This finding may be useful when considering consumption methods that allow more functional components to be consumed to enhance the health-promoting potential of Matcha. Furthermore, we firstly reported the correlation of miRNAs with main bioactive components in Matcha including polyphenols, epigallocatechin-3-*O*-gallate (EGCG), theanine, and caffeine. These results provide new insights into the potential functionality of Matcha[1)].

Matcha has been reported to improve lipid metabolism and reduce inflammation; however, the functional components remain largely unknown. Therefore, we focused on the effect of Matcha-derived miRNAs on metabolic dysfunction-associated steatohepatitis (MASH), which is primarily caused by lipid accumulation and inflammation and induces fibrosis, an important pathological feature leading to cirrhosis and hepatocellular carcinoma. One representative Matcha-derived miRNA inhibited the hepatic stellate cell activation, which is a key event contributes to the progress of liver fibrosis. Administration of this miRNA ameliorated liver fibrosis in a MASH model mouse. These findings are the first to demonstrate the functional potential of Matcha-derived miRNAs.

1) Huang *et al*., ***Food Chem. Mol. Sci***., 10, 100265, 2025.

氏　　名 ： 西野 大地

論文題名 ： Studies on Epigenetic Mechanisms Underlying Long-Term Effects of Developmental Metabolic Programming on Grass-Fed Beef Productivity in Japanese Black Cattle
（黒毛和牛のグラスフェッドビーフ生産性に影響する発達期代謝プログラミングとそのエピジェネティック制御機構に関する研究）

区　　分 ：甲

論 文 内 容 の 要 旨

牛肉生産は、ウシが植物繊維を高品質なタンパク質へ変換するプロセスであり、ヒトにとって重要な食料供給源である。しかし、近年、生産性向上を目的とし、肥育期を重視した穀物依存型の飼養モデルが主流となっている。一方、生物の代謝や体質の基盤は胎仔期および新生仔期に形成されることが知られ、これらの時期の栄養環境が個体の表現型に長期的に影響することが報告されている。牛肉生産においても、妊娠期の栄養環境が出生仔ウシの成長に影響を与え、新生仔期の栄養制御が粗飼肥育における生産性を改善する可能性が示唆されている。これらにはエピジェネティクスに基づく代謝プログラミングの関与が想定されるが、その制御機構は未解明である。本研究では、黒毛和牛を用い、胎仔期および新生仔期の栄養環境が表現型に及ぼす影響を調査し、骨格筋における代謝産物・DNA メチル化・遺伝子発現の連関をマルチオミクス解析により特定した。これにより、初期の代謝プログラミングが生産性に及ぼす影響を明らかにし、そのエピジェネティックな制御機構を解明するとともに、粗飼料飼養の改善に向けた新たなアプローチを検討した。

筋内脂肪の形成に重要な時期である新生仔期に着目し、この時期の高栄養飼養が、粗飼料肥育牛の生産性に及ぼす長期的影響を筋組織化学、ルミノロジー、メタボロミクス、エピジェネティクスの観点から多角的に解析した。生後 10 ヵ月齢（mo）まで粗飼料を給与する群と穀物主体の高栄養を給与する群（EHN 群）を設定し、10mo 以降は同一の粗飼料で肥育を行った。30mo 時点において、EHN 群の胸最長筋では脂肪細胞のサイズおよび脂肪形成関連遺伝子の発現が増加し、脂肪を主要なエネルギー基質とするI型およびIIA 型筋線維の割合が減少した。ルーメン内のプロピオン酸濃度は、EHN 群で新生仔期のみならず肥育後期にも増加しており、脂肪形成の基質供給が持続していることが示唆された。また、マルチオミクス解析により、EHN 群の胸最長筋では、ミトコンドリアの機能および生合成がエピジェネティックに低下していることが示された。特に、β 酸化能低下を示す複数のアシルカルニチンが蓄積し、ミトコンドリア機能に関与する遺伝子領域にメチル化変動が認められ、これらの遺伝子群の発現が減少していた。先行研究では、EHN 群の胸最長筋内脂肪蓄積割合が有意に高いことが確認されている。これらの結果は、新生仔期プログラミングによる筋内脂肪蓄積の増加が、①早期脂肪形成の亢進、②プロピオン酸産生型のルーメン発酵プログラミング、③DNA メチル化を介した筋ミトコンドリアの機能低下およびそれに伴う筋線維型の変化に起因することを示唆している。

骨格筋、特に筋線維の基盤形成における胎仔期の重要性に着目し、妊娠期の低栄養が妊娠 260 日目の胎仔胸最長筋に及ぼす影響を解析した。妊娠期の低栄養は、細胞発達、神経発達、タンパク質合成に関連する DNA メチル化状態および遺伝子発現に変化を引き起こすことが明らかとなった。さらに、妊娠期に低栄養または十分な栄養を給与する 2 群を設定し、出生後 10mo まで同一の高栄

養で飼養することで、胎仔期の低栄養が新生仔期プログラミングに及ぼす影響を検討した。低栄養群仔ウシの胸最長筋では、ミトコンドリアでのエネルギー産生に必要な代謝産物や遺伝子の発現が有意に減少し、タンパク質を構成するアミノ酸の蓄積および神経伝達物質の減少が認められた。さらに、妊娠期に低栄養処理を受けた胎仔骨格筋において観察された細胞発達、神経発達、タンパク質代謝に関連する遺伝子群の DNA メチル化および遺伝子発現の変動が 10mo の仔ウシの胸最長筋でも確認された。これらの結果は、胎仔期の低栄養環境が、筋でのエネルギー産生を抑制してタンパク質合成や筋内神経発達へのエネルギー消費を抑える「倹約的な体質」をプログラミングし、それが長期的に維持されることを示唆している。また、これらの制御機構に DNA メチル化が関与している可能性が示された。

本研究により、妊娠期における十分な栄養供給が骨格筋の基盤形成を強化し、新生仔期の高栄養給与が DNA メチル化を介した代謝プログラミングを誘導することで、筋内脂肪の蓄積能力を最大化できる可能性が示唆された。したがって、本研究の知見は、ウシの潜在能力を効率的に引き出し、粗飼料肥育における生産性向上を目指す新たな牛肉生産システムの構築に貢献するものである。

Name : MOHAMMAD HAMAYOON WARDAK
（モハマド ハマヨーン ワーダック）

Title : Sustainable Preservation Solution for Fruit and Vegetables: Edible Films and Partial Coating Technologies

（持続可能な青果物保存技術としての可食フィルムと部分コーティング）

Thesis Summary

This dissertation focuses on the developments and application of innovative edible films and coating, with particular attention to a groundbreaking partial coating technique to maintain the postharvest quality and enhance shelf-life of fresh fruits. As global demand escalates for sustainable and environmentally friendly alternative to traditional plastic packaging, significant research has focused on natural biopolymer-based coating. These innovative materials not only function as protective barriers but also provide bioactive properties such as antimicrobial and antioxidant properties.

The first study introduces a novel partial coating approach that regulates that gas exchange and moisture transfer by covering the main route of transpiration in perishable fruits. A composite film formulation – comprising citric acid-crosslinked starch reinforced with cellulose nanofiber (CNF) – was applied to tomato fruits. This technique addresses critical limitations of conventional full coating, such as prolong drying time and safety concerns of consumers, while effectively reducing respiration and moisture transpiration. Incorporating CNF enhanced the film's mechanical, thermal and barrier performance. Storage trials revealed no significant differences between the partial and full coatings, in terms of weight loss, firmness and color parameter, though both coating methods were outperformed uncoated fruits.

The second study explores the optimization of an edible coating composed of sodium alginate and kiwifruit seed essential oil (KSO) to improve the postharvest quality of persimmon fruits. The

formulation was optimized to achieve enhanced film barrier, mechanical, thermal, structural integrity, and bioactive retention. The partial coating strategy was designed to allow selective coverage, maintaining physiological balance and extend the shelf life of persimmon. This work underscores the potential of the innovative approach of partial coating with SA and KSO coating materials to preserve the quality of persimmon fruits during storage and suggests that it can extend the shelf-life and marketability of this product.

Building on these findings, the third study evaluates how drying conditions influence the structural and functional characteristics of sodium alginate films enriched with kiwifruit seed essential oil (KSO). This research demonstrates that drying temperature plays a vital role in determining film morphology and performance, with moderate temperatures promoting uniform oil dispersion and film integrity while higher drying temperature causes phase separation and structural discontinuity. The study provides valuable insights for optimizing manufacturing protocols to ensure consistent quality in biopolymer-based packaging, reinforcing the potential of sustainable food preservation technologies.

Collectively, these studies demonstrate a comprehensive approach to edible coating development, integrating innovation formulation, process optimization, and targeted application. The research underscores the advantages of combining natural biopolymers such as starch, sodium alginate, and cellulose nanofibers with bioactive plant-based oils to create coatings that are not only safe and eco-friendly but also effective in prolonging shelf life of fruits. The novel partial coating technique introduced in this work offers a promising alternative to conventional methods.

Name : TRAN THI VAN

（チャン　ティ　ヴァン）

Title : Impact of Nano-Biomaterials and Essential Oils on Carboxymethyl Cellulose-Based Edible Films and Emulsions
（ナノバイオ材料および精油がカルボキシメチルセルロース基材の可食フィルムおよびエマルションに与える影響)

Thesis Summary

Nanomaterials were used to improve the characteristics of edible coating based on carboxymethylcellulose (CMC) as the link between the based materials and other ingredients to form stronger bond structure. This helps to retain the structure in the hardship environment or conditions, making them suitable for prolonging the shelf life and quality of objectives such as fruits or foods. These coating helps to cover the surface of objectives and protect them of respiration process. The purposes of this study were to prepare films/coatings for fresh fruit storage using nanomaterials as a sub-material combined with CMC and essential oils. The study was divided into three points as follows:

1) Effect of edible coating incorporating cellulose nanofibers and self-produced Mandarin Oil based on sodium carboxymethyl cellulose on strawberries

2) Effect of coatings incorporating cellulose nanofibers and 1-methylcyclopropane or mandarin peel extract on the freshness and metabolic profiles of cold stored strawberry

3) Application of green pomelo peel essential oil-based carboxymethylcellulose coatings reinforced with nano chitosan and nano cellulose fibers during the drying process on dried silkworms

In the first step, edible coatings were prepared using a mixture of 1.0 % (w/v) sodium carboxymethyl cellulose (CMC), 0.5 % (w/v) cellulose nanofibers (CNF), and varying concentrations of mandarin oil (MO) at 0.05, 0.1, 0.2, 0.3, or 0.4 % (w/v). The research investigated the physicochemical characteristics and antifungal activities of the films on *Botrytis cinerea* (*B. cinerea*). CMC/CNF/MO coatings were used to preserve the strawberries (Amaou). The changes in quality and antifungal activity on the strawberries were assessed during storage at 5 °C and 85 ± 5% relative humidity (RH). Results showed the major components of MO include β-pinene, limonene, and methyl palmitate. The addition of MO into coatings indicated a significant effect on the properties of coating solutions as well as the film characteristics as shown in b^* and roughness values increased strongly. The coatings contained MO also represented a marked effect on fruit weight loss and antifungal activity against *B. cinerea* strain both in vitro and in vivo. The addition of MO at a concentration of 0.05% illustrated the significant effect in controlling weight loss and antifungal activity on strawberries.

Further study, the application of coatings is a strategy for maintaining the freshness of

highly perishable fruits. This research aimed to evaluate the quality indices of strawberries (Amaou) coated with new coatings based on the sodium carboxymethyl cellulose (CMC) and cellulose nanofibers (CNF) with incorporated mandarin peel extract (ME) or 1-methylcyclopropene (1-MCP) during storage at 20 days at 5 °C and 85% relative humidity (RH). Dissolving the coating solution containing ME in 1-MCP maintained its color for up to 50 days. Coating enhanced with ME and/or 1-MCP maintained fresh strawberries more effectively than the control, reducing weight loss and maintaining firmness, total soluble solids (TSS), citric acid, colour, and total phenolic content. The CCM2-2 coating solution showed superior effects on the weight loss and relative percentages of strawberry metabolites compared to the other coatings, as confirmed by the different components.

Lastly, the application of edible coatings to dried products such as silkworms (*Bombyx mori*) is an innovative method for preserving nutrients during drying process. These coatings are made from safe, natural ingredients such as carboxymethylcellulose (CMC), nano cellulose fiber (NCF), nano chitosan (N-Ch), and varying concentrations of green pomelo peel essential oil (GPO). The results revealed that the addition of GPO into coating/film significantly changed the pH, viscosity, and TSS values of the coating solutions while reducing film weight loss, particularly in the CCG3 film (61.93%). CCG3 also showed the strongest antibacterial effect against "*Staphylococcus aureus*" and maintained lower silkworm moisture content compared to other samples. Metabolic analysis revealed that certain compounds, such as 2-Hydroxyglutaric acid-3TMS and malic acid-3TMS, were more robust in CCG3. X-ray CT image analysis using image processing technology showed high accuracy, with the porosity area in the CCG3 sample being greater. These findings highlighted the potential application of edible coating in drying process to protect the nutrients of dried products.

To summarize, using different nano material combinations based on CMC can greatly extend the shelf-life of fruits and keep quality of dried silkworms, this combination is expected to become a new direction in food preservation technology.

Name : モハメド サミール モハメド アボ エルフェト エル テルバニー
(MOHAMED SAMIR MOHAMED ABO ELFETOH EL TELBANY)

Title : Study on isolation, characterization of bacteriophages against multidrug-resistant enterococci, and their synergy with bacteriocins and endolysins
（多剤耐性腸球菌に対するバクテリオファージの単離、特性評価、ならびにバクテリオシンおよびエンドリシンとの相乗作用に関する研究）

Thesis Summary

Enterococci are gram-positive, cocci-shaped, facultative anaerobic bacteria. Enterococci are commonly found in various foods, including vegetables, fermented sausages, and cheeses. Furthermore, they are considered undesirable organisms in processed meats, as they can lead to food spoilage. They are characterized by their ability to survive in harsh conditions like high salt concentrations (6.5%) and a wide range of temperatures and pH levels. *E. faecalis* and *E. faecium* are the main enterococcal species responsible for various life-threatening infections, including urinary tract infections, bacteremia, nosocomial infections, and endocarditis. They may also be associated with foodborne illness due to their enteric habitat and capacity to enter the food chain, as well as their resistance to a broad spectrum of antibiotics, virulence genes, and ability to form biofilms on both biotic and abiotic surfaces.

Firstly, *E. faecalis* used was isolated from various Egyptian and Japanese food sources, including raw milk and vegetables. These isolates were characterized and showed resistance to a wide range of antibiotics like kanamycin, gentamicin, rifampicin, and ciprofloxacin. Furthermore, all isolates showed the ability to form biofilm and were classified as strong, moderate, and weak biofilm producers. Five bacteriophages (phages) were isolated from different food samples, including cow faeces, compost, and raw milk. A phage, named vB_EfKS5, was selected and further characterized based on its wide host range, high reproducibility, and productivity within different enterococcal hosts. This phage lysed 70.85% of *E. faecalis* tested isolates and 42.85% of *E. faecium*. Phage vB_EfKS5 showed high reproducibility with an efficiency of plating of 0.5 or higher, which showed the potential of this phage in controlling different hosts of *E. faecalis*. The genome of phage vB_EfKS5 is double-stranded DNA, 59,246 bp in length, and has 125 open reading frames. It does not contain any virulence, resistance, or lysogenic genes. Four major groups were identified and had functions related to DNA replication and protein regulation, DNA packaging, structure, lysis of the host, and additional functions. This phage showed stability at different temperatures up to 70°C and pH ranging from 3 to 12. It could reduce the biofilm biomass of *E. faecalis* with different multiplicities of infection. The combination of phage vB_EfKS5 with nisin reduced the total viable counts of *E. faecalis* in broth and milk, revealing synergistic actions.

Secondly, phages vB_EfKS5 and PEF9 are lytic phages with lytic activities against

Enterococcus spp. and encode endolysins designated as LysEf-5 and LysEf-9, respectively. LysEf-5 and LysEf-9 consist of 714 aa and 724 aa, and their molecular masses are 26.4 and 26.5 kDa, respectively. Both belong to the *N*-acetylmuramyl-L-alanine amidase family of endolysins. LysEf-5 and LysEf-9 showed 99% identity. LysEf-9 was selected for further characterization and analysis. The modular structure of LysEf-9 consisted of two basic structures: the binding domains, which are responsible for binding to the host surface receptors, and the enzymatically active domain, which plays the main role in the lysis activity of endolysin. LysEf-9 was able to reduce the turbidity of *E. faecalis* JCM 7783 with different concentrations, and the concentration of 125 μg/ml reduced the OD_{600} from 0.6 to 0.3. The host range experiment showed the wide spectrum of LysEf-9 as it infected 12 out of 15 *E. faecalis* and 3 out of 6 *E. faecium*. Also, it showed specificity to enterococci, as it did not lyse any other tested species.

Thirdly, a bacteriocin-producing *Enterococcus* strain was isolated from raw milk collected from the Kyushu University farm. The culture-free supernatant, crude bacteriocin, was isolated and partially purified using the ammonium sulfate precipitation method. The resistant mutant of phage vB_EfKS5 was isolated and used as a host strain, besides *E. faecalis* JCM 7783 and *E. faecium* FHC 26. The crude bacteriocin showed lytic activity against 17 out of 20 tested enterococci and an isolated phage-resistant mutant. Using LysEf-9 with the crude bacteriocin reduced the viable count of *E. faecalis* JCM 7783 by 2.45 log after 24 h. In addition, LysEf-9 with phage vB_EfKS5 retarded the growth of the phage-resistant mutant. LysEf-9 lowered the viable count of *E. faecalis* JCM 7783, *E. faecium* FHC26, and the phage-resistant mutant in the 48-h biofilm by 3 log, 2 log, and 1.5 log, respectively. The combination of LysEf-9 with crude bacteriocin reduced the total viable count of the indicator strains by at least 3 logs.

Finally, a phage, named vB_EfS_C1, was isolated against *E. faecium.* The genomic analysis of the phage revealed that its length is 86,777 bp and it contains lysis-related genes like endolysin, holin, lysozyme, and endopeptidase. A competent cell from *E. faecalis* JH2-2 was prepared with high transformant efficiency. Two plasmids, pMG3c-pCas-9 and pRNSA-pCas-9, were constructed using Gibson assembly and transformed into *E. coli* BL21. A spacer consisting of 20 bp downstream of the PAM site was introduced into the plasmid and transformed into *E. coli* BL21 and then into *E. faecalis* JH2-2 competent cells. The site-specific mutation in the phage tail spike protein and recombinase protein was tested using a CRISPR plasmid and mutagenesis, such as ultraviolet (UV) and chemical mutagens. These trials aimed to enhance the phage lysis efficiency and expand its host range.

This study successfully isolated and characterized phages, endolysins, and crude bacteriocins against drug-resistant enterococci and phage-resistant mutants and provided a framework for improving phage-based control through genomic characterization and modification.