第２４回農学部賞　受賞者

・DAO TRANG THU
　国際コース　生物化学研究室

第２４回生物資源環境科学府賞
受賞者

・SONG TIANYU　（修士課程）
環境農学専攻　　数理モデリング学分野
「**A Study of Call-based Bird Recognition Based on Deep Learning**」

・YAN XIRUI　（博士後期課程）
環境農学専攻　　農産食料流通工学分野
「**Development of chitosan based bioactive coating enriched with trans cinnamaldehyde essential oil for enhancing postharvest quality of fresh produce**（トランス シンナムアルデヒド精油添加キトサンベースコーティングによる生鮮農産物の品質保持）」

・RUAN YANG　（博士後期課程）
環境農学専攻　　森林圏環境資源科学分野
「**Bioactive Hydrophobic Components from Hericium erinaceus Fruiting Body and Their Mechanism of Anti-inflammatory and Neuroprotective Activities**（ヤマブシタケ子実体由来の疎水性生理活性成分とその抗炎症および神経保護作用のメカニズムの解明）」

・AYE THIDA MAUNG　（博士後期課程）
生命機能科学専攻　　食品衛生化学分野

「**Study on characterization of antimicrobial resistance Listeria monocytogenes and their biocontrol by using specific bacteriophages and natural antimicrobial substances**（ポリフェノールの食中毒病原体およびその毒素に対する作用機構に関する研究）」

A Study of Call-based Bird Recognition Based on Deep Learning

SONG TIANYU

1. Motivation

Bird calls serve as crucial ecological indicators, uniquely identifying various species and contributing to the assessment of ecological diversity and environmental conservation efforts. Analyzing bird calls facilitates the detection of population dynamics and trends within avian communities. This research explores call-based bird classification using the PSA-DenseNet model. Our model integrates DenseNet with an attention mechanism and exhibits exceptional accuracy metrics, surpassing established models like DenseNet, MobileNet V2, ResNet, and EPSANet. Provides new possibilities for bird calls and other sound recognition.

2. Method

The dataset used in this research is a public dataset, which has a total of 14,311 audio clips, each lasting 2 seconds, the dataset includes recordings from 20 distinct bird species. In the bird call feature extraction stage, we use Mel-Frequency Cepstral Coefficients (MFCC) as the feature extraction method.

In terms of the model structure, this experiment uses the DenseNet-121 architecture as the backbone network. Then, based on the backbone, we used a specific attention mechanism called PSAModule. It mainly extracts the spatial information of multi-scale features through grouped convolution and establishes long range channel dependencies through channel weights.

3. Results

Our model achieves impressive accuracy metrics: 95.8% accuracy, 94.7% macro F1 score, and 94.8% recall, surpassing existing models. Moreover, except for Western Water Rail and Teal whose classification accuracy is 85.29% and 89.74% respectively, each category achieves classification accuracy exceeding 90%, highlighting the model's resilience to diverse bird call types. Notably, Gray Heron, Woodcock, and Gray Partridge achieve perfect 100% classification accuracy.

4. Conclusion

In this study, we use the PSA-DenseNet model for bird sound classification. The results underscore the model's ability at assimilating crucial information from extracted audio features to accomplish recognition objectives. This suggests that it may be applicable to a wider range of audio feature learning tasks in future research. However, it is worth noting that this model does face challenges associated with its extensive parameter count and prolonged training duration. Consequently, future research may explore the development of lighter models based on this foundation.

Name : 闫 僖芮（Yan Xirui）ヤン　シルイ

Title : Development of chitosan-based bioactive coating enriched with *trans*-cinnamaldehyde essential oil for enhancing postharvest quality of fresh produce
(トランス-シンナムアルデヒド精油添加キトサンベースコーティングによる生鮮農産物の品質保持)

Category：Kou

Thesis Summary

Edible polysaccharide coatings have been used to improve the shelf life of postharvest products by regulating gas permeability through the formation of strong hydrogen bonds with solvents. Meanwhile, the preparation process of coating/film packaging depends on the properties of the ingredients, the preparation method and the final commercial value. The aim of this study was to prepare bioactive films/coatings for fresh fruit storage using chitosan (CS) as a substrate in different combinations (blending, cross-linked). The study was divided into three points as follows:

1) Diepoxy- (ethylene glycol) (PEG) was prepared by cross-linked method and then combined with chitosan (CS)/*trans*-cinnamaldehyde (CIN) to form a polymer composite coating to prolong the shelf-life of postharvest bananas.

2) Bioactive films of chitosan (CS)/ polyvinyl alcohol (PVA)/*trans*-cinnamaldehyde (CIN) were prepared by co-blending and the effects of different concentrations (0.5%, 1.0% and 1.5%) of CIN on the physicochemical properties of the ternary films were investigated.

3) To improve the storage life and regulate reactive oxygen species (ROS) metabolism of these tomatoes by utilizing chitosan (CS), polyvinyl alcohol (PVA), and *trans*-cinnamaldehyde (CIN) water barrier coatings and the potential mechanisms were investigated.

In the first work, development of bioactive coatings containing 1% (w/v) chitosan (CS), 0.6% (w/v) diepoxy-polyethylene glycol (PEG), and *trans*-cinnamaldehyde (CIN) was achieved. The tensile strength, light transmission, water vapor permeability (WVP), and antibacterial properties were enhanced by the incorporation of CIN. The CIN-containing films appeared compact and rough, as observed using scanning electron microscopy (SEM) and atomic force microscopy (AFM). In addition, the quality attributes of the bananas were evaluated at room temperature for 24 days, and the results showed that the CS/PEG/CIN coating delayed the respiration peak, weight loss, sugar content loss, and maintained firmness, color, total soluble solids (TSS), titratable acid (TA), and the appearance of the bananas. Principal component analysis (PCA) revealed that the bioactive coating significantly affected the respiration rate and weight loss of bananas.

Further work, bioactive films of chitosan (CS) /polyvinyl alcohol (PVA) /*trans*-cinnamaldehyde (CIN) were prepared by co-blending, and the impact of varying concentrations (0.5, 1.0 and 1.5 %)

of CIN on the physicochemical properties of the ternary films was investigated. The ATR/FT-IR analysis revealed that the bioactive film is modulated by Schiff base (C=N) and hydrogen-bond interactions of CS, PVA, and CIN. Inclusion of CIN into the film improved mechanical properties with tensile strength increased from 0.5 % (68.52 MPa) to 1.5 % (76.95 MPa). The presence of CIN within the CS/PVA film also remarkably affected oxygen permeability and improved light transmittance. Additionally, the water barrier and contact angle properties were improved with increasing CIN content. The morphology of the CIN-containing films appeared non-stratified and dense when observed by SEM and AFM. Moreover, spore germination and in vitro assays confirmed strong antifungal activity of the CIN-containing film against *P. italicum* (~90 %) and *B. cinerea* (~85 %). The ternary films also exhibited excellent antioxidant activity, as evidenced by DPPH radical scavenging activity (31.43%) and ferric reducing power ($OD_{700\ nm}$ = 0.172) at the highest CIN concentration tested.

Lastly, aimed to extend the shelf life of tomatoes while controlling reactive oxygen species (ROS) metabolism through the application of water barrier coatings containing chitosan (CS), polyvinyl alcohol (PVA), and *trans*-cinnamaldehyde (CIN). The results showed that the addition of CIN increased the structural density, water barrier properties, and *B. cinerea* resistance of the coatings. The CIN-containing coating significantly improved water loss, firmness, titratable acid (TA) value, color, and appearance; decreased lipid peroxidation; and modulated the ROS scavenging system. Principal component analysis and correlation revealed that the CIN-coated material, characterized by water-barrier properties, contributed positively to the preservation of tomatoes during refrigeration. Water barrier coating facilitates the enzymatic ROS scavenging system in tomatoes, thereby extending their senescence.

To summarize, different treatment combinations based on CS can greatly extend the shelf-life of fruits and vegetables and delay post-harvest senescence, which is expected to become a new preservation technology.

Name ：阮　暘 (RUAN YANG) ルウアン　ヤン

Title ：Bioactive Hydrophobic Components from *Hericium erinaceus* Fruiting Body and Their Mechanism of Anti-inflammatory and Neuroprotective Activities (ヤマブシタケ子実体由来の疎水性生理活性成分とその抗炎症および神経保護作用のメカニズムの解明)

Category：Kou

Thesis Summary

This study focuses on the medicinal and edible mushroom - *Hericium erinaceus*, a well-known species in China and Japan widely utilized in the food industry. Many hydrophobic components in natural products have excellent biological activity (such as flavonoids) and can easily cross biofilms to play a role (such as the blood-brain barrier). However, current bioactivity research on *H. erinaceus* primarily concentrates on hydrophilic components, with limited studies on hydrophobic and unique components such as hericenones. Therefore, this research aims to isolate hydrophobic components, establish a different level of research material perspectives (extract and compound), investigate their traditional activities (anti-inflammatory and neuroprotective), and uncover their potential mechanisms through data mining and pharmacological research. This research is divided into four parts (Figure 1).

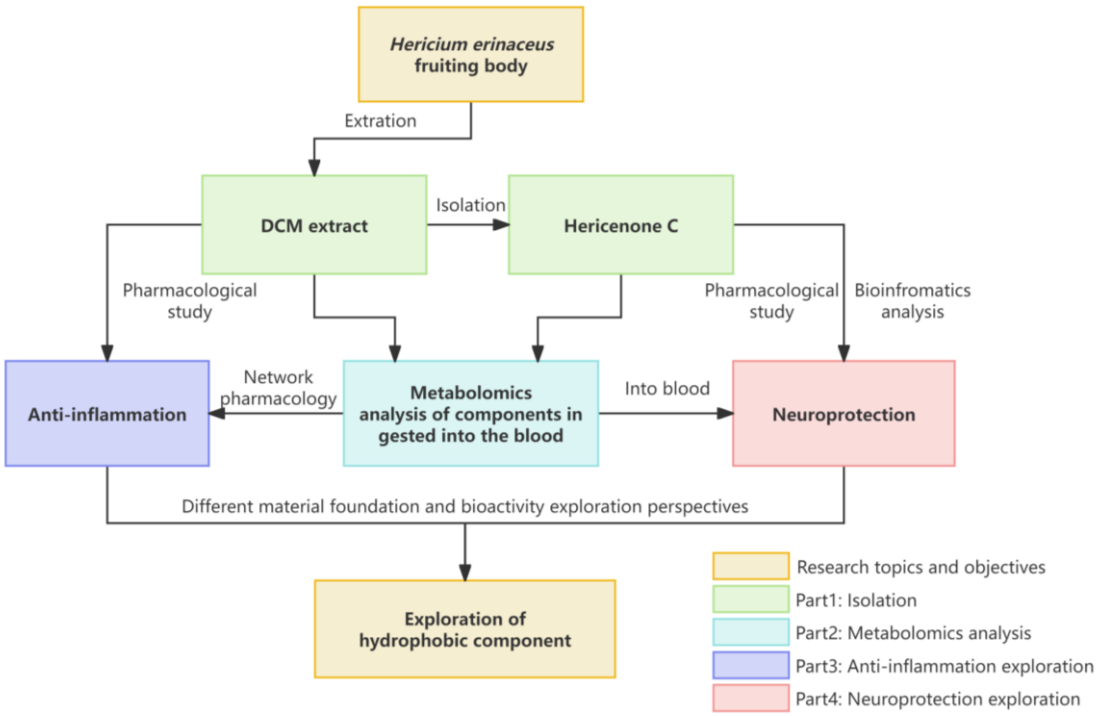

Figure 1. Research flow.

The first part focused on isolation. Dichloromethane (DCM) was used to extract *H. erinaceus* to obtain *H. erinaceus* DCM extract (HEDE), and the HEDE was separated and purified by column chromatography to obtain four novel natural compounds, hericenone O, hericenone P, hericenone Q, and hericenone R, along with eleven known compounds. Due to the most abundance of hericenone C in the hericenone derivative,

which significantly exceeded that of other related derivatives, the further research pursued with hericenone C. This step laid the material foundation for subsequent activity experiments.

The second part studied the metabolomics of the components ingested into the blood in mice. Plasma was collected from mice by continuous gavage of HEDE and hericenone C for 7 days, and subjected to metabolomics analysis by LC/MS. By comparing ion fragments from secondary mass spectrometry to databases and standard samples, result showed some compounds in HEDE with a high abundance which can enter the bloodstream, and the metabolomic analysis also indicated that hericenone C can enter the bloodstream in its original form. These findings provide a basis for exploring the active ingredients of HEDE in vivo and for the oral use of hericenone C for therapeutic purposes.

The third part evaluated the anti-inflammatory activity of the HEDE using lipopolysaccharide (LPS)-induced mice model of Acute lung injury (ALI). ALI results from both local and systemic inflammatory responses and is also one of the serious complications of COVID-19's high morbidity and mortality, over the past few years, COVID-19 has caused widespread impact around the world. Pretreatment with HEDE demonstrated potent anti-inflammatory activity to LPS-induced ALI in this study. The results of metabolomics analysis in part 2 combined with network pharmacology showed that HEDE can potentially ameliorate the inflammation of ALI through the TLR4/NFKB1/STAT3/HIF1a pathway. This provides the basis for the potential functional food development of HEDE.

Finally, by establishing an $A\beta_{25-35}$-induced Alzheimer's disease (AD) cell model, it indicated that hericenone C can improve $A\beta_{25-35}$-induced neuronal cell death. Aging is the top risk related to AD and telomeres are closely related to aging. Past research has shown that hericenone C has the activity of promoting nerve growth factor synthesis in vitro and a mixture rich in hericenone C can improve cognition in aging animals. Therefore, the bioinformatics study of telomere aging was carried out based on the transcriptomics of human AD patients, and it was found through molecular biological methods that hericenone C can play a neuroprotective role through the telomere aging-related targets and pathway (NGF/TrkA/ERK/CREB) on $A\beta_{25-35}$-induced AD cell model. This part revealed the potential of hericenone C as a pharmaceutical candidate for AD.

This study combined column chromatography, metabolomics, network pharmacology, and bioinformatics. This study established an evaluation system based on mouse and human neural cells. It verified the anti-inflammatory activity of hydrophobic extracts and the neuroprotective activity of unique compounds from *H. erinaceus*, elucidating their possible mechanisms of action from a molecular biology perspective. It offers new perspectives and scientific evidence for the development of *H. erinaceus* as a potential pharmaceutical or functional food. In addition, the natural product development approach adopted in this study can effectively explore potential targets for natural product therapy through metabolomic analysis of components ingested into the blood after oral administration of natural products and bioinformatics analysis of publicly available transcriptomic data.

Name ：Aye Thida Maung

Title ： Study on characterization of antimicrobial resistance *Listeria monocytogenes* and their biocontrol by using specific bacteriophages and natural antimicrobial substances
（ポリフェノールの食中毒病原体およびその毒素に対する作用機構に関する研究）

Thesis Summary

Listeria monocytogenes is a serious foodborne pathogen and widely distributed in environments. This bacterium causes listeriosis, most of the cases are associated with non-heat processed or undercooked foods and linked to serotypes 1/2a, 1/2b, and 4b. The presence of its virulence factors and biofilm formation constitutes a potential public health risk. Due to the extensive use of antibiotics, the emergence of antimicrobial-resistant pathogens including *L. monocytogenes* has become a global public health problem in the treatment of infectious diseases in animals and humans. Bacteriophages and natural antibacterial compounds, such as plant-based essential oils, are alternative tools for controlling harmful bacteria in the food sector. Therefore, this study was conducted to characterize the antimicrobial-resistant *L. monocytogenes* and investigate their biocontrol using specific bacteriophage and essential oils in a single or combination.

Firstly, *L. monocytogenes* was isolated using 85 raw chicken and viscera samples collected from supermarkets in Fukuoka in 2022. Nine samples (10.6%) were positive, and 17 strains were isolated. Characterization in serotyping indicated 41.2%, 29.4%, 23.5%, and 5.9% were serotypes 1/2b, 3a, 3b, and 1/2a. All the isolates were identified and clustered by Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry. Antimicrobial susceptibility testing demonstrated the isolates were susceptible to various antibiotics except cefoxitin, oxacillin, and fosfomycin. In the comparison of 5-year differences surveillance, the prevalence of *L. monocytogenes* in 2022 was significantly ($p < 0.05$) lower than those in 2017 (24%) and 2012 (52.9%), and pathogenic serotype distributions decreased over time. Antimicrobial resistance (AMR) and multi-drug resistance rates of isolates in 2022 were reduced compared to those in 2017 and 2012. The virulence genes (*hlyA, plcA, clpC, and inlA*) were detected in most of the isolates in different years. The biofilm formation of the isolates in 2022 was significantly ($p < 0.05$) weaker than those in previous isolates. Despite the low levels of contamination in chicken meats and AMR of the isolates, virulent *L. monocytogenes* contamination in food should still be acknowledged.

Secondly, 11 *L. monocytogenes* phages from raw chicken samples and 15 phages from Kyushu University's cattle farm samples were isolated. According to host range determination, most phages exhibited lytic activities against *L. monocytogenes*, *L. innocua*, *L. seeligeri*, *L. welshimeri*, and *L. grayi*. Characterization of three selected phages was performed. All the phages were stable at pH ranging from 4 to 10, and temperatures ranging from 4-50 °C excluding phage vB_LmoS-PLM34 survived at 4-40 °C. The transmission electron microscopy of the broadest host range phage vB_LmoS-PLM34 showed an icosahedral head (diameter~60 nm) and noncontractile tail

(length~230 nm) that belongs to the *Siphoviridae* family. The whole genomic sequencing analysis demonstrated all phages were the absence of tRNA, virulence, or antimicrobial resistance genes and the presence of endolysin genes. Phages vB_LmoS-PLM9 and vB_LmoS-PLM34 composed N-acetylmuramoyl-l-alanine amidases, whereas phage vB_LmoS-PLM26 included peptidoglycan l-alanyl-d-glutamate endopeptidase endolysins. However, only phage vB_LmoS-PLM9 was recognized as a lytic phage since it did not contain lysogenic-related genes. One-step growth curve analysis of lytic phage vB_LmoS-PLM9 indicated a latent period of 70 min and a burst size of 51.2 ± 2.1 PFU/cell. This study suggested bacteriophages against *L. monocytogenes* were successfully isolated and characterized.

Thirdly, 8 kinds of essential oils including clove, cinnamon bark, cinnamon cassia, ginger, turmeric, basil, lemon, and lemongrass were evaluated for their antibacterial efficiency. Cinnamon bark and cinnamon cassia showed the strongest anti-listerial activities, followed by clove and lemongrass oils when tested with 10% essential oils. The effective oils against *L. monocytogenes* and various *Listeria* spp. were observed. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of both cinnamon bark and cassia oils indicated 0.0625 and 0.125%, and clove oil showed 0.25 and 0.5%. The application of essential oils and/or phage vB_LmoS-PLM9 in individual and combination was investigated in broth and milk. The combined treatments were more effective than single treatments. The combined treatments of phage (MOI of 10) and both cinnamon oils (0.03%), and phage (MOI of 1) and clove oil (0.125%) reduced the viable counts of *L. monocytogenes* and inhibited the regrowth of resistant cell populations in broth at 30 °C. Furthermore, treatment with the combination of phage (MOI of 100) and cinnamon oil (0.125%) was effective in milk, especially at 4 °C by reducing the viable count to less than the lower detection limit. The combination of phage (MOI of 100) and clove oil (0.5%) decreased the surviving cells and continuously inhibited the regrowth of cells in milk at 4 °C. These results suggest combining phage and essential oil is a potential approach for controlling *L. monocytogenes* in milk.

Finally, biocontrol of pathogenic *L. monocytogenes* in various solid foods and its biofilm using phage vB_LmoS-PLM9 and cinnamon/ clove oils were examined. Phage and cinnamon/ clove oils showed higher effectiveness than single treatments particularly at 4 °C. Phage (5×10^8 PFU/mL) and cinnamon bark (1%) on ham, phage (5×10^8 PFU/mL) and cinnamon bark (0.5%) on chicken meat, cheese, mixed salad, and coleslaw reduced the viable counts compared with the single treatment, but not in smoked salmon. The combined treatment of phage (5×10^8 PFU/mL) and cinnamon bark (1%) decreased the viable cells less than the detection level in chicken meat after 4 d storage at 4 °C. In combined phage (10^9 PFU/mL) and clove oil (1%) on ham, mixed salad, and coleslaw, surviving cells were inhibited or reduced at a low-temperature refrigeration at 4 °C. Moreover, phage (5×10^9 PFU/mL) and 0.125% of cinnamon or clove oils decreased the viability of *L. monocytogenes* on the removal of 24-h preformed biofilm at 30 °C. These results suggested that combined phage and cinnamon or clove oil might be a promising candidate for controlling pathogenic *L. monocytogenes* in the food industry.

Overall, these studies successfully isolated and characterized the antimicrobial-resistant *L. monocytogenes* and investigated their biocontrol using isolated bacteriophage vB_LmoS-PLM9 and cinnamon or clove oils.