跨領域永續研究整合型計畫:

臺灣國立大學系統年輕學者創新性合作計畫執行報告格式

申請單位	國立彰化師範大學
總計畫主持人	國立彰化師範大學周睿鈺
總計畫名稱 (中文)	轉危為機：透過微生物達成永續性的 PLA 和 PHB 生產。
總計畫名稱 (英文)	Transforming Harmful into Favorable: Achieving Sustainable PLA and PHB Production by Microbes
子計畫主持人	國立彰化師範大學周睿鈺(Jui-Yu Chou)教授、國立中興大學陳彥伯(Yen-Po Chen)助理教授、國立嘉義大學何尚哲(Shang-Tse Ho)助理教授。
中文摘要	本計畫探討與聯合國可持續發展目標(SDGs)相符的永續解決方案，特別是 SDG 12(永續消費與生產)，並透過循環經濟原則將農業廢料和有害氣體轉化為有價值的資源。我們的研究結果在三個關鍵領域獲得了顯著進展：**(1):可持續生物塑料生產(子計畫1)**—我們開發了一種創新的生物塑料生產方法，利用來自低成本農業來源的嗜澱粉芽孢桿菌發酵乳酸生產乳酸內酯(LAC)。使用 FT-IR、NMR、TGA 和 XRD 對 LAC 進行結構分析，證實了成功的二聚化過程，並且微生物 LAC 在熱穩定性和結晶度(32%-42%)方面優於商業 LAC。此外，我們從稻田和甘蔗田中分離了產聚羥基丁酸(PHB)的細菌，其中 *Pseudomonas xanthomarina* 產量最高(7.5 g/L)。PHB-澱粉薄膜在30天內顯示出25%的生物降解，顯示其作為環保材料的潛力。**(2): 大豆優格替代物(yogurt substitute)開發(子計畫2)**—我們分離了具有產 β-葡萄糖苷酶、α-半乳糖苷酶和 γ-氨基丁酸(GABA)能力的乳酸菌，來提高大豆豆漿優格替代物的營養價值。發酵過程將大豆異黃酮苷元轉化為可被人體吸收的苷元，並產生 GABA，從而改善消化性和營養價值。乳酸菌的 α-半乳糖苷酶活性還幫助水解棉子糖，減少大豆產品通常會引起的脹氣。最終發酵的豆漿優格替代物在 pH、酸度、乳清排出度和黏度等理化性質上有所改善，為傳統牛奶優格提供了一個可行且可持續的替代品。**(3): 代謝組學分析(子計畫3)**—我們成功地將代謝組學分析應用於研究由海藻糖木聚糖(GXG)和海藻酸鈉(SA)生物高分子製成的尿素包埋水凝膠對 *Brassica juncea* (一種重要的油菜和蔬菜作物)所引起的代謝變化。LC-MS 分析揭示了尿素對 *B. juncea* 的代謝反應，並指出了使用水凝膠控制釋放尿素與直接施用尿素之間的代謝反應差異。這項發現強調了代謝組學在農業和生物技術應用中的潛力，並證實了其作為分析工具的可靠性。這些研究結果與全球可持續發展努力目標一致，通過提供創新的解決方案來促進生物塑料的發展、可持續食品生產和有效的永續農業實踐。通過減少對石化產品的依賴並提升植物食品的營養品質，本計畫支持實現可持續和淨零碳排放的目標。
英文摘要	This project investigates sustainable solutions aligned with the United Nations Sustainable Development Goals (SDGs), particularly SDG 12 on sustainable consumption and production, by leveraging circular economy principles to transform agricultural waste and harmful

	gases into valuable resources. Our findings highlight significant progress in three key areas: **(1): Sustainable Bioplastics Production (Subproject 1)—**We developed an innovative approach to bioplastic production by utilizing Bacillus amyloliquefaciens-fermented lactic acid derived from low-cost agricultural sources to produce lactide (LAC). Characterization using FT-IR, NMR, TGA, and XRD confirmed successful dimerization, with microbial LAC showing higher thermal stability and crystallinity (32%-42%) compared to commercial LAC. Additionally, polyhydroxybutyrate (PHB) was produced by isolating bacteria from paddy and sugarcane fields, with *Pseudomonas xanthomarina* yielding the highest PHB production (7.5 g/L). PHB-starch thin films demonstrated 25% biodegradation in 30 days, showcasing their potential as eco-friendly materials. **(2): Soy-Based Yogurt Substitute Development (Subproject 2)—**We isolated lactic acid bacteria (LAB) capable of producing β-glucosidase, α-galactosidase, and γ-aminobutyric acid (GABA) to enhance the nutritional value of soy-based yogurt substitute. The fermentation process converted isoflavone glycosides into bioavailable aglycones and produced GABA, improving digestibility and nutritional benefits. LAB activity also hydrolyzed raffinose, reducing flatulence associated with soy products. The resulting soy yogurt substitute exhibited improved physicochemical properties, offering a viable, sustainable alternative to traditional cow's milk yogurt. **(3): Metabolomic Analysis of Urea Release (Subproject 3)—**We successfully applied metabolomic analysis to explore the metabolic changes in *Brassica juncea* (a vital oilseed and vegetable crop) induced by controlled urea release through urea-encapsulated hydrogels made from galactoxyloglucan (GXG) and sodium alginate (SA). LC-MS analysis revealed metabolic alterations in *B. juncea*, highlighting differences in responses to controlled urea release via hydrogels versus direct urea application. This work underscores the potential of metabolomics to provide insights into complex metabolic processes and confirms its reliability as an analytical tool in agricultural and biotechnological applications. These findings align with global efforts towards sustainable development by providing innovative solutions for bioplastics, sustainable food production, and effective agricultural practices. By reducing reliance on petroleum-based products and enhancing the nutritional quality of plant-based foods, this project supports the goals of achieving a sustainable, net-zero carbon future.
執行方法及步驟	**Subproject 1: Microbial strain selection for LA and PHB production in Taiwan, and synthesis of PLA and PHB polymers:** This research project focuses on the isolation, characterization, and application of microorganisms for lactic acid (LA) and polyhydroxybutyrate (PHB) production, as well as their transformation into sustainable biopolymers. Briefly, here is the procedure for our experiment: (1) Isolation and Identification of LA-Producing Microbes: LA-producing lactic acid bacteria (LAB) were primarily isolated from traditional and indigenous fermented foods, including fermented sausages, sourdough bread, soy sauce, rice wine, and traps using milk with glucose as a carbon source. Fructophilic LAB (FLAB) were isolated from fructose-rich niches such as flowers, fruits, and bee gastrointestinal tracts using milk with fructose as a carbon source. *Rhizopus* fungi were isolated from sources including traditional Indonesian foods fermented with *Rhizopus oligosporus* and jiuqu, a winemaking starter culture. Microbial LA production was estimated using spectrophotometric methods. (2) Effect of Sugarcane Molasses on LA Production: Sugarcane molasses was investigated as a low-cost carbon source for maximizing LA production. Pilot-scale tests focused on optimizing the conversion of complex sugars to glucose using yeast-derived invertase. Chromatographic techniques were employed to analyze sugar conversion ratios. (3) Characterization of LA:

LA production was optimized using response surface methodology (RSM), with the product characterized by pH measurement, spectrophotometry, UV-visible absorption, Fourier transform infrared spectroscopy (FTIR), and high-performance liquid chromatography (HPLC). (4) Synthesis and Characterization of PLA: Produced LA was polymerized into polylactic acid (PLA) via ring-opening polymerization (Fig. 1). PLA characterization employed FTIR, NMR, HPLC, GC-MS, SEM, and AFM. Biodegradable PLA-blended packaging materials were further evaluated using GPC, TG/DSC, and biodegradability tests. (5) Biodegradability Testing: PLA-blended films were tested under laboratory conditions to evaluate soil biodegradation, with CO2 evolution monitored to quantify degradation levels. (6) Isolation of PHB-Producing Microorganisms: Samples from freshwater and rhizosphere soils in Taiwan were screened for PHB-producing strains (Fig. 2). Positive strains were identified via 16S rRNA sequencing, with PHB production optimized in modified nitrogen-limited salt media (NLSM). PHB characterization was conducted using UV-vis spectrophotometry, FTIR, NMR, TGA/DSC, XRD, and SEM.

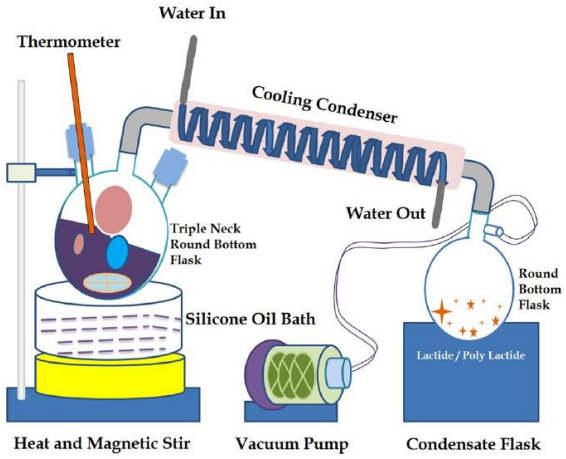


Fig. 1. Schematic representation of dimerization of LA to LAC.

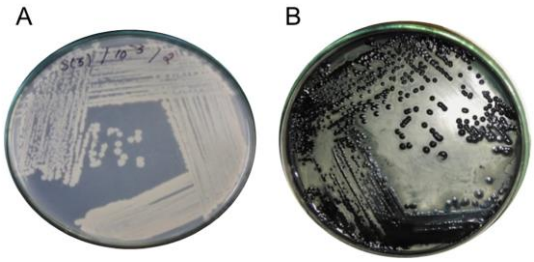


Fig. 2. Sudan black screening of bacterial isolates – a) control (Non PHB-producing bacteria) b) PHB-producing bacterial isolates.

Subproject 2: Evaluating bacterial fermentation byproducts for potential probiotic and postbiotic benefits through cell and animal models:

This subproject proposal aims to harness byproducts and microbes for the production of bioactive compounds with notable health benefits, including immune modulation, anti-allergenic, anti-inflammatory, anti-colitis, anti-aging, skincare, renal protection, and detoxification properties. This initiative aligns with the growing demand for sustainable and cost-effective approaches to producing bioactive components with diverse health-promoting attributes. Briefly, here is the procedure for our experiment: (1) Collection of Potential Fermentation Byproducts or Bioactive Compounds from Other Subprojects: Fermentation byproducts, including microbes and cell-free fermentation supernatants (CFSs), will be collected from Subproject-1 following fermentation processes. Probiotics will be prepared as live microbes, while postbiotics will be developed through heat inactivation and sonication of microbial cells, as well as CFSs. Additionally, identified potential compounds will be supplied from Subproject-3. All samples will be prepared at indicated concentrations before use in in-vitro and cell model evaluation platforms. (2) Evaluation of Probiotic or

Postbiotic Benefits by *in-vitro* Methods: Selected microbes from Subproject-1 will undergo comprehensive *in-vitro* probiotic characterization, including safety evaluation, antibiotic sensitivity testing, acid and bile salt tolerance assessment, surface hydrophobicity analysis, and intestinal epithelial binding ability. Further evaluations will assess antioxidant activity, angiotensin-converting enzyme inhibition, and detoxification of aflatoxin and uremic toxin precursors by candidate microbes or fermentation byproducts. (3) Evaluation of Probiotic or Postbiotic Benefits by Cell Models (Fig. 3, 4): Cell-based models will be employed to evaluate the health-promoting potential of candidate probiotics and postbiotics. The models include: (a) Intestinal epithelial and enteroendocrine cells to assess cytokine production, intestinal barrier function, and recovery following chemical, toxin, and oxidative stress damage; (b) Immune cells such as macrophages and dendritic cells for immunomodulation analysis via cytokine profiling and cell activation; (c) Neural cells to evaluate neuroprotection and anti-neuroinflammatory effects; (d) Reproductive and renal cells for assessing damage caused by oxidative stress, aflatoxin, and other toxins; and (e) Keratinocytes to evaluate barrier function and recovery after exposure to chemical, toxin, and oxidative stress. (4) Evaluation of Probiotic or Postbiotic Benefits by Animal Models: Based on *in-vitro* and cell evaluation results, appropriate animal models will be selected to assess health-promoting effects in vivo. These include: (a) Immune and inflammation models such as chemical-induced colitis, allergic, and dermatitis mouse models to study barrier function, inflammation, histopathology, intestinal microbiome, and underlying mechanisms; (b) Aging models involving oxidation-induced or specific mouse strains to evaluate brain and reproductive system damage; (c) Obesity models induced by a high-fat diet to assess obesity characteristics, insulin sensitivity, and glucose regulation; and (d) Chronic kidney disease models induced by a high-adenine diet to evaluate biochemical parameters, uremic toxin accumulation, histopathology, intestinal microbiome, and related mechanisms.

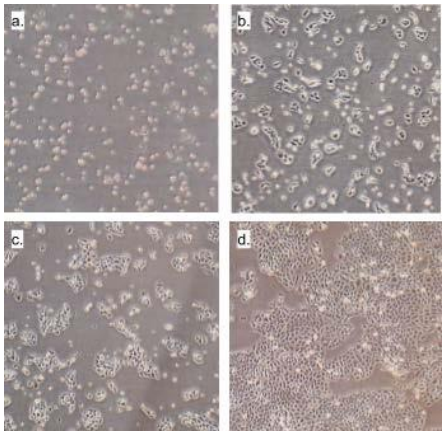


Fig. 3. Overview of HaCaT cell culture morphology and confluency during 6-day period of incubation. (a) Initial phase of growth; (b) Cell lines after 1 day of incubation with 10% confluency, (c) Cell lines after 3 days of incubation with 25% confluency, and (d) Cell lines after 6 days of incubation with 70% confluency.

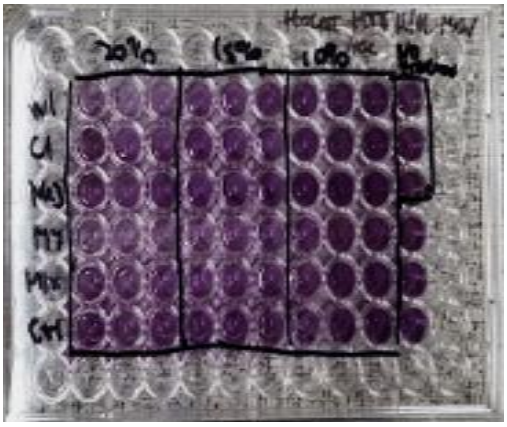
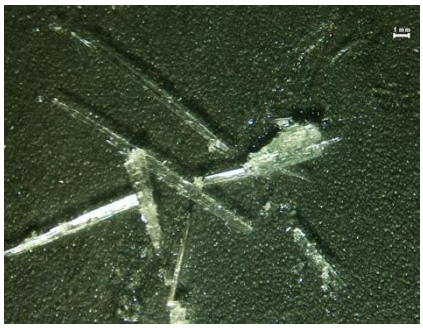
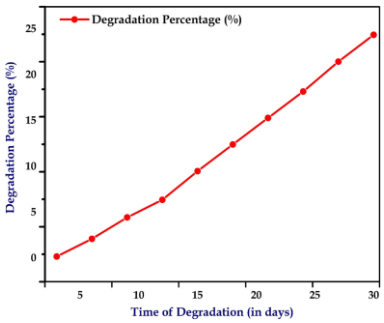


Fig. 4. Appearance of the MTT assay indicating HaCaT cell viability. The MTT assay measures cell viability by assessing the reduction of MTT to formazan crystals in metabolically active HaCaT cells. The intensity of the purple color reflects cell viability, with a stronger color indicating higher cell activity and number.

Subproject 3: Metabolomic analysis of bacterial pathways synthesizing lactic acid and polyhydroxybutyrate:

This project utilizes metabolomics analysis to investigate the small-molecule metabolites produced during the fermentation processes in autotrophic and chemoheterotrophic systems. Mass spectrometry (MS) enables both qualitative and quantitative assessments of intracellular and diverse samples, offering dynamic data on a wide range of metabolites. Metabolomics, being more closely associated with biological phenotypes compared to genomics, transcriptomics, and proteomics, facilitates the identification of small-molecule metabolites that reflect the biological phenotype and can serve as potential biomarkers. While there has been progress in Lactic Acid (LA) and Polyhydroxybutyrate (PHB) production, current yields still fall short of market demands. Therefore, a deeper understanding of the metabolomic pathways involved in LA and PHB production through microbial fermentation is essential to meet these demands. Following the strain screening experiment led by Subject 1 team, selected strains will undergo metabolomic analysis as outlined below. Briefly, here is the procedure for our experiment: (1) Sampling and Quenching of Intracellular Metabolites: For each sample, 20 mL of bacterial broth will be collected in triplicates from the fermentation culture. The sample will be centrifuged at 8500 rpm for 15 minutes at -4°C, and the bacterial cell pellets will be washed twice with 10 mL of 1% saline solution to remove debris. The filtrate will be transferred immediately into a 50 mL centrifuge tube containing 20 mL of pre-chilled 60% methanol at -80°C. Metabolic quenching will occur within 10-20 seconds, followed by centrifugation at 8500 rpm for 5 minutes at -4°C. The resulting pellet will be stored at -80°C until further analysis. (2) Extraction of Intracellular Metabolites: After 48 hours, the cell pellet will be retrieved from -80°C and resuspended in 2.5 mL of ice-cold methanol-water solution (1:1). The suspension will be thawed in an ice bath for 5 minutes, vortexed thoroughly, and frozen again at -80°C for 30 minutes. This freeze-thaw cycle will be repeated three times. Afterward, the sample will be centrifuged at 10,000 rpm for 20 minutes at -20°C. An additional 2.5 mL of ice-cold methanol-water solution will be added to the cell pellets, and the supernatant will be combined with the previous one. The resulting supernatant will be lyophilized at -88°C under a 1.000 Pa vacuum for 24 hours before being subjected to LC-MS and GC-MS analysis. (3) Derivatization of Intracellular Metabolites: The samples will undergo a two-step derivatization process. In the first step, 200 µL of methoxyamine pyridine hydrochloride will be added to the sample and incubated at 37°C for 90 minutes. In the second step, 200 µL of N,O-bistrifluoroacetamide will be added, followed by incubation at 70°C for 30 minutes, with a final incubation at room temperature for 30 minutes. After derivatization, the samples will be transferred to a GC-MS vial for analysis. (4) GC-MS Analysis: Gas chromatography-mass spectrometry (GC-MS) will be performed using the Shimadzu QP 2020 system. Helium will be used as the carrier gas with a flow rate of 1.20 mL/min at a pressure of 68.1 kPa. A split ratio of 10.0 will be used for sample injection (1 µL). The initial column temperature will be set at 50°C for 20 minutes, with a linear increase in temperature to 280°C over 40 minutes and a 2-minute hold at 280°C. Mass spectra will be recorded from 50 to 500 m/z at an acquisition rate of 0.30 seconds per spectrum. Data analysis will be performed by matching the obtained spectra with the NIST14.LIB and WILEY8.LIB standard libraries. (5) LC-MS Analysis: Liquid chromatography-mass spectrometry (LC-MS) will be conducted using an Agilent LC/Q-TOF 6530 system with an electrospray ionization (ESI) source. Methanol will be used as the mobile phase at a constant flow rate of 0.4 mL/min. A 10 µL sample (2 mg/mL) will be injected, and the analysis will be conducted under a capillary

	voltage of 4000 V, a pressure of 50 psi, and a nebulizer flow rate of 10 mL/min. The nebulizer gas temperature will be maintained at 300°C using nitrogen. Functional metabolites will be analyzed based on the KEGG database, and a heat map will be generated using Hemi 2.0 software. (6) Statistical Analysis: Statistical analysis will be carried out using Origin 8.5, with principal component analysis (PCA) performed using OriginPro 2022b software.
成果與績效	**Results of subproject 1:** Our findings highlight sustainable bioplastic production using microbial fermentation. First, we produced lactide (LAC) from *Bacillus amyloliquefaciens*-fermented lactic acid, using low-cost agricultural sources (Fig. 5). Characterization by FT-IR, NMR, TGA, and XRD confirmed successful dimerization, with microbial LAC showing higher thermal stability and crystallinity (32%-42%) compared to commercial LAC. Second, we isolated three bacteria, *Acinetobacter baumannii*, *Enterobacter hormaechei*, and *Pseudomonas xanthomarina*, from paddy and sugarcane fields to produce polyhydroxybutyrate (PHB). *P. xanthomarina* produced the highest PHB (7.5 g/L). PHB-starch thin films showed 25% degradation in 30 days, demonstrating their biodegradability (Fig. 6).  Fig. 5. LAC produced through the dimerization process  Fig. 6. Degradation of PHB-starch blended thin film as analyzed by thermogravimetric analysis (TGA). **Results of subproject 2:** Our findings focus on isolating lactic acid bacteria (LAB) with the ability to produce β-glucosidase, α-galactosidase, and γ-aminobutyric acid (GABA) for the development of soy-based yogurt substitute. Soybeans, rich in fiber, phytochemicals, and isoflavones, were used as a sustainable alternative to cow's milk for yogurt production. The fermentation process with selected LAB strains enhanced the nutritional value of the soy yogurt by converting isoflavone glycosides into bioavailable aglycones via β-glucosidase, while also producing GABA from glutamate precursors in soybeans. Additionally, α-galactosidase activity in LAB helped hydrolyze raffinose, reducing flatulence typically associated with soy products. The soy-based yogurt substitute was fermented for 24 and 48 hours, with the resulting products showing improved physicochemical properties (pH, acidity, syneresis, and viscosity), making it a viable alternative to traditional cow’s milk yogurt with enhanced digestibility and nutritional benefits.

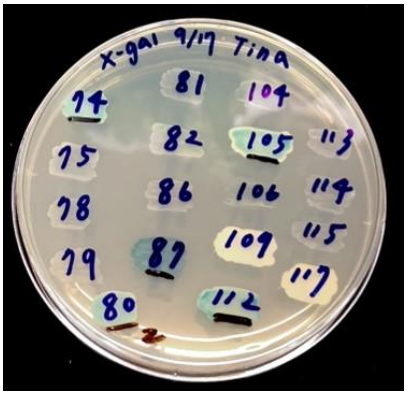


Fig. 7. X-Gal Assay Verification for α-Galactosidase Activity in LAB Strains.

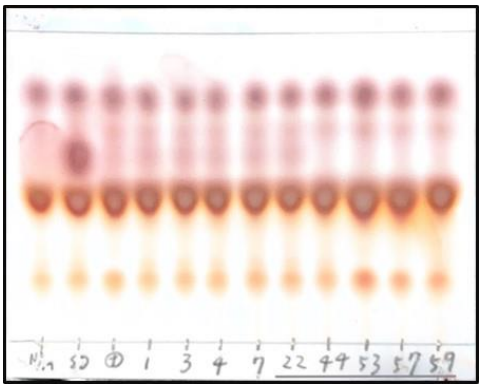


Fig. 8. Thin Layer Chromatography (TLC) screening of GABA-producing LAB strains.

Results of subproject 3: Our findings demonstrate that the metabolomic analysis is an effective tool for understanding complex metabolic processes. We successfully applied this approach to investigate the metabolic changes associated with controlled urea release through urea-encapsulated hydrogels made from galactoxyloglucan (GXG) and sodium alginate (SA) biopolymers. LC-MS analysis revealed the metabolic effects of urea and identified altered intracellular metabolites in *B. juncea*, highlighting significant differences in metabolic responses between controlled urea release via hydrogels and direct urea application. Hierarchical cluster analysis further confirmed the abundance of metabolomic profiles. Additionally, our ongoing metabolomic analysis of bacterial pathways involved in synthesizing lactic acid and polyhydroxybutyrate is progressing well, with the data expected to be completed in the second and third years of our three-year project. These results underscore the reliability and effectiveness of our metabolomic analysis approach.

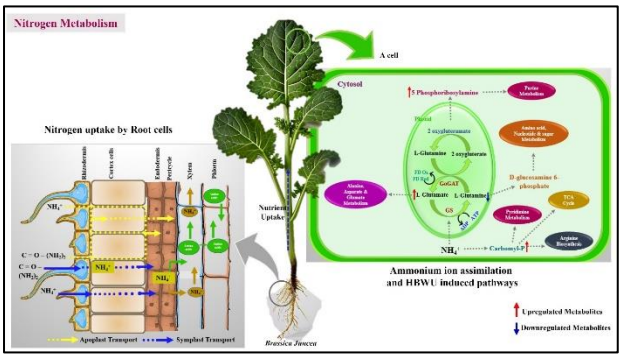


Fig. 9. A schematic representation of the proposed hydrogel beads with urea-induced metabolic pathways and nitrogen metabolism in *Brassica juncea*, a vital oilseed and vegetable crop, based on LC-MS analysis to elucidate the metabolic effects of urea and identify altered intracellular metabolites in *B. juncea*.

Based on our results from the first year and with the support of this project, **we have already published three papers, with one currently under revision**. Below, we briefly describe our key findings, along with the details of the journals where the papers were published:

1. In 2024, the principal investigator, **Dr. Jui-Yu Chou**, in collaboration with sub-project leader Professor **Yen-Po Chen** and Professor Jothi Basu Muthuramalingam from Alagappa University, published a review article exploring advancements in the production of polylactic acid (PLA). The article highlights PLA as a biodegradable plastic with growing market demand, which has driven the development of lactic acid (LA). LA derived from microbial sources via enzymatic polymerization is an environmentally friendly synthesis pathway. However, its production faces challenges such as high culture medium requirements, susceptibility to bacteriophage infections, and low yields due

to by-product generation. Given PLA's potential as a sustainable alternative to petrochemical polymers, the authors recommend that researchers focus on exploring LA-producing microorganisms in diverse environments. They further suggest integrating genetic engineering and metabolic regulation technologies in future studies to optimize microbial functionality and production efficiency, addressing increasing industrial demands and promoting the development of sustainable materials.

Reference: Balasubramanian, V.K., Muthuramalingam, J.B., **Chen, Y.P.**, & **Chou, J.Y.*** (2024). Recent trends in lactic acid-producing microorganisms through microbial fermentation for the synthesis of polylactic acid. **Archives of Microbiology**, 206(1), 31.

2. In 2024, the principal investigator, **Dr. Jui-Yu Chou**, in collaboration with sub-project leader Professor **Shang-Tse Ho** and Professor Jothi Basu Muthuramalingam from Alagappa University, submitted a research paper to show hydrogels, three-dimensional polymeric networks capable of absorbing and retaining significant amounts of aqueous solution, offer a promising platform for the controlled release of desired compounds. In this study, we synthesized urea-encapsulated hydrogels using galactoxyloglucan and sodium alginate biopolymers and evaluated their effects on the phenotypic and metabolic responses of *Brassica juncea*, a vital oilseed and vegetable crop. Experiments were conducted with four treatments: control (without hydrogel beads and urea), direct urea supplementation (U), hydrogel beads with urea (HBWU), and hydrogel beads without urea (HBWOU). HBWU-treated plants exhibited significantly improved growth, highlighted by a chlorophyll content of 11.06 mg/0.1g compared to 3.67 mg/0.1g in the control and 6.41 mg/0.1g in the U-treated group. Metabolic analysis using LC-MS revealed 17 major intracellular metabolites involved in nitrogen metabolism, with the HBWU treatment markedly enhancing nitrogen assimilation through the upregulation of nine metabolites. Hierarchical cluster analysis highlighted distinct metabolic responses between controlled urea release via hydrogels and direct urea application. A proposed schematic diagram further elucidated the HBWU-induced metabolic pathways and nitrogen metabolism in *B. juncea*. These findings demonstrate the potential of hydrogel-based controlled-release systems to enhance plant growth and nitrogen assimilation, paving the way for sustainable agricultural practices. The study has been submitted to the journal Molecular Omics, which focuses on leading research in multi-omics and single-omics technologies. It is currently in the revision stage.

   Reference: Balakrishnan Muthumari, Balasubramanian Vignesh Kumar, Murugan Kavitha, John Kennedy John Praveen Kumar, Subashri Dhanasekaran, Shih-Feng Fu, **Shang-Tse Ho**, Muthuramalingam Jothi Basu * and **Jui-Yu Chou***. (2024) Unraveling the phenotypic and metabolic responses induced by urea-encapsulated hydrogel beads on *Brassica juncea* (L.) Czern & Coss. **Molecular Omics** (Revision)

3. In 2024, the principal investigator, **Dr. Jui-Yu Chou**, in collaboration with Professor Jothi Basu Muthuramalingam from Alagappa University, published a research paper to reported that Lactic acid (LA) is a versatile molecule with a wide range of applications across numerous industries and is produced through both biological and chemical processes. As part of our project, we focused on converting LA into lactide (LAC), the precursor for polylactic acid (PLA), which is considered a sustainable alternative to petroleum-based products. Despite its potential, the high production costs of PLA remain a significant challenge, primarily driven by the costs associated with LA and LAC production. To address this, we developed a cost-effective method by utilizing low-cost agricultural sources for microbial LA production, followed by its conversion into LAC

	through dimerization. The produced LAC was analyzed using FT-IR, NMR, TGA, and XRD techniques. FT-IR confirmed the successful dimerization of LA to LAC, while NMR demonstrated the alignment of methine and methyl groups in the produced LAC. TGA revealed that the microbial LAC exhibited greater thermal stability compared to commercial LAC, and XRD analysis indicated that the produced LACs were crystalline with crystallinity levels of 32% and 42%. To our knowledge, this study is the first to describe the production of LA through microbial fermentation and its subsequent conversion to LAC via dimerization, paving the way for more cost-effective PLA production. **Reference**: Balasubramanian, V.K., Balakrishnan, M., Murugan, K., John Kennedy, J.P.K., **Chou, J.Y.**, & Muthuramalingam, J.B. (2024). Synthesis and characterization of lactide from *Bacillus amyloliquefaciens* brewed lactic acid utilizing cheap agricultural sources. **3 Biotech**, 14(1), 13. 4. In 2024, the principal investigator, **Dr. Jui-Yu Chou**, in collaboration with Professor Jothi Basu Muthuramalingam from Alagappa University, published a research paper to reported that Polyhydroxybutyrate (PHB) is a microbial polyester belonging to the polyhydroxyalkanoate (PHA) family. In this study, three Gram-negative bacteria—*Acinetobacter baumannii*, *Enterobacter hormaechei*, and *Pseudomonas xanthomarina*—were isolated and cultured in nitrogen-limited minimal salt media to produce PHB. The pH and culture conditions were optimized using various concentrations of glucose and peptone as carbon and nitrogen sources, respectively, to enhance PHB production. Characterization of the PHB produced was performed using FTIR, UV–vis, NMR, TGA, and XRD. The optimal pH for PHB production was determined to be 6.5 for *Acinetobacter baumannii* and 7.5 for both *Enterobacter hormaechei* and *Pseudomonas xanthomarina*, with *P. xanthomarina* achieving the highest yield of 7.5 g/L in optimized conditions. Additionally, PHB-starch thin films were prepared, characterized by XRD, and subjected to biodegradability testing. The degradation study revealed that 25% of the PHB-starch blended thin film degraded within 30 days, highlighting its potential for environmentally sustainable applications. **Reference**: Balasubramanian, V.K., Chellapandi, R., Balakrishnan, M., Murugan, K., Kennedy, J.P.K.J., Murugan, S., Khumalo, M.V., Sarangi, P.K., **Chou, J.Y.**, & Muthuramalingam, J.B. (2024). Biosynthesis of bioplastic polyhydroxybutyrate (PHB) from microbes isolated from paddy/sugarcane fields and fabrication of biodegradable thin film. **Process Safety and Environmental Protection**, 187, 1178-1188.
對永續議題之貢獻	These first-year results of the project contribute significantly to sustainability through the development of eco-friendly materials, optimization of production processes, and efficient utilization of agricultural resources: **(1): Advancing biodegradable materials—**The first study examines the progress in polylactic acid (PLA) production, highlighting microbial fermentation for lactic acid (LA) synthesis as an environmentally friendly alternative. By integrating genetic engineering and metabolic regulation, the study aims to enhance microbial efficiency for industrial-scale production. This establishes PLA as a viable and sustainable replacement for petrochemical polymers, reducing reliance on fossil fuels and encouraging resource recycling. **(2): Optimizing nitrogen fertilizer use in agriculture—**The second study introduces galactoxyloglucan-sodium alginate hydrogels for controlled urea release, which significantly enhances plant growth and nitrogen assimilation in mustard (*Brassica*

	juncea). This approach minimizes nitrogen fertilizer waste, mitigates environmental pollution, and lays the groundwork for precision and sustainable agricultural practices. **(3): Reducing biomaterial production costs—**The third study leverages low-cost agricultural by-products, such as molasses, to produce LA via microbial fermentation and subsequently synthesize lactide (LAC). By reducing production costs, this innovation facilitates the broader adoption of PLA in various industries, including packaging, thereby advancing green economic growth. **(4): Developing bioplastics as sustainable alternatives to petrochemical plastics—**The fourth study focuses on producing polyhydroxybutyrate (PHB) from microbes and fabricating biodegradable thin films. This research underscores PHB's potential as a sustainable bioplastic by enhancing its production efficiency and verifying its biodegradability, thus addressing long-term plastic pollution. Collectively, these studies advance eco-friendly material technologies, optimize resource utilization, and promote circular economies, offering innovative solutions and technical advancements to address global sustainability challenges such as plastic waste and agricultural pollution.
結論與建議	This project has significantly fostered academic research collaboration, establishing a sustainable development platform and new models for higher education in Taiwan. It has enhanced the scope and diversity of research, nurtured young scholars, and strengthened cross-university collaboration. These efforts have secured external funding and increased Taiwan's national university system's (臺灣國立大學系統) international visibility. By addressing key issues in bioplastics, microbial fermentation, and agriculture, the project has advanced scientific knowledge and created a more interconnected research environment within Taiwan's academic community. The success of this initiative highlights the importance of continued funding to ensure the completion of the three-year project and to further extend its impact. We strongly recommend ongoing support for this project, enabling us to fully realize its goals and potential. Additionally, it would be beneficial to consider increasing the funding for projects involving international collaboration, as this would enhance global partnerships and increase the diversity of research outputs. Providing support for inviting international scholars to Taiwan, such as funding for their visits or travel expenses, would further foster global exchange and ensure Taiwan remains at the forefront of sustainable academic research and innovation. In conclusion, this project has not only contributed to Taiwan's academic development but also aligned with global sustainability goals, especially in the context of bioplastics and food innovation, thus playing a crucial role in elevating Taiwan's international standing in both research and sustainability efforts.
附件	Please visit the websites of our published papers, which are based on the results of this project support 1. https://link.springer.com/article/10.1007/s00203-023-03745-z 2. https://link.springer.com/article/10.1007/s13205-023-03855-x 3. https://www.sciencedirect.com/science/article/pii/S0957582024005317

備註：

1. 本報告內容以5至10頁為限。
2. 報告繳交時請提供**電子檔各1份**至總計畫主持人所屬學校之研發處承辦人、子計畫研發處負責人及本案承辦學校承辦人