食品微生物的美麗新世界

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微生物應用在生物技術與食品加工、製造過程中擔任重要的角色，除可為產品提供良好之氣味 (風味)，並可改善食物之消化性及營養價值，將食品之風味、功能性與附加價值提升。另一方面，微生物亦為影響食品安全的主要影響因素之一，多數引起食品中毒的主要原因為病原菌所引起，造成國家社會經濟上的重大損失。由食品產製與消費的觀點來看，安全的食品必須建構在健康與風味之上。建構安全的生產制度將是食品或生技產業最重要的一環。緣此，本研究室針對『有益處』及『有害處』的微生物在食品科學的角度進行深入的探討，由基本的微生物檢驗鑑定、分類、醱酵生產製程及功效性等試驗的『點』，漸漸擴展至以快速篩選技術及流行病學統計為主食品安全風險性評估等較應用之研究、微生物相之分析 (包含口腔、腸道等)、生物反應器之開發及高效率之功效性篩選等方面之的『線』，最終將所發展及開發之技術應用於食品或生技產業，提高生產效率與功效性之附加價值的『面』，由前端的技術開發研究延伸至產業應用的實踐。本研究室近年來研究成果簡述如下。

一、益生菌醱酵保健食品之基礎研究與開發應用

為因應科技發展與產業需求，本研究室藉由微生物及其醱酵所產生之有益代謝物，應用在保健食品的開發。乳酸菌為人體腸道與口腔中廣泛存在之益生菌，已被證實具有許多保健功效，在食品工業上經常被運用。開發乳酸菌新型態與多重功效 (multi-function) 保健功效之相關保健產品是未來重要發展方向。過去致力於自各式醱酵蔬菜中篩選乳酸菌進行醱酵，其中由臺式泡菜中篩選獲得之胚芽乳酸桿菌 TWK10 (*Lactobacillus plantarum* TWK10) 具有許多良好的特性且醱酵產物中可提供更多的應用性。

1. 益生菌醱酵豆奶及其機能性成分對高血壓及其伴生之血管性失智症之改善

黃豆因原料價廉、營養且適合微生物生長，乃以豆奶為醱酵基質進行醱酵，期望瞭解乳酸菌醱酵豆奶對於高血壓的調節及對高血壓所伴生之血管性失智症改善之效果。初期以細胞培養模式探討乳酸菌醱酵豆奶對促進血管舒張的影響，並藉此探討作用機轉；進一步以自發性高血壓大鼠模式探討乳酸菌醱酵豆奶對高血壓的調節機制；分離、純化並鑑定乳酸菌醱酵豆奶中具調節血壓的成分為尿嘧啶 (uracil) 與甘油的 1:3 混合物，亦首次證實另外一個在胚芽乳酸桿菌 TWK10 乳酸菌醱酵豆奶中特有的成分-馬雌酚 (equol) 具有調節血壓的效果，並利用 DOCA-salt 誘導之高血壓大鼠模式，證實上述成分具有改善血管性失智症 (輕度認知功能障礙) 的效果。

2. 益生菌醱酵豆奶抑制黑色素生合成之美白機制之探討

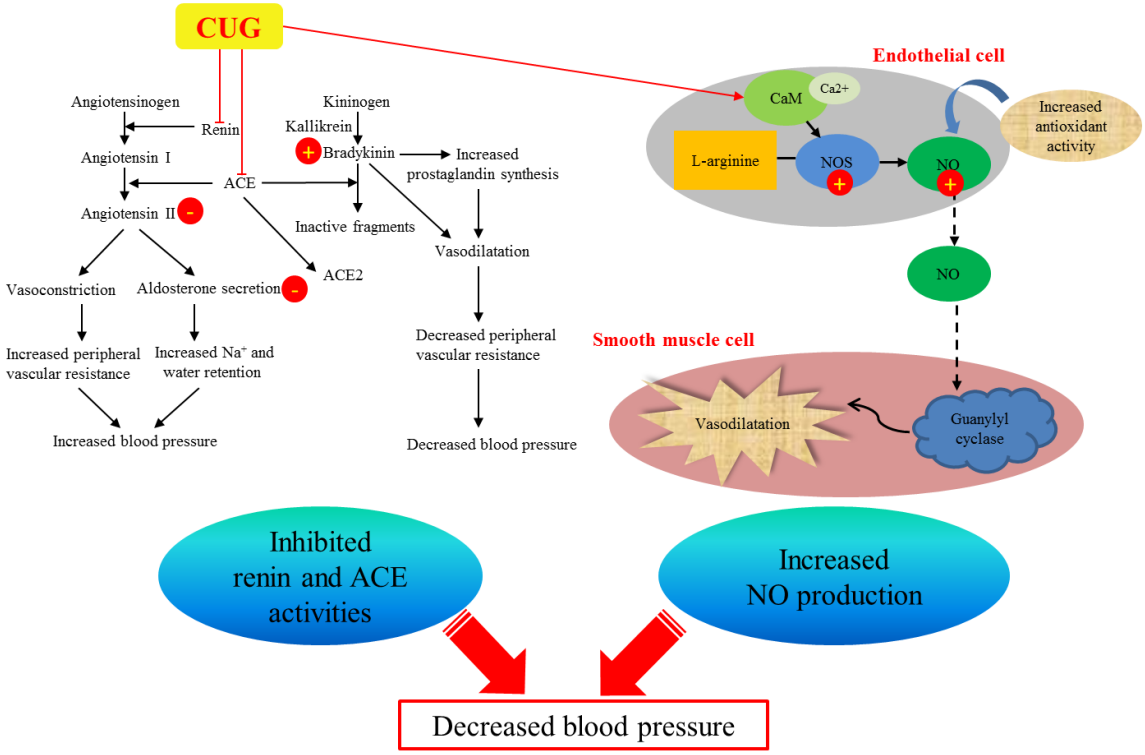
為因應未來保健市場趨勢，進行具區隔性之產品開發可有效刺激新產品衍生，其中以美白相關之保健產品是未來業界重要發展方向。我們將乳酸菌醱酵豆奶為基質，以產業化為目標進行機制的探討與測試，透過學理上的證實瞭解保健食品的功能與作用機制，使開發之保健食品功效指標化、產品科學化的立論基礎，因此我們藉由細胞培養模式，探討乳酸菌醱酵豆奶抑制黑色素生合成的機制，並自乳酸菌醱酵豆奶中分離與鑑定有效成分為尿嘧啶及馬唑酚，並完整地確認這兩種成分對於黑色素細胞黑色素生合成之可逆性的抑制機制，藉此成果可應用在保養品及化妝品的開發。

3. 胚芽乳酸桿菌 TWK10 改善運動能力之表現

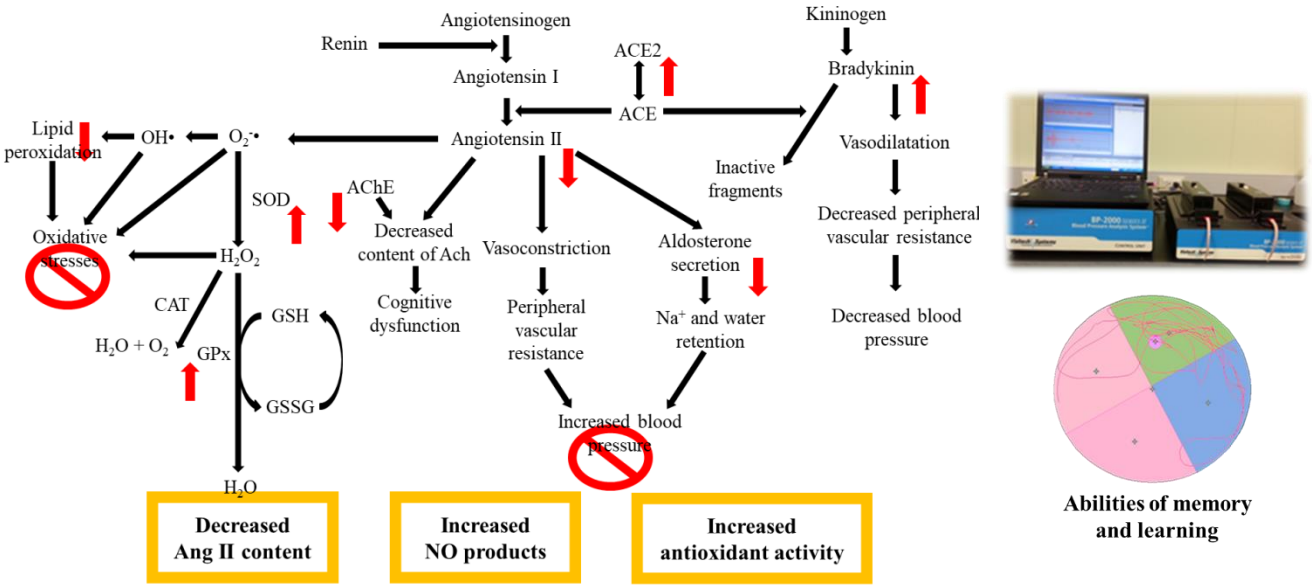
人體腸道內微生物是影響宿主代謝、能量平衡和身體組成的關鍵，對人體內能量收割的轉換與利用起著至關重要的因素，而這恰恰也是影響運動表現的要素。本研究室與國立體育大學運動科學研究所黃啟彰教授研究團隊共同研究，探討以胚芽乳酸桿菌 TWK10 評估對小鼠運動表現、抗疲勞相關生理指標及腓腸肌肌纖維之影響，小鼠經連續餵食 TWK10 六週後可顯著增加小鼠運動能力且藉由改善小鼠腸道微生物組成，改變體內的能量代謝，分解短鏈脂肪酸、丁酸、葡萄糖等分子，提高能量收割、增加運動耐力時間。進而減少小鼠血乳酸及肌胺酸激酶含量並增強其運動表現，達到抗疲勞之功效。

二、紅藜啤酒之製程開發與最適化條件之研究

台灣藜 (*Chenopodium formosanum Koidz*) 為台灣原生種穀類植物，其甜菜色素可作為天然著色劑和抗氧化劑，且含有豐富之營養成分如膳食纖維及其他微量元素等，在食品加工中常被用於飲料、飯糰、米酒和泡芙等產品製程中。過去兩年，本研究室投入紅藜啤酒的製程開發，從紅藜穀物帶殼與不帶殼的選用、紅藜發芽時間與芽長、糖化條件、酵母菌種篩選、殺菌、後熟及紅藜啤酒醱酵成品之品評等進行探討，同時也探討帶殼紅藜啤酒中機能性成分的分析。藉此增進產業技術之創新與經濟之發展，並增加傳統在地農產品之附加價值。



圖一、尿嘧啶與甘油混合物抑制高血壓之機制。



圖二、TWK10 發酵豆奶萃取物改善高血壓及其伴生失智症之機制。

圖三、紅藜啤酒的發酵過程。

圖四、應用生物科技研究室研究團隊。

[未來展望]：

本研究室的研究成果除發表研究論文之外，亦進一步嘗試以產學合作、技術轉移與專利布局等方式將研究成果應用於產業界，合作模式由廠商先行就所從事之行業別進行產品大方向的市場調查及規劃，再與具統計調查專長之學研界進行消費者使用產品之行為與認知之評估，共同形成欲開發產品之概念，進一步由學研團隊進行生技產品安全性與功效性之探討；藉由市場評估、功效確立、產品設計、品管指標訂定等研究開發過程，進行雛型產品開發。此外，我們會同時進行小分子生理活性物質之分離純化，將醱酵基質中的有效成分鑑定與活性確認，希望透過學理上的驗證開發具有多重保健功效的保健產品，除了可在學術上達到研究的成效，並期望可做為廠商在產品開發與功效宣稱方面強而有力的學理後盾，讓保健產品更具有功效指標化、產品科學化的立論基礎。

Brave New World of Food Microbiology

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Microorganisms play a major role in biotechnological applications, food manufacturing, and processing. Not only do they impart a good taste to food products but also improve their functionality, digestibility, and nutritional values. In addition, microorganisms are one of the most important factors affecting food safety. Most foodborne illnesses are caused by pathogenic fungi, bacteria, and virus, leading to significant economic losses. Building a food safety production system is the most important processes in the food or biotechnology industry. From the consumer perspective, the purpose of food production should be to produce the safest and highest quality foods. For this reason, our research team conducts in-depth investigations of the "beneficial" and "harmful" microorganisms with the field of Food Science. Research is performed on microbial identification, classification, fermentation and functionality, along with food safety, risk management and assessment of the outcome of basic rapid screening technology, and epidemiologic evaluation. Applied research is conducted by analyzing bacterial diversity in different microbial ecosystems including the oral and gut microbiota, development of bioreactors, allowing for a highly efficient functional ingredients screening. Finally, technology development leads to an improvement in the productivity and functionality in the food or biotechnology industry. Research conducted in our research team during the past decade is briefly described below.

1. Basic research and applications of fermented functional foods based on probiotics

In response to technological innovation and industry needs, our research team focused on the development of functional foods fermented with microorganisms, leading to the production of beneficial metabolites by microbial bio-conversion. Lactic acid bacteria (LAB) presents in the human intestinal tract and oral cavity is commonly used as a probiotic. It has been shown to benefit hosts (humans) significantly and is often used in the food industry for application. Therefore, the development of a new, multi-functional strain of LAB is an important direction of research of food science. Our research team had screened several LAB strains obtained from various fermented vegetables. *Lactobacillus plantarum* TWK10 (TWK10) exhibiting potential probiotic properties was isolated from Taiwanese fermented cabbage and used as the target to investigate.

1-1. TWK10-fermented soy milk and its functional ingredients in the improvement of hypertension and its associated vascular dementia (VaD)

Soybean is an inexpensive source of protein and possesses superior nutritional and functional values, which are suitable for microbial cultivation and growth. Hence, we investigated the effects of TWK10-fermented soy milk on the improvement of hypertension and its associated VaD. Initially, the effects of soy milk fermented with TWK10 on nitric oxide-mediated vascular relaxation factors in cell model systems were determined. Further, we used spontaneously hypertensive rats (SHRs) as experimental models to examine the physiological effects of TWK10-fermented soy milk on blood pressure. Bioactive angiotensin-converting enzyme inhibitors were isolated and identified from TWK10-fermented soy milk. A combination of uracil and glycerol, one of the bioactive enzyme inhibitors present in 1:3 ratio (uracil/glycerol) exhibited antihypertensive activity. Moreover, for the first time, we published that the unique components in the TWK10-fermented soy milk was equol, which lowered the blood pressure and its associated VaD. Thus, such bioactive compounds found in the fermented soy milk can lead to a reduction in hypertension and associated VaD.

1-2. Study on the whitening mechanism of probiotics-fermented soy milk on inhibiting melanogenesis

To respond to future healthcare market trends, the development of food segmented products could effectively stimulate the derivatization of new products. Among such products, the development of whitening healthcare products is an important directive in the cosmetic industry. We used LAB-fermented soy milk as a target and industrialization as an experimental approach to investigate these mechanisms. Theoretical verification was performed to understand the function and effective mechanisms of the developed health products, investigate their functionality, and elucidate their scientific basis. We investigated the inhibitory effects of LAB-fermented soy milk on melanogenesis in a cell model and confirmed the mechanism of reversible inhibition of melanogenesis in the melanocytes by the bioactive compounds, uracil and equol, which were isolated and identified from LAB-fermented soy milk. Based on the above results, it can be used in the development of skin care products or cosmetics.

1-3. *Lactobacillus plantarum* TWK10 (TWK10) improves athletic abilities

Gut microbiota in the human body affects host metabolism, energy balance, and body composition. It is a vital factor affecting energy conversion and use, which affects exercise performance. Our research team and Professor Chi-Chang Huang from the National Taiwan Sport University jointly studied the effects of TWK10 on exercise performance, anti-fatigue-related physiological markers, and gastrocnemius muscle fibers in mice. Mice that were continuously administered TWK10 for 6 weeks exhibited a significant increase in exercise capacity and changes in the gut microbiota, subsequently leading to changes in their *in vivo* energy metabolism, such as the catabolism of short-chain fatty acids, butyrate, and glucose, improved energy harvesting, and increased exercise endurance. This reduced blood lactic acid and creatine kinase levels in mice, thereby increasing their exercise performance and achieving anti-fatigue effects.

2. Study on the optimal conditions and process developments of djulis (*Chenopodium formosanum* Koidz) beer

Djulis (*Chenopodium formosanum* Koidz) is a species of indigenous cereal plants in Taiwan. Betalain is known as a natural pigment and antioxidant used in food industry. It is rich in nutrients such as dietary fiber and other trace elements. Djulis is used in food processing to make beverages, rice balls, rice wine, and puffs. In the last two years, our research team has worked on the development of beer brewed with djulis by investigating djulis with and without husks, germination time of djulis and bud length, mashing conditions, screening yeast strains, sterilization, and aging. Sensory evaluation of the beer brewed with djulis was performed as well. At the same time, we also analyzed the functional components in beer brewed with husked djulis. This would enhance the innovation and economic development in industrial techniques and increase the added value of traditional local agricultural products.

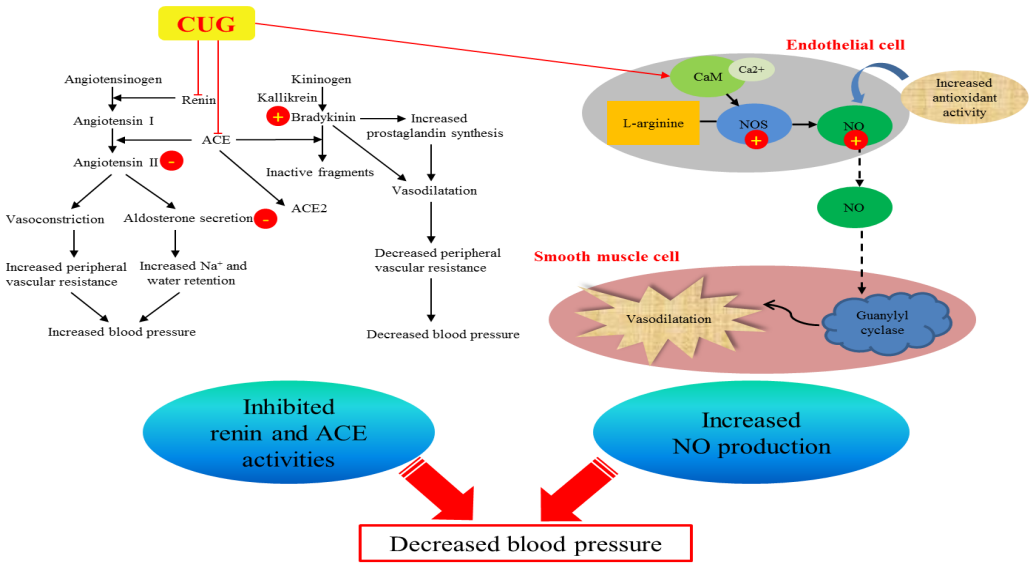

Figure 1. Mechanism of antihypertensive effect of combined uracil and glycerol treatment.

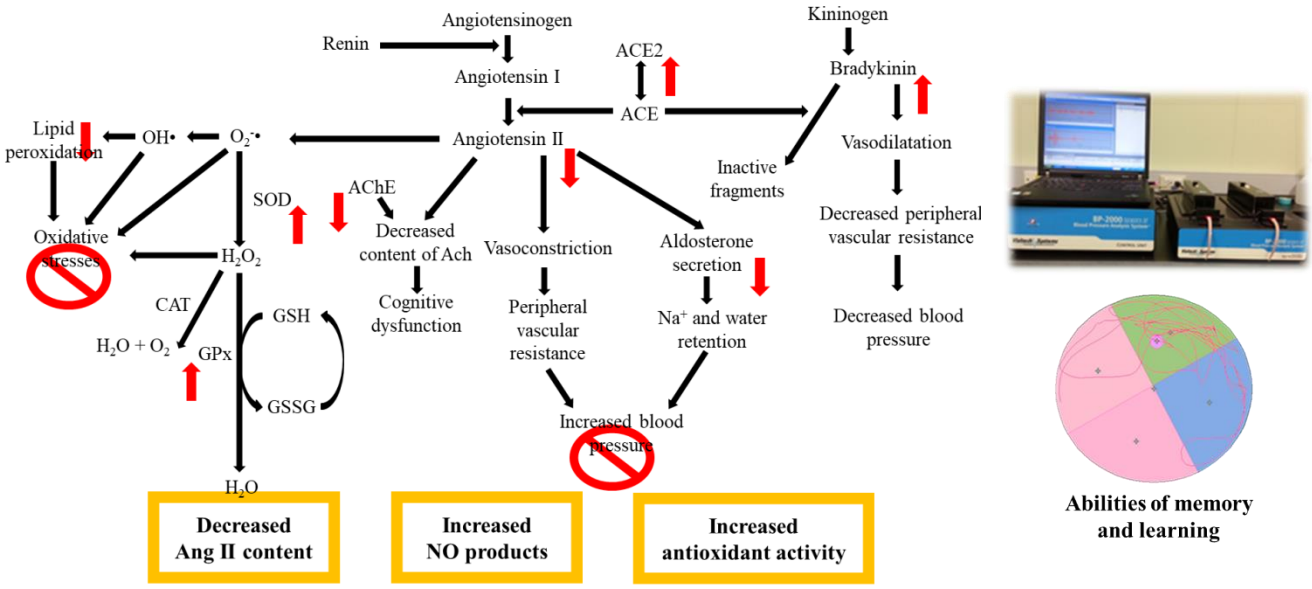

Figure 2. The proposed mechanism of administration of extracts from TWK10-fermented soy milk to improve hypertension and its associated dementia.

Figure 3. The fermentation process of djulis (*Chenopodium formosanum* Koidz) beer.

Figure 4. The research team of Laboratory of Applied Biotechnology.

[Briefly share your research achievement and development of the future:]

In addition to publications, the research findings of our research team would be used for industrial-academic collaborations, technology transfer, and patent portfolio, thus supporting industrial usage. Such collaborations would be first based on market survey and planning of the general direction of the product, before academic evaluations on consumer behavior and product recognition are performed through statistical survey experiments. This would jointly form a product concept to be developed. Furthermore, the academic research team would evaluate the safety and function of the biotechnology (food) product. Thereafter, market assessment, function evaluation, and the determination of functional ingredients for quality management would be conducted along with designing the product for the development of a prototype. At the same time, we would simultaneously conduct the separation, purification, and identification of functional compounds in the fermentation products. We hope that more health products could be developed based on theoretical verification. In addition to the research findings, we hope to provide a valuable theoretical basis for product development and efficacy claims, providing a foundation for setting efficacy markers, and a scientific basis for the product.