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Bridging the Gaps between R&D, Practical Application, and Commercialization of Biofertilizers and Biopesticides in Taiwan

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ABSTRACT

Under the goals of global environmental protection, sustainable production, and food safety, Taiwan's current agricultural policy focuses on reducing the use of chemical products and encouraging the use of environmentally friendly agricultural products. Furthermore, the applications of beneficial microbial products in the nutritional and health management of agricultural production (including crops, livestock, and fisheries) have become an important R&D objective for both the academia and industry. To meet the product standards of various agricultural production systems and the demands encountered by the industry, the Agricultural Technology Research Institute (ATRI) in Taiwan has formed a collaborative team across the fields of crop, fishery, poultry, and livestock production to develop market competitive agricultural biotechnology products. ATRI collaborates with universities and agricultural research & extension stations to develop new products and complete a series of R&D progress from microbial strain identification, fundamental studies, fermentation mass production, efficacy studies, formula adjustments, to implementation techniques. Core technologies such as fermentation mass production process, formula adjustments, and manufacturing are the keys to shortening the commercialization process and reducing uncertainty risks in the technology transfer process. The test samples were produced according to product standards for efficacy evaluation, and conducting on-site efficacy testing, and verification can reduce differences in the efficacy of final products. The industry has recognized the team's efforts through technology transfer, and related commercial products have been launched in different fields. Furthermore, our team also has an animal toxicology laboratory that complies with OECD Good Laboratory Safety (GLP) standards. The safety assessments of development targets were conducted to establish supporting documentation and raw material production capabilities required for product registration. These efforts bridge the gap among R&D institutions and industries to enhance the quantity and variety of indigenous agricultural microbial products in Taiwan.

Keywords: beneficial microbial products, fermentation mass production, formula adjustments, efficacy studies, and implementation techniques

INTRODUCTION

Agricultural chemical products such as pesticides, chemical fertilizers, veterinary drugs, fishery drugs, and environmental agents are effective solutions for many production problems frequently used in conventional agriculture practices (Bernardes *et al*., 2015; Aktar *et al*., 2009; Fenik *et al*., 2011; Strassemeyer *et al*., 2017). However, the dosage, frequency, and time of use should be strictly managed, as if care is not taken, side effects such as food safety and contamination caused by residues become a critical issue for all mankind. Therefore, reducing the use of agricultural chemicals and finding more environmentally friendly alternatives have become a consensus in agricultural policy around the world (Commission *et al*., 2022; Gamage *et al*., 2023; Brunelle *et al*., 2024). To stabilize the food supply and reduce the amount of chemical usage, microorganisms are often used as alternative solutions for protection (biopesticides, veterinary and fishery drugs), nutrition supply (biofertilizers, feed additives), and growth promotion (biostimulants) in agricultural production (Palaniyandi *et al*., 2013; Saarela *et al*., 2000; Verschuere *et al*., 2000). Furthermore, environmentally sustainable development is another important task of agricultural action, and how to maximize the resource recycling have become important subjects for researchers. Agricultural waste is recognized as wastes left over after cultivating and processing agricultural products like crops, poultry, and livestock. Annual production of agricultural waste was estimated at around 998 million tons (Nishikant *et al*., 2023). Agricultural waste can be recycled back into agricultural practices if properly treated. However, if not handled properly, they can become a source of pollution. Animal manure from livestock such as pigs, goats, cattle, and chickens can be utilized to make fertilizer as it contains nutrients required for crop growth. However, the risk of animal manure being contaminated with drug residues and microbial contaminations is high (Iglesias *et al*., 2006). In addition, secondary contamination of agricultural products and the environment may occur (Zurfuh *et al*., 2016), while drug-resistant strains of microorganisms that accelerate the spread and succession of disease are also a concern (Jechalke *et al*., 2014; Zheng *et al*., 2017). The potential pollution risks of animal manure need to be addressed in circular agriculture and resource regeneration. Varieties of commonly promoted and unitized beneficial microorganisms, such as *Lactobacillus* spp., *Bacillus* spp., and *Streptomyces* spp., which help regulate intestinal and rhizosphere microbial communities, could offer effective solutions to eliminate or reduce contamination risks in recycling applications (Ayala *et al*., 2017; Donato *et al*., 2017; Maloney *et al*., 1993).

Once researchers identify the functions and applications of beneficial microbes in agriculture, microbial mass production is required for field experiments. Typical laboratory equipment, such as plates, shakers, and bench-top fermenters can produce several liters or kilograms of microbial cultures for preliminary efficacy testing, however, this mass production process is unsuitable for field experiments or commercial product manufacturing (Adekunle *et al*., 2001; Rayhane *et al*., 2019). The microbial culture process involves complex factors such as growth, metabolism, and sporulation. Samples from the laboratory multiplication process potentially have several issues including large batch variation, unstable quality, high labor intensive, high cost, and scale-up limitation (Singh *et al*., 2007; Hsu and Wu *et al*., 2002; Xia *et al*., 2021). It may also lead to potential problems such as inconsistent experimental results, failure to verify the quality, and incompatibility with market demand. Appropriate culture propagation procedures are important for a microbial agent to become a commercialized and approved product (Wang *et al*., 2024; Xia *et al*., 2021). Furthermore, before launching microbial products, finding formulas suitable for different uses, such as biopesticides, biofertilizers, animal feed additives, environmental agents, among others, is another key step (Singh *et al*., 2024; Behl *et al*., 2024; Nagpal *et al*., 2021).

To assist microbial agents' R&D program in academics and industries, the Agricultural Technology Research Institute (ATRI) in Taiwan has established collaborative teams across crops, fisheries, poultry, and livestock production to bring the R&D results to the market. Core technologies include a series of R&D progress from microbial strain identification, fundamental studies, fermentation production, efficacy studies, formula adjustments, phenotype-based screening tests, manufacturing, and implementation techniques, etc. This one-stop customized technical service is the key to shortening the commercialization process, reducing uncertainty risks in the technology transfer process, and meeting the product standards of each agricultural production system and the demands encountered by the industry. In recent years, ATRI has cooperated with different R&D institutions such as universities, institutions affiliated with the Ministry of Agriculture (MOA), and Academia Sinica to develop various functional products from *Bacillus subtilis* for applications such as fertilizers, fishery and poultry feed additives, and rice stress-resistant cultivation (Shih *et al*., 2013; Tu *et al*., 2024). Meanwhile, solid-state fermentation mass production models were also established for entomogenous fungi, such as *Streptomyces* spp., *Beauveria* spp., *Metarhizium anisopliae*, and *Paecilomyces farinosus*, etc., and the formulae for foliar spraying and extended storage period were also developed (Desgranges *et al*., 1993; Pham *et al*., 2010; Sala *et al*., 2023; Mascarin *et al*., 2024). The collaborative model of simultaneous formula calibration and on-site efficacy testing has successively enhanced the feasibility of industrial application using academic research results.

CORE TECHNOLOGIES AND PROCESSES

Integrated production from trial production to mass production for microbial agents

The first step in the mass production of beneficial microbes is establishing important basic preparation information such as inoculum sources, excipients, and nutritional ingredients. The previously established mass production technology patent (US 7632493 B2) uses a 10 L desktop fermentation tank system to test four commonly used bacterial culture medium formulae and regularly sample to obtain the inoculum growth concentration curve to optimize the incubation period and the initial inoculation concentration. In addition, this 10 L inoculum culture system was used to test the affinity of the defoamer agent, oxygen demand, and other fermentation culture conditions to obtain parameters and the basic inoculation conditions for preparing the next step of fermentation in the 50L experimental-grade fermentation tank. Based on the requirements and characteristics of inoculum, a laboratory-grade 50 L/100 L fermentation tank was used to verify the primary fermentation process and test basic nutritional demands, ventilation conditions, stirring rates, and appropriate defoamer agents and strategies. During the fermentation process, key parameters such as temperature, pH, dissolved oxygen (DO), and stirring speed are continuously monitored in real-time. Additionally, the generation and elimination of forms in the tank are routinely observed, while samples are also taken to assess bacterial proliferation and evaluate the overall fermentation status.

Development of liquid fermentation mass production process for beneficial microbial agents

ATRI's agricultural microbial products plant has the lowest commercial scale 1,500L mass production fermentation tank (Figure 1), which is used for fermentation process verification and qualitative testing. Based on the verified information of the primary fermentation process of the 50/ 100 L fermentation tank, an efficient fermentation process of the 1,500 L fermentation tank is established. Parameters for mass production parameters have also been adjusted to meet the demands of industrial mass production costs and process optimization. Samples produced by this mass production process can be directly used as functional biopesticide/ biofertilizer products or microbial agents for different product types through other processing technologies such as spray drying, fluidized-bed granulation, seed coating spraying, among others.

Figure 1. 100 L fermentation (left) and 1,500 L mass production fermentation tank (right).

Two-phase fermentation system for microbial mass production

Some beneficial microbes are filamentous and multicellular and require an interface between air and medium for sporulation. To improve the production efficiency, the two-phase mass production system was established by combining liquid fermentation and solid-state ventilation processes (Figure 2). A *Streptomyces* HLA44 stain was used as a model strain to test this mass production system. The inoculum source was multiplied through a 10 L desktop fermentation tank system and then homogenized. Meanwhile, the solid fermentation medium is packaged in culture bags with a specifically designed lip for better ventilation and then sterilized through a 400 L horizontal cylindrical pressure steam sterilizer. Aliquot the liquid inoculum into bags containing cooled and sterilized medium and place in a walk-in environmentally controlled incubation chamber at 30 °C. After 10-14 days, when the spores completely cover the solid substrate and no water condensation is observed, they can be collected and placed in a 6-8 °C refrigerator for subsequent processing into product formulation.

Microbial product formulation processing

Depending on the application requirements, microbial products, such as biopesticides, biofertilizers, animal feed additives, and environmental agents, need to find suitable formulations that are convenient for use, more effective, and affordable. Microbial product formulations involve the direct use of microbial culture media derived from either liquid or solid fermentation process. Spray-dried powder, is another type of microbial formula, which is created by incorporating excipients and/or protective agents, followed by drying the mixture in a spray dryer using high-temperature sterile air at 100-140 °C. Various microbial products, including water-dispersible granules, feed additives, soil amendments, and seed coating (Figure 3) are manufactured using the fluid bed granulation process. This method involves suspending particles in an airstream while spraying liquid culture onto the surface of excipients' and/or protective agents' particles.

Figure 2. Two-phase fermentation system for microbial mass production.

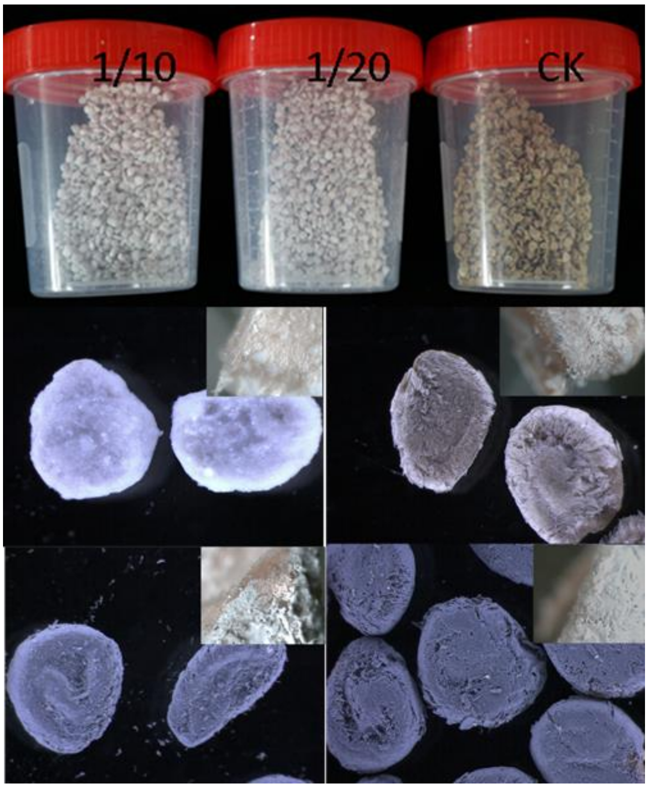

Figure 3. Application of microbial agents in seed coating.

Microbial product implementation techniques and efficacy verification

Numerous species and strains of beneficial microbes have been found to have various functions, including plant protection, nutrition supply, crop growth promotion, animal health boosters, pollutant removers, and reducing contamination risks in recycling waste. The main objective of our R&D team is to develop products and implementation techniques that meet the specific demands of various fields. Therefore, prototype microbial products corresponding to crop cultivation, animal husbandry, and aquaculture are produced, and the team's experts in various fields simultaneously analyze, optimize, and verify the efficacy. A high-throughput evaluation technology based on a spectral imaging system was also developed to optimize formulations, evaluate performance, and adjust application methods for microbial products in crop protection and cultivation. Furthermore, feedback on formula design and direction adjustment will be provided based on relevant application results to improve the actual application effect of R&D products. The results not only serve as supporting information for product registration documents but also provide the industry with actual estimates of the application value and production costs. These will solve industrial technology bottlenecks and market expansion difficulties, and accelerate the development of probiotic products in the agricultural, livestock, and aquatic product markets.

Product safety assessment and supporting documentation

In Taiwan, agricultural microbial products are regulated by agencies under the Ministry of Agriculture (MOA), specifically the Agricultural and Food Agency (AFA) and the Animal and Plant Health Inspection Agency (APHIA). Registering a new microbial product requires detailed production information, including production technology, efficacy data, and animal safety assessment data. The Animal Toxicology Laboratory in ATRI is GLP-certified by the National Certification Foundation (TAF, registration number GLP0025) and offers technical services for acute oral and pulmonary toxicity/pathogenicity studies.

ACHIEVEMENTS IN MICROBIAL PRODUCT DEVELOPMENT

ATRI collaborates with universities and agricultural research & extension stations to complete a series of R&D progress of microbial products. For example, in developing stress-tolerant microbial agents for rice cropping systems, mass production was adopted by testing different commercial culture media. The differences in carbon type, nitrogen type, and concentration have a significant impact on the growth potential of this microbial strain. The results show that this microbial strain has good proliferation efficiency in both 523 and TSB culture media (Figure 4). Based on the media selection results, the defoamer agent was tested for its affinity to the cultured strains. Three DuPont food additive defoamer products were used and all tests showed good affinity. Defoamer agent No. 15 was selected as the foam control material due to its ability to increase oxygen transfer rates and promote growth (Figure 5). After analyzing the above basic test results, a commercial production fermentation test was conducted with a 50 L/100 L experimental-grade fermentation tank. Suitable commercial production media is an important factor in controlling the cost of commercial mass production. In terms of nutritional content, three different specifications of yeast products were analyzed. Changes in dissolved oxygen values and microbial growth have the most significant impact on fermentation parameters (Figure 6). Expanding to ton-scale mass production and improving product quality both are important for product development. A 1,500L factory-level fermentation tank was used to verify and optimize continuous commercial production conditions (Figure 7).

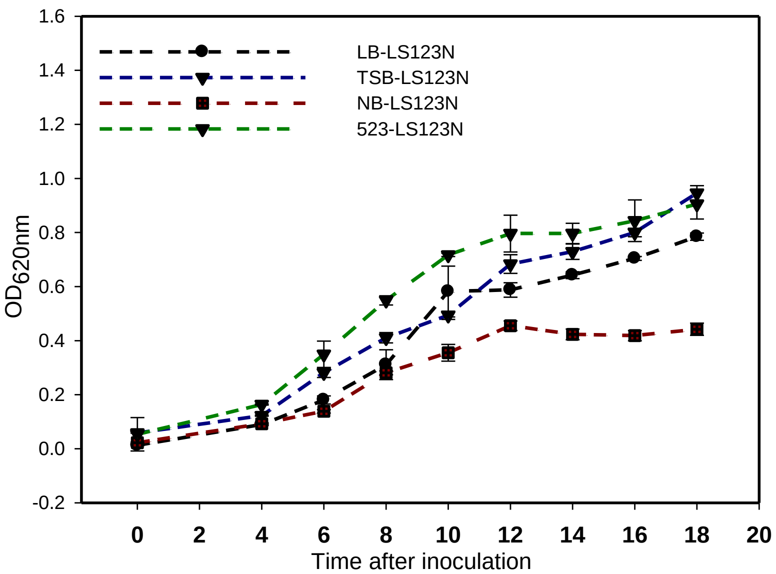

Figure 4. The growth rate of microbes in four different conventional culture media.
(LB-Lysogeny broth、TSB-Tryptone Soy Broth、NB-Nutrient Broth、523-Bacteria screening medium 523)

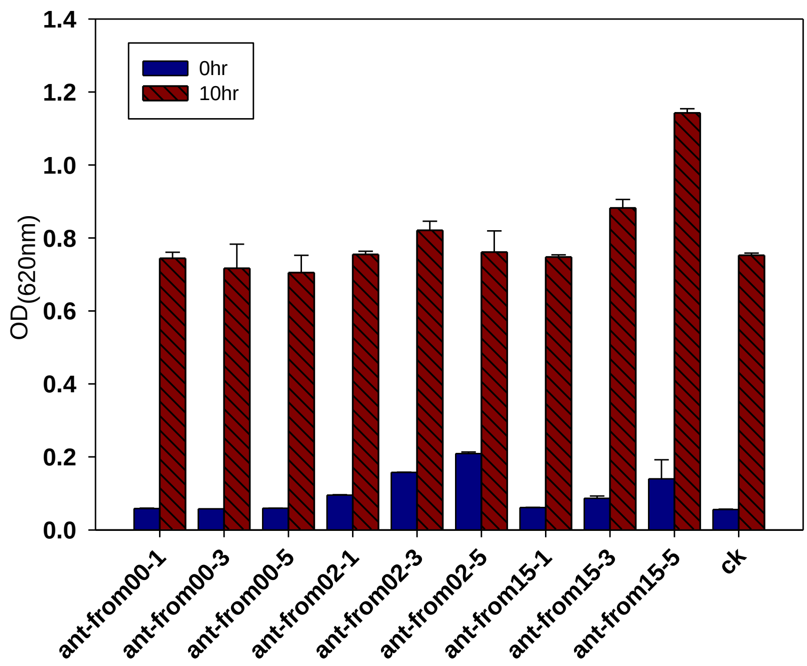

Figure 5. Effect of three XIAMETER™ Series defoamer agents on microbial growth potential.

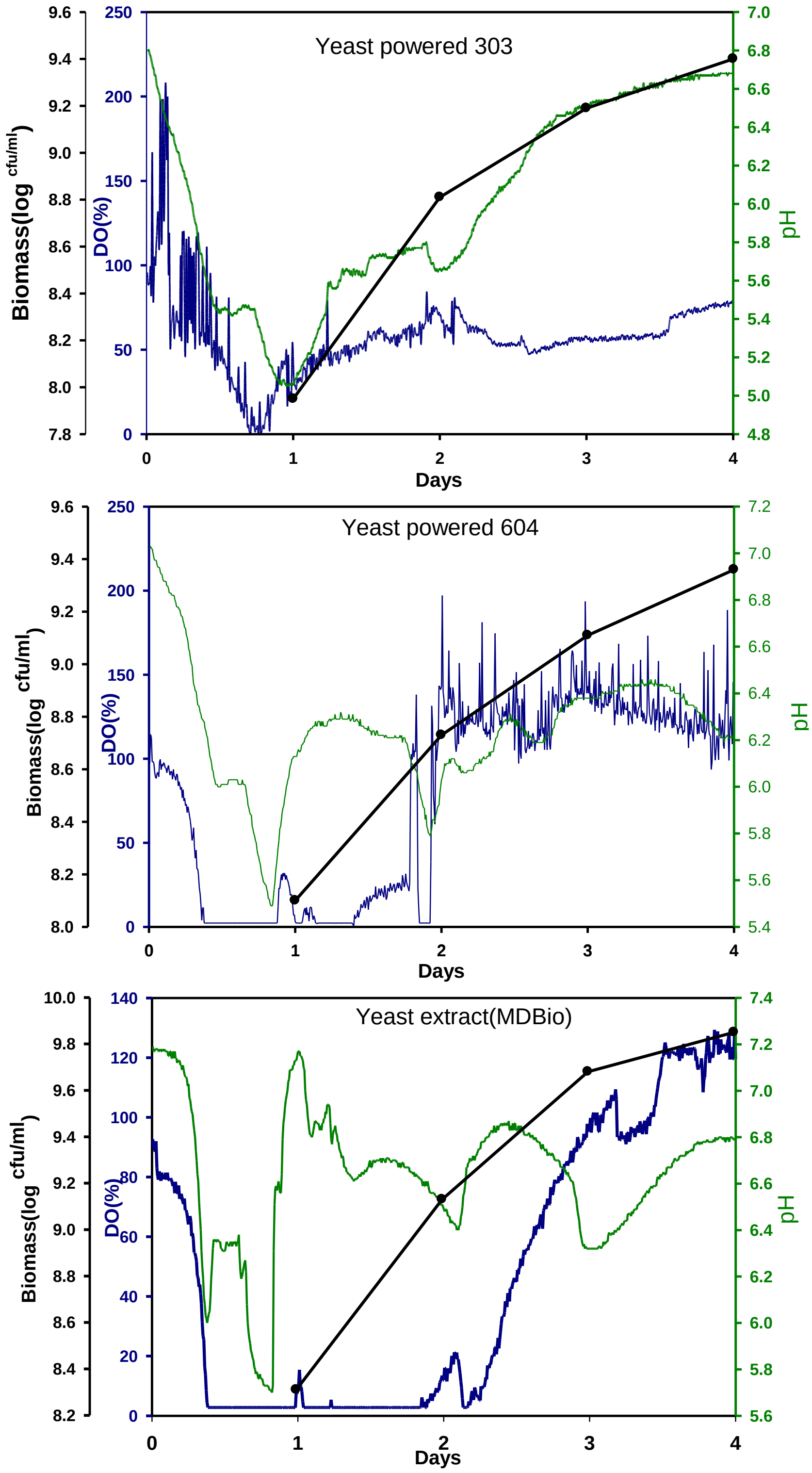

Figure 6. *Bacillus* sp.LS-123N fermentation test was conducted with a 50 L/100 L experimental-grade fermentation tank.

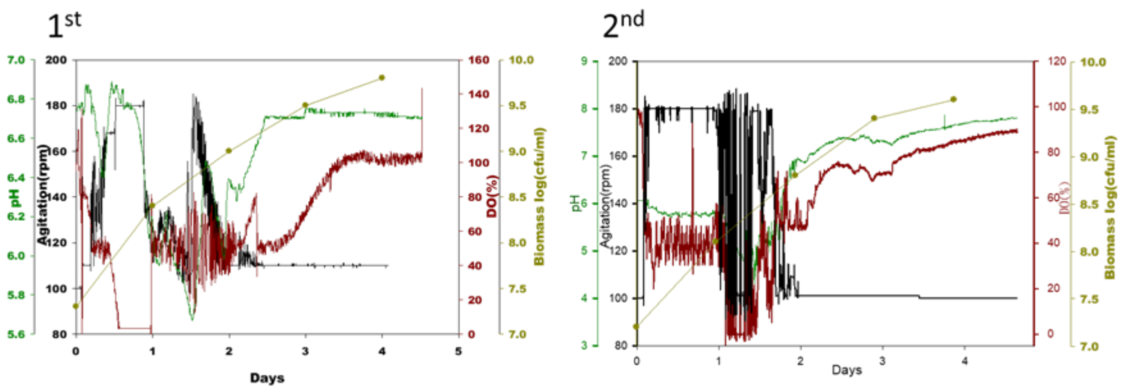

Figure 7. Verification of 1,500 L fermentation tank microbial mass production process (left，first time; right，second time).

The R&D team produces test samples according to product standards for efficacy evaluation and conducts on-site efficacy testing at the same time. Efficacy tests were conducted on multiple product prototypes and final products (Figure 8) in various fields such as crop protection, livestock and poultry, and aquaculture (Figure 9). Practical application test results provide great feedback for formula design and direction adjustment, helping to improve products. The fermentation mass production process, formula adjustments, and manufacturing are the keys to shortening the commercialization process and reducing uncertainty risks in the technology transfer process. The industry has recognized the team's efforts through technology transfer, and related commercial products have been launched in different fields (Figures 10, 11). Furthermore, our team also has an animal toxicology laboratory that complies with OECD Good Laboratory Safety (GLP) standards. The safety assessments of development targets were conducted to establish supporting documentation and raw material production capabilities required for product registration. These efforts bridge the gap among R&D institutions and industries to enhance the quantity and variety of indigenous agricultural microbial products in Taiwan. These will solve industrial technology bottlenecks and market expansion difficulties, and also accelerate the development of probiotic products in the agricultural, livestock, and aquatic product markets, and achieve the goals of developing natural, green, and sustainable agriculture, ensuring food safety and being eco-friendly.

Figure 8. Different microbial prototypes and products for various applications

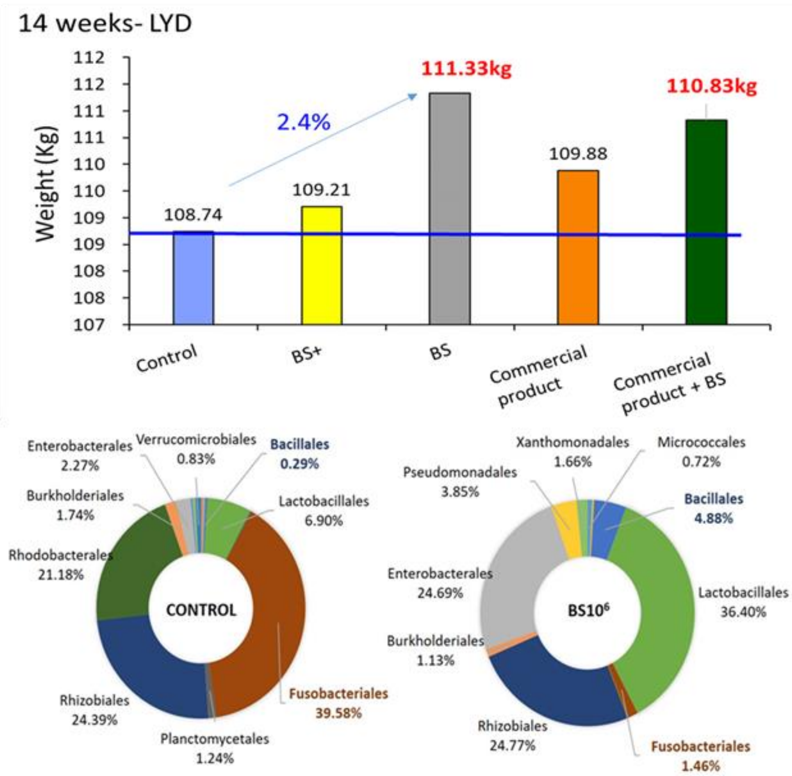

Figure 9. Evaluation of the efficacy of multifunctional microbial agents in livestock (upper) and fishery farming (down).

Figure 10. Microbial product application in strawberry cultivation – Biofertilizers.

Figure 11. Microbial R&D result – Agricultural resource recycling application and biofertilizer.

CONCLUSION

The current agricultural policy focuses on reducing chemical product usage and promoting eco-friendly materials in Taiwan. The Ministery of Agriculture (MOA) has set a ten-year target for halving the use of pesticides by 2027 in Taiwan. In order to achieve this goal, the development of biopesticides have been promoted and many microbial species and strains have been discovered that are beneficial to agriculture. The development of a microbial product is a long R&D process, including strain screening tests, functional identification, fundamental studies, different degrees of mass production technology, formula, implementation techniques, efficacy verification, and safety assessment. To achieve the product standards of different agricultural production systems and the needs encountered by the industry, ATRI has formed a collaborative team across the fields of crop, fishery, poultry, and livestock production to develop market-competitive agricultural microbial products. ATRI’s core technologies include integrated production technology (trial to mass production), ton-scale liquid fermentation mass production, two-phase fermentation system, formulation process, implementation techniques, efficacy verification, and product safety assessment. These core technologies offered by ATRI provide a comprehensive one-stop service platform for microbial product development, facilitating the industrialization of new microbial agents and reducing the time required for their market launch.

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AUTHORS' CONTRIBUTIONS

Win-De Huang designed the research, collected and analyzed the data. Yu-Hsuan Lin organized, strengthened and performed the final paper.

CONFLICT OF INTEREST

All the two authors, Win-De Huang, and Yu-Hsuan Lin declare that they have no conflict of interests.