Chapter

Application of Novel Technology in Aquaculture

Chung-Lun Lu, Shiu-Nan Chen and Shao-Wen Hung

Abstract

Aquaculture continues to be the fastest-growing food production field that has a lot of potential to meet the aqua-protein needs. The scientific and business communities are responding to the many challenges and opportunities inherent in the growing aquaculture field. Advancements in production and detection of harmful material systems and technologies are contributing to aquaculture industry's expansion and sustainability. All of these production system technologies are benefitting from expanding information and communication systems, which are enabling advances in every stage of production. In the future, the new farming operation of friendly environment will focus on the use of nonecology destructive substances, no antibiotics, and the natural probiotics or novel immunomodulatory substances to match the physiological regulation of cultured organisms and the management of aquaculture. The future scientific-based innovation will contribute toward meeting increasing food demands, while improving social, environmental, and financial sustainability of the global aquaculture industry.

Keywords: aquaculture, immunomodulatory, prevention management, probiotics, surface-enhanced Raman scattering

1. Introduction

As the catch of marine fisheries is decreasing year by year, the demand for aquaculture products in human food supply sources is increasing year by year. However, the rapid spread of aquatic diseases, reduced area of farmed land, or the decreased need of environmental conservation caused the deterioration of the global cultivation of aquatic animal environment. Now, the emphasis on ecological environmental protection and the risk of biological threats after the use of aquatic animal drugs are gradually proven and discovered.

In the future, the new farming operation of friendly environment will focus on the use of nonecology destructive substances, no antibiotics, and the natural probiotics or novel immunomodulatory substances to match the physiological regulation of cultured organisms [1] and the management of aquaculture [2–4], resulting in a good immune disease strategy for breeding organisms. Furthermore, rapid disease screening technology reduces the risk of disease spread that is prone to occur in manual operations.

2. Probiotics establish a balanced aquaculture environment management

2.1 Probiotics in aquaculture

The biggest challenge faced by the aquaculture industry is the malignant changes in the farming environment, whether it is changed by man-made or natural conditions. Aquaculture farming environmental degradation often leads to the accumulation of toxic substances or the dominance of pathogenic microorganisms. Since the industrial operator has only paid attention to the improvement of technology and production yield for a long time, and neglected how to maintain the ecological balance of the culture pond and the surrounding environment, when a disease breaks out, the first strategy is to apply animal drugs, especially antibiotics; undeniably, drug treatment for a disease is the most direct and immediate practice [5], but its damage to the environment is difficult to estimate. In the aquaculture industry, the negative impact of the application of drugs on the environment has received attention increasingly. In addition, drug residues cause food safety concerns and may lead pathogenic microorganisms to become drug resistant [5], which has an irreversible effect on nutrients in the culture environment [6].

So, the main purpose of adding probiotics or microbial products in the aquaculture pond is to decompose organic substances and at the same time to inhibit the growth of pathogenic microorganisms, reduce the risk of disease occurrence [7], convert toxic substances, reduce the accumulation of organic matter, stabilize the water color, and balance ecosystem in water. In the early days, the definition of probiotics was limited to microorganisms that were beneficial to the health of the organism. With the continuous revision, the current broad definition of probiotics not only increases the health of the organism but also improves the survival rate and the quality of the water, improving the immune mechanism of animals and even stabilizing the balance of the bacteria in the body [8], which are all functions of probiotics, and these benefits are very important for the aquaculture process, especially when the fry of aquatic animal are incubated, using probiotics as microbial additives to improve the final aquaculture production yield has important benefits [9].

Microbial additives usually do not have only a single strain. A single strain will have some disadvantages in the treatment of the aquaculture environment. A single strain usually only has the ability to handle certain substances, and the organic components in the aquaculture water are extremely complicated. Although the single strain treatment will change the figures or water quality numbers in the research, the improvement of the overall water quality is not great, so the addition of compound strains has become one of the important concepts of microbial additives. It is one of the important goals of microbial additives to treat different wastes in aquaculture environment with different strains to achieve the goal of purifying and stabilizing the whole culture system. The classification of probiotics in culture is distinguished by the currently widely used probiotics, which can be distinguished by their efficacy and timing of use: as follows: (i) probiotics for fry incubation (as show in **Table 1**); (ii) probiotics for cultured adult or subadult organisms (as show in **Table 2**); (iii) antipathogenic probiotics screened in the environment (as show in **Table 3**); and (iv) probiotics that increase the immune mechanism for aquatic animals.

The immune mechanisms of many aquatic animals are not as complete as those of higher mammals, and most aquatic products rely heavily on nonspecific immune mechanisms due to the lack of an active mechanism for antibody immunity. In the case of shrimps, in general, nonspecific immune mechanisms mainly include phagocytosis in the blood cells, nodule formation, encapsulation, and the interaction of other substances in the plasma, which include the cytotoxicity of cytotoxins, complement activated by the action of lectin, and the role of proPO system and

Species of probiotics	Aquaculture animal
Thalassobacter utilis PM4	Shrimp (*Penaeus monodon*)
Trosomonas sp.	Shrimp (*Penaeus monodon* and *Penaeus penicillatus*)
Nitrobacter sp.	Shrimp (*Penaeus monodon* and *Penaeus penicillatus*)
Bacillus sp.	Shrimp (*Penaeus monodon* and *Penaeus penicillatus*)
Thalassobacter utilis PM4	Crab (*Portunus trituberculatus*)
Bacillus toyoi	Turbot-via rotifers (*Brachionus plicatilis*)
Bacillus sp. spores	Turbot-via rotifers (*Brachionus plicatilis*)
Lactic bacteria	Turbot-via rotifers (*Brachionus plicatilis*)
Lactobacillus plantarum	Turbot-via rotifers (*Brachionus plicatilis*)
Lactobacillus helveticus	Turbot-via rotifers (*Brachionus plicatilis*)
Lactobacillus balgaricus	Turbot-via rotifers (*Brachionus plicatilis*)
Streptococcus lactis	Turbot-via Artemia
Alteromonas sp.	Oyster *(Crassostrea gigas)*
Roseobacter sp.(BS107)	Scallop (*Pecten maximus*)

Table 1.
Probiotics for aquatic animal fry incubation.

antibacterial protein/peptides [10, 11]. The biggest disadvantages of these effects are in two parts: one is the lack of memory, and the biggest disadvantage of the mechanism of nonspecific immunity is this. Due to the lack of memorable specificity, the phenomenon of repeated infection becomes a big burden in the aquaculture breeding industry. The second disadvantage is that some immune functions usually do not start real time and they need some special activating substances, such as the

Species of probiotics	Aquaculture animal	Effect
Lyoohilized *Carnobacterium divergens*	Atlantic salmon	Decrease *Vibrio anguillarm* strain infection
Lyoohilized *Carnobacterium divergens*	Atlantic salmon	Decrease *Aeromonas salmonicida* infection
Carnobacterium strain K1	Flounder	Inhibition the proliferation of *V. anguillarm* and *A. salmonicida*
Carnobacterium	Flounder	Inhibition the proliferation of V. anguillarm

Table 2.
Probiotics for cultured adult or sub-adult aquatic organisms.

Species of probiotics	Aquaculture animal	Effect
Nitrosomonas sp.	Shrimp fry	Increase survival rate
Nitrobacter sp.	Shrimp fry	Increase survival rate
Bacillus sp	Fry and adult fish	Increase survival rate
Spray-dried *Tetraselmis suecica*	Atlantic salmon adult fish	Increase the survival rate after poly-pathogenic infection

Table 3.
Anti-pathogenic probiotics screened in the environment.

participation of immune-stimulator to initiate the original immune state, so the reaction time is much delayed. This is also one of the reasons for the sudden death of the aquaculture animals.

Recently, many studies have found that the addition of probiotics can improve the immunity of cultured organisms. Such a phenomenon is very helpful for aquaculture organisms. Especially for the cultured organisms of shrimps and crabs, because they mainly rely on nonspecific immune mechanisms, how to activate their nonspecific immune system and improve their efficacy has become an important issue in aquaculture management. According to the report, using the source of immune activation, substances such as β-glucan can significantly stimulate the nonspecific immune mechanism of many water-generating substances, and these related polysaccharides are separated from the secondary metabolites of microorganisms. Such results have led to many ideas for making related microorganisms into biological additives, and many studies have confirmed that active yeasts and polysaccharides produced by beneficial bacilli do have positive effects on improving the survival rate and yield of cultured organisms. Feed was added with probiotics or directly added with polysaccharides, and positive benefits in improving the disease resistance and survival rate of cultured organisms have been found [12, 13].

2.2 Effects of probiotics on aquaculture environmental indicators

At present, probiotics are being used in the treatment of industrial wastewater, domestic sewage, animal waste, soil improvement, etc., and their functions in the aquaculture environment are as follows: (i) Inhibiting the occurrence of diseases: beneficial microorganisms can inhibit the growth of pathogenic bacteria by secreting inhibitory substances, competing nutrients, and competing for attachment sites. (ii) Maintaining good water quality: adding beneficial microorganisms can promote the decomposition of organic matter or the conversion of toxic substances, reduce the accumulation of organic matter in the culture pond, and convert toxic substances into nutrient compounds with low toxicity or even reusability, such as ammonia. It is converted to nitrous acid by nitrification and then converted to nitric acid, which can be used for the growth and utilization of plant plankton, which has the effect of stabilizing water quality and balancing the ecosystem. (iii) Improving health and growth: probiotics can improve the bacterial phase in the digestive tract of cultured organisms, promote the digestion and absorption of cultured organisms, help the health and growth of cultured organisms, and increase the survival rate to increase production yield. (iv) Increasing natural bait: the use of photosynthetic bacteria to culture animal plankton can increase the survival rate of brine shrimp, and the added bacteria can be used as a food for protozoa, and the bacteria themselves are a good source of bait to reduce the use of artificial feed.

Probiotics are used in aquaculture environments in the following ways: (i) combined with artificial feed, (ii) put in culture pond water, (iii) immersion, (iv) via live baits, and (v) via medium release. The usage timing can be divided into preculture and postculture. The former is used as the whole pool rearranges with the hope of utilizing the benefit bacteria phase, while the latter is used as the maintenance of the stable culture environment. The additives in feed can be divided into mixed and mixed for fermentation. It is added after fermentation to increase the digestion and absorption rate of the organisms, thereby reducing the excretion and pollution of the water. The addition of biological wastewater treatment system can be regarded as the microbial planting in the water system, which can ensure the stability of the water quality of the system and increase the effect of treatment. The easiest way to use probiotics to treat water quality is to put the microbial products directly into the culture pond. However, this kind of externally introduced probiotics must be made into a dominant species to function, so it must be continued every other day. To achieve the desired results, the probiotics mass and quantity of the products used should be especially confirmed. In addition, the effect of probiotics is quite susceptible to environmental factors such as salinity, temperature, and pH value. Therefore, it is best to activate the probiotics first and

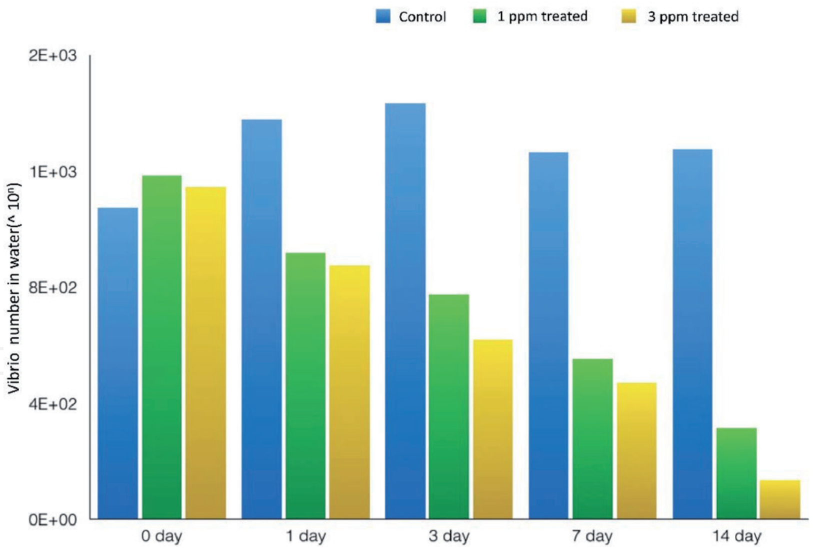

Figure 1.
Effectiveness of photosynthetic bacteria applied in white shrimp culture. Change in the total amount of Vibrio *in the culture pond within 14 days of continuous use of photosynthetic bacteria in the white shrimp culture process. The results showed that in the experimental group with more than 1 ppm of photosynthetic bacteria added continuously, the total amount of* Vibrio *was significantly reduced, which proved that the use of photosynthetic bacteria can effectively reduce the number of* Vibrio *in water.*

Water quality index	Before use	After use
BOD	82 ppm	16 ppm
COD	59 ppm	7 ppm
NH4-N	8 ppm	1.5 ppm
H2S	1.5 ppm	0.03 ppm

Table 4.
Probits stabilizes the water quality, but also reduces the accumulation of residual.

adjust the temperature of the mixture to be similar to the temperature of the pool water, and because the probiotics themselves are live bacteria, they should not be mixed with antibacterial agents, sulfonamides, antibiotics, and disinfectant water or in high temperature process.

An example of our use in white shrimp (*Litopenaeus vannamei*) culture farms, we use a variety of special strains such as Photosynthetic bacteria *Rhodobacter capsulatus* and *Micrococcus luteus*. Photosynthetic bacteria can utilize the hydrogen sulfide at the bottom of the pool as a nutrient to remove harmful substances and absorb heavy metals and inhibit the growth of Vibrio (**Figure 1**), while *M. luteus* can rapidly decompose proteins and increase the speed of nitrogen circulation in water to effectively reduce the occurrence and damage of sediments at the bottom of the pool. The interactive use of different strains not only effectively stabilizes the water quality but also reduces the accumulation of residual bait and organic matter in the shrimp culture process (**Table 4**) and greatly inhibits the proliferation of pathogenic bacteria such as Vibrio in the water. Such technology has been widely used in aquaculture industry in Taiwan and in Surabaya, Indonesia, and Thailand. Photosynthetic bacteria have become one of the most beneficial microbial strains in today's aquaculture industry (**Figure 2**).

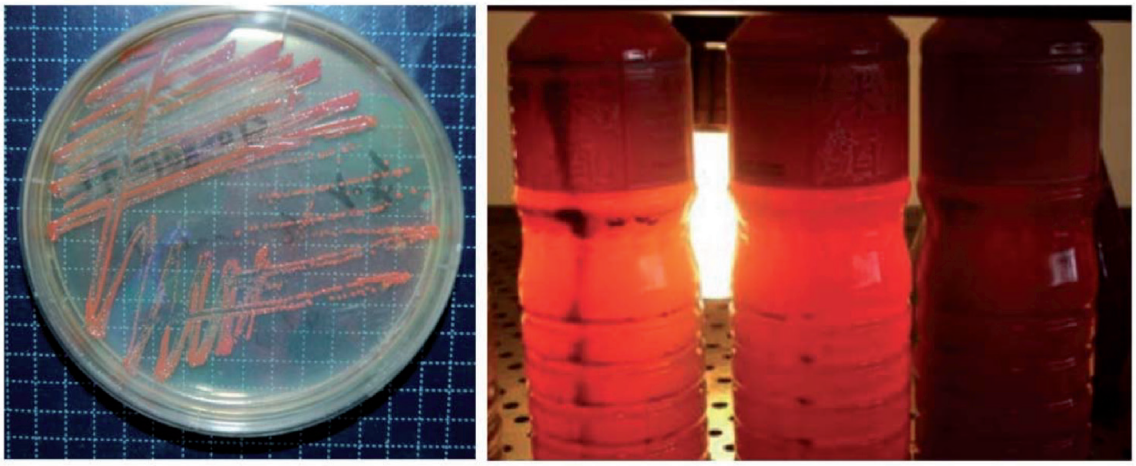

Figure 2.
Photosynthetic bacteria culture and mass production. Purification of photosynthetic bacteria on agar plate (left). Commercial mass production of photosynthetic bacteria (right).

3. Strategies for cultured aquatic animals' immunization and immune regulation

3.1 Aquatic animals' immunity and immune regulation

Animal congenital immune cells or other nonimmune cells, such as epithelial cells that secrete cytokines, mononuclear cells, macrophages, dendritic cells, and natural killer cells, have many nonspecific pattern recognition receptors, targeting identification and binding of antigen-related molecular patterns on invading pathogens. Complement receptor type 3 (CR3 receptor) on these cells is a receptor for the recognition of β-glucan [14], and when β-glucan binds to CR3 receptor, it triggers a series of signaling cascades to activate transcription factors, which translates cellular transcription into cytokines that trigger inflammatory responses, and incrementally regulates the expression of the major histocompatibility complex (MHC) of the antigen. In turn, other immune cells are activated to achieve immunomodulatory functions [15].

The polysaccharide extracted from the yeast (*Saccharomyces cerevisiae*) was orally administered for 4 weeks in dogs. The expression of total IgA, IgM, and IgG in serum and lacrimal gland was detected, and the expression of IgA in serum was found to be significantly decreased and IgM increased significantly, while IgG did not change significantly; the amount of IgA in the lacrimal gland was also significantly reduced. In the second part, the Bordetella vaccine was injected subcutaneously, and the specific IgA, IgM, and IgG showed the same trend as in the first part. Oral administration of β-glucan to Miguel dog changed the expression of IgA and IgM in serum, and there was no difference in the expression of IgG, indicating that the intestinal mucosal immune response was stimulated through this way. Glucan enters the gut-associated lymphoid tissue from M cells and binds to dentin 1 and TLR-2 on macrophages and dendritic cells, secreting IL-12 and TNF-α and other cytokines, which alter the cytokine microenvironment to stimulate the homologous transformation of B cells, affecting the secretion of immunoglobulins of different isoforms [16]. Dendritic cells are one of the important antigen-presenting cells in the immune system. Lu et al. used a polysaccharide extracted from *Antrodia camphorate* to carry out cell experiments and cocultured it with immature dendritic cells and T cells isolated from healthy human blood to investigate the maturation of dendritic cells and T-cell proliferation. The result showed polysaccharides promote maturation of dendritic cells to stimulate T-cell proliferation and IFN-γ expression [17]. Coculture of polysaccharides with macrophages can promote macrophage secretion of immune-related factors and cytokine gene expression, such as nitric oxide (NO), tumor necrosis factor-α (TNF-α), IL-1β, IL-6, etc., and promote macrophage activity [18].

According to the description of the fish immune system, we believe that fish do have a memory-specific immune function, so the administration of the vaccine should be quite feasible, but it is considered that the vaccine must be applied to the suitable stage and subject that can cause the immune reaction, and also regular additional injections to enhance the memory effect for the immune response is necessary. However, the application of additional injections is a slight inconvenience for the user who works on the breeding aquaculture farms. Therefore, the immunomodulatory substances can be used complementarily to enhance the nonspecific immune function and to resist pathogens quickly in the farmed fish. Currently, we are investing in the research and development of immunomodulatory substances, in order to solve the dilemma of the decline of biological and physiological ability of breeding. In recent years, research on immunomodulatory technology or the development of immunomodulatory products has gradually become a prominent feature in aquaculture disease control and health management strategies. Many studies have found that once the nonspecific immunity and specific immune function of the cultured organisms are effectively improved, the survival rate, growth rate, and disease resistance of the cultured organisms are significantly improved. It is a pity that many immunomodulatory substances incur excessively high production costs or inherent unitability that it was difficult to obtain similar benefits as the research results in farm operations. In order to solve such problems, our research team spent more than 10 years of painstaking efforts to find the best solution in the macromolecular polysaccharides extracted from mushroom. We overcame the bottleneck of poor stability and high production cost of polysaccharide products in the past and made the immunomodulatory additives to be more easily applied in aquaculture.

3.2 Immunomodulatory additives in aquaculture

Mushroom glucan (MBG) is a kind of natural polymeric substance (polysaccharide), which is an immunomodulatory substance, and therefore does not cause an uncontrolled increase in immunity in fish, but certain drugs, such as levamisole, may cause undesirable side effects.

In a case study on eel cultured in fresh water, we have found that MBG can properly regulate the cellular immunity; the head, kidney, and spleen are the most important hematopoietic tissues of eel, and when the fish is infected with bacteria or viruses, blood cells from hematopoietic tissues will suffer damage, its number will also be significantly reduced, and the results show that the polysaccharide applied will significantly activate the hematopoietic system of the eel, effectively increasing the number of leukocytes. After 48 hours of using the MBG, the phagocytic cells with phagocytic ability in the body can be increased by about 4–6 times; experimental observations show that the phagocytosis ability is also significantly increased by the polysaccharide-activated phagocytic cells. Studies have confirmed that after the use of polysaccharides, they can significantly enhance the cellular immune function of eels, so that the fish body can resist pathogen infection, which is effective for improving health and improving disease resistance.

The immune stimulatory effects of immunostimulants like glucan, chitosan, and other polysaccharides have been widely studied in fish and crustaceans and were reviewed by Sakai [19]. When fish received immunomodulatory substance, macrophage undergoes phagocytosis, and the rate of intracellular oxygen consumption also increases at the same time, resulting in a so-called respiratory burst phenomenon, and the reaction of oxygen and NADPH into a reactive oxygen species with bacteriostatic ability via NADPH oxidase such as superoxide anion (O_2^-) and then will be converted to hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD). The produced hydrogen peroxide can be transferred to hypochlorous acid (HOCl), etc., by myeloperoxidase. The cells can use these reactive oxygen intermediates (ROIs) to avoid the invasion of pathogens [20].

In the study of cobia (*Rachycentron canadum*) fish, it was found that after treatment with polysaccharides, the leukocyte phagocytosis rate, phagocytic index, superoxide ion yield, and lysozyme-like immune-related index were significantly improved in the 2–4 days after treatment. The activity of superoxide dismutase was highest in 4–8 days, or even up to 30 days, similar to that in the untreated group in the physiological index, thus demonstrating the efficient use of polysaccharide in the culture of cobia. It can effectively stimulate macrophages in a short period of time, improve the immunity of fish, and provide a continuous supply of immune-stimulating activity through the addition of feed, which is very helpful for the defense against disease and disease outbreak stage. And long-term treatment has no adverse effects on the fish. For the spot grouper, which is currently the main cultured seawater economic fish in Taiwan, the research has similar effects, and polysaccharides can be used for a long term and be increased in the treatment with 0.8 g/kg and 1.6 g/kg feed. The effect of phagocytes and other related nonspecific immune responses also increases significantly.

In invertebrates' culture industry, the abalone culture process often suffered problems in low rate of attachment and poor resistance to foreign pathogens. In abalone immune system, pattern recognition proteins (PRPs) play an important role in innate immunity by recognizing common epitopes on the surface of invading microorganism. In a study of pathogenic challenge experiment of abalone, the test groups were injected with Vibrio, lipopolysaccharides (LPS), and beta-1,3-glucan, respectively, and then compared to controls. Abalone PRP increase can recognize different pathogen-associated molecular patterns (PAMP) and may activate different genes involved in the defense against these pathogens. It acts as an acute inducible protein that could play an important role in the abalone immune defense mechanism [21].

The shrimp immune system has to be activated by pattern recognition proteins such as lipopolysaccharide, β-glucan, and peptidoglycan-binding proteins [22, 23]. Once these proteins are bound to their particular targets, they activate hemocytes to release their contents and trigger different biochemical mechanisms [24]. Immunostimulants increase the immune responses in several shrimp species by promoting phagocytosis, bactericidal activity, proPO activity, and respiratory bursts and enhancing resistance against pathogens [25, 26].

The use of immersion, injection, and feeding with polysaccharides to improve the nonspecific immune response for aquaculture organisms has become popular. This polysaccharide efficacy was found in the administration with freshwater carp, catfish, trout, eel, tilapia, or seawater salmon, flounder, grouper, cobia, etc. Finally, although the effects of polysaccharide function on aquatic animal-specific immunity are not directly stimulated and promoted, in other animal experiments, it has been found that the use of polysaccharides as an adjuvant for animal vaccines can increase the vaccine potency, stimulating the production of antibodies. The vaccine of fish has also been widely studied in recent years. With the aid of polysaccharides, it is believed that more research and certification are needed. Therefore, it is hoped that this will be another culture technology in the near future prospect.

4. Novel disease screening technology

The Raman spectrometer (RS) is an instrument that measures the Raman scattering light spectrum. Raman scattered light was discovered by Indian scientists in 1928. Unlike the general laser scattered light, the wavelength of Raman scattered light is slightly different from the wavelength of the original incident light. The reason for the difference is that the collision occurs after the molecules are hit by photons. Increasing or reducing photon energy causes the effects of these changes due to molecular bonding and structure [27]. Therefore, this technology is widely used in the detection of high molecular polymers, nanomaterials, electrochemistry, semiconductors, thin films, mineralogy, carbides, etc.

Surface-enhanced Raman scattering (SERS) has been proven to be highly sensitive for trace chemical detection. The power of SERS detection is the label-free detection capabilities, due to the highly specific Raman scattering fingerprint spectrum (RSFS) and combined with the high sensitivity. SERS has been used to detect a wide range of targets including bio- and nonbiomaterials (DNA, proteins, pesticides, and metabolites). RSFS is a narrow band signal with highly specific characteristic to imply that it can be used to detect multiple analytes simultaneously. Therefore, SERS may finally realize its potential as a highly sensitive and specific analytical technique for trace chemical and biomolecule detection [28].

In recent years, due to the advancement of CCD and laser, scientists have gradually pulled RS to use in the detection of bioassays and medical drugs [27, 29]. At present, SERS is used to enhance the signals needed for specific analysis. It has been widely used in life science research. Common applications include pharmaceuticals, clinical trials, cellular research, immunology, proteomics research, genetics, genetic engineering, plastic surgery, biomedical materials, environmental engineering, and biosafety. In the field of life science research, SERS is able to identify samples with single cell-level accuracy. In the future, instruments combining Raman spectroscopy and microscopic imaging technology will bring great benefits to the life science community.

With the intensive and increasing breeding scale of pigs in various countries, the problem of pig diseases is becoming more and more complicated, and mixed infections or secondary infections are also increasing. Therefore, the diagnosis and prevention of diseases have increasingly shown their importance. In addition, due to the use of antibiotics and vaccines, the symptoms of bacterial (porcine mycoplasma pneumonia and Streptococcus suis infection) or viral (porcine reproductive and respiratory syndrome and porcine type 2 circovirus infection) infections are atypically present. They also often cause serious economic losses for the livestock industry. If they are diagnosed by relying only on clinical symptoms, it will be difficult to confirm the diagnosis. Additionally, the need to strengthen laboratory diagnosis is currently the primary choice. Therefore, it is necessary to develop a nonintrusive real-time image monitoring system. In our laboratory, we tried to characterize and evaluate a SERS-based diagnostic system for the detection and identification of bacteria in the gnotobiotic mice for rapid detection of two common kinds of bacteria in rodent (*Staphylococcus aureus* and *Pseudomonas aeruginosa*)

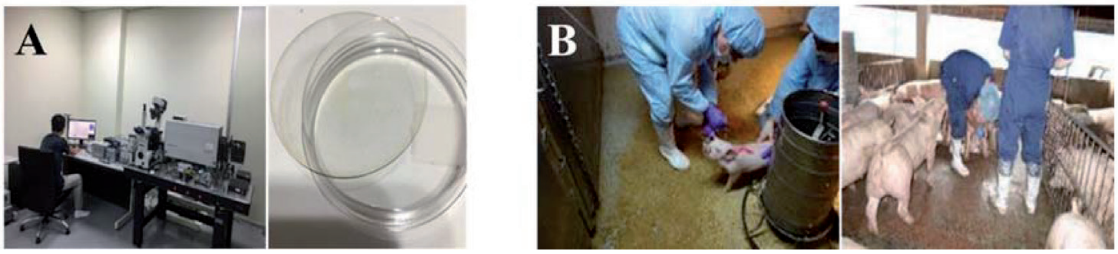

Figure 3.
SERS-based detection system and pig sample collections. (A) the SERS-based detection system. (B) Sample collections from the traditional pig farms.

Figure 4.
SERS spectra obtained with (A) Mycoplasma hyopneumoniae, *(B)* Streptococcus suis, *(C) Staphylococcus aureus, and (D) Pseudomonas aeruginosa. Bacteria recovered from pooled swine urine: (E) swine urine, (F) M. hyopneumoniae, and (G)* S. suis. *Bacteria recovered from pooled swine serum: (H) swine serum, (I) M. hyopneumoniae, (J)* S. suis, *(K) M. hyopneumoniae, and* S. suis. *Bacteria recovered from pooled swine feces: (L) swine feces, (M) M. hyopneumoniae, (N)* S. suis, *(O) M. hyopneumoniae*, and S. suis. *Colonization of gnotobiotic mice with the known bacterial strain: 0 day (feces specimens) (P) S. aureus, (Q) P. aeruginosa, (R) S. aureus*, *and P. aeruginosa. 7 days after colonization (feces specimens) (S) S. aureus, (T) P. aeruginosa, (U) S. aureus*, *and P. aeruginosa. 0 day (serum specimens) (V) without colonization. 7 days after colonization (serum specimens) (W) S. aureus, (X)* P. aeruginosa, *(Y) S. aureus*, *and P. aeruginosa. Arrows indicate the highly specific peaks of Raman scattering fingerprint spectrum for pathogens.*

and two kinds of pathogenic bacteria in swine (Mycoplasma hyopneumoniae and Streptococcus suis). SERS spectra of bacteria recovered from sera, feces, and urine were compared to pure culture bacteria. These results indicated that successful detection, identification, and classification of these bacteria from mice specimens (sera and feces) and swine specimens (sera, feces, and urine) using a SERS-based system were demonstrated. SERS is shown to offer reproducible molecular spectroscopic signatures for analytical applications. The approach may be a new and powerful tool for real-time surveillance of animal bacterial disease pathogens in clinics (**Figures 3** and **4**) [28].

This shift away from traditional aquaculture farming to large-scale intensive methods has resulted in the increasing cases of complex, mixed infections, or secondary infections. The early diagnostic and disease prevention becomes more important for aquaculture farm management. However, antibiotic treatment caused atypical diseases symptoms might be interfered clinical diagnosis. In order to perform accurate pathological diagnosis, we need to combine the strengthening laboratory diagnosis and traditional diagnostics. SERS has recently been shown to be a potentially powerful whole-organism fingerprinting technique and is used for the rapid identification of bacteria. Biosensors based on SERS hold great promise as a platform for sensitive and rapid detection of bacterial pathogens and decrease time of diagnosis [30].

In spite of these capabilities, SERS has been limited to research labs due to stationary equipment and high cost substrates. The instruments combining Raman spectroscopy and microscopic imaging technology will continuously be developed. At present, a highly sensitive and rapid method of SERS combining with electrochemical preconcentration in detecting malachite green in aquaculture water has been established [31]. However, application of SERS in the aquaculture pathogen detections is lacking now. In the future, the application of SERS in the rapid detection for aquaculture pathogens or veterinary medical drugs in the aquaculture farms is very important and needed.

5. Conclusions

Aquaculture is the fastest-growing food production field. The scientific and business communities are responding to the many challenges and opportunities. The new farming operation of friendly environment will focus on the use of nonecology destructive substances, no antibiotics, and the natural probiotics or novel immunomodulatory substances to match the physiological regulation of cultured organisms and the management of aquaculture. Finally, R&D of the novel pathogen detection technology in the aquaculture is also very important and needed.

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Conflict of interest

The authors declare no conflict of interest.

Author details

Chung-Lun Lu[1], Shiu-Nan Chen[2] and Shao-Wen Hung[3]*

1 Aquatic Technology Laboratories, Agricultural Technology Research Institute, Hsinchu, Taiwan

2 Institute of Fisheries Science, National Taiwan University, Taipei, Taiwan

3 Animal Technology Laboratories, Agricultural Technology Research Institute, Hsinchu, Taiwan

*Address all correspondence to: 1032169@mail.atri.org.tw

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