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Preface of the Proceeding of
The 3rd International Conference on Science and Technology 2018

Bismillaahirrahmaanirrahiim
Assalaamu'alaikumwarahmatullaahwabarakaatuh.
Peace be upon us.

Praise always we pray to God Almighty for giving us the abundance of grace, guidance and inayah, so that we all could meet in Lombok, a beautiful island "the Island of Thousand Mosques" in West Nusa Tenggara Province. Our Lombok island known to its many natural and cultural diversity where you can enjoy cuisines, beaches, waterfalls, mountain, traditional villages and handicraft of many ethnics in this Island.

On Behalf the Committee, I would like to thank you all attendee of the "3rd International Conference on Science and Technology (ICST) 2018" on December 10th 2019 and shared impressive ideas, knowledge and experiences through the article to build network for possible future collaboration, therefore the proceedings can be realized.

This proceeding published 43 articles from 107 presenters that came from various universities and research institutions in Indonesia and from overseas. Research papers already reviewed on the basis of a full length manuscript that accepted based on quality, originality and relevance.

At this moment, the organizing committee would like to express our gratitude to the all keynote speakers and presenters who have submitted for article and also to all participants to share their acknowledged works, your effort and contribution to the conference are absolutely valuable. Our special gratitude also goes to the Rector of the University of Mataram and Head of Institute for Research and Community Services) University of Mataram, West Nusa Tenggara, Lombok, Indonesia, who have been highly supporting the conference.

Last but not least, I would like to thank the organizing committee as well as all other supporters and participants, as without their effort, commitment and hard work, the publication of this proceeding will be hardly achieved. Critics and suggestions on the improvement of this proceeding will be highly appreciated. Hopefully the next ICST can be better prepared based on this recommendation.

Wassalamu'alaikum warohmatullahi wabarakatuh.

Chairman of 3rd ICST 2018

Dr.rer.nat. Lalu Rudyat Telly Savalas, M.Si.

TABLE OF CONTENT

Committee i

Preface ii

Table of Content iii

Keynote Speeches vi

P_ICST2018_01	First Record of Some Amblyopine and Oxudercine Gobies (Teleostei, Gobiidae) in The Lesser Sunda Islands Yuliadi Zamroni	01-16
P_ICST2018_02	Somatotype of Sasak Children at Different Altitude in Lombok Island Novita Tri Artiningrum, Bambang Suryobroto, Tetri Widiyani	17-23
P_ICST2018_03	Forecasting of Tourist Visits Probabilities using Diagonalization Transition Matrices of Markov Chain in West Lombok Regency Desy Komalasari, Baiq Triastiti Rosalina	24-27
P_ICST2018_06	From an International Standard School to A Regular School: English Primary School Teachers' Agentic Responses toward a Changed Policy of English Teaching and Assessment Santi Farmasari	28-35
P_ICST2018_07	Chemical, Microbiological, and Sensory Properties of Pickled Pindang's Eggs Affected by *Lactobacillus plantarum* Alfine Rijaldie Malik, Nazaruddin, Wiharyani Werdiningsih	36-45
P_ICST2018_10	Zonation and Analysis of Drainage Network Capacity for Better Flood Control in The Unus Drainage System, Mataram City Agustono Setiawan, Lalu Wirahman W, Bambang Harianto, IDG Jaya Negara, Zaedar Gazalba	46-56
P_ICST2018_11	Yield Components of Various Varieties of Peanut Relay Intercropped Between Rows of Maize Inoculated with Arbuscular Mycorrhiza under Two Nitrogen Levels Wayan Wangiyana, I Komang Damar Jaya, Herman Suheri	57-62
P_ICST2018_12	The Antibacterial Activity of Tomato (*Lycopersicum esculentum L*) Extract on Bacillus Cereus Causes of Food Poisoning Earlyna Sinthia Dewi	63-67
P_ICST2018_14	Kinetics Modelling of Change on Brix Value of Lime (*Cytrus amblycarpa*) while Storage Erni Romansyah, Suwati, Muliatiningsih, Asmawati	68-71
P_ICST2018_15	Analysis of Garbage Transportation System in Mataram City using Dynamic Programming with GIS-Based Data DM Ayu Lestari, Teti Zubaidah, Buan Anshari	72-78
P_ICST2018_16	Determination of Success Factors Smallholders Livestock in Realizing Beef Self Sufficiency in West Tenggara Barat Mashur, Kholik, Kunti Tirtasari, Dina Oktaviana	79-100
P_ICST2018_17	The Effect of Volume Faction of Coconut Fiber with Sengon Core Powder on The Strength of Hybrid Sandwich Composites Salman, A Alit Triady, Muhammad Hazmi	101-108
P_ICST2018_19	Reciprocal Teaching on Students' Reading Comprehension Achievement Sopian Saori, Pathul Indriana	109-115
P_ICST2018_20	Effect of Glass Cover Gap on The Performance of Flat Plate Collectors with Granite Stone Absorber Made Wirawan, Mirmanto, Dody Tantawin	116-123

P_ICST2018_24	Innovation to Increase the Tensile Strength of Sade Woven Yarn using Sizing Method Gita Mardialina, Siti Alaa, Susi Rahayu, Dian W. Kurniawidi	124-128
P_ICST2018_25	Comparison of the Effectivity between Jackknife and Bootstrap for Parameter Estimation in Regression with Assumption Violation Uny Madya binti Wasio, Mustika Hadijati, Nurul Fitriyani	129-132
P_ICST2018_26	Drying Kinetics of *Moringa oleifera* Leaves on Antioxidant Activities and Nutrition Content Rucitra Widyasari, Yeni Sulastri, Rini Nofrida, Zainuri, M. Zaini Abbas, Taufikul Hadi	133-137
P_ICST2018_27	High-Throughput In Vitro Construction of Protein Library: A Potential Technology for Containing An Emerging Pandemic Avian Influenza Muhamad Ali and Yunita Sabrina	138-144
P_ICST2018_28	Anticipating Building Damages due to Earthquake using Averaged Shear Wave Velocity in The Upper 30 M Depth (VS30) in Central Java Rita Desiasni, Ade Anggraini, Wiwit Suryanto, Amirin Kusmiran	145-153
P_ICST2018_29	Mechanical Fatigue Life Analysis of Steel Leaf Spring Structure using Finite Element Method Amirin Kusmiran, Zainal Abidin, Muhamad Hidayat, Rita Desiasni, Nurulia Shinta Rahmani	154-162
P_ICST2018_31	The Effect of Monosodium Glutamate Administration to the Prostaglandin Levels on Pregnant Albino Wistar Rats Fitri Apriyanti, Dian Soekmawaty Riezqy Ariendha, Arni Amir, Defrin	163-166
P_ICST2018_33	Determinants of Student Development to Achieve the Vision of the Faculty of Mathematics and Natural Sciences, Universitas Mataram Agus Kurnia, Nurul Fitriyani, Sigit Ary Wijayanto	167-170
P_ICST2018_34	Review: Application of Probionts to Enhance Growth Rate and the Immune Systems in Terrestrial and Aquatic Species Muhamad Amin, Muhammad Ashari, Muhamad Ali	171-182
P_ICST2018_37	The Characteristics of Physical Properties of SAP (*Arenga pinnata Merr.*) during Cooking Using Semi-Automatic Machine Stirrer Hary Kurniawan, Yeni Sulastri, Rucitra Widyasari	183-186
P_ICST2018_38	In Vitro Regeneration of Agarwood Plant (*Aquilaria filarial*) Baiq Erna Listiana, Sumarjan, Ulrich Schurr, Tri Mulyaningsih	187-194
P_ICST2018_39	Improvement of Independence Character and Student Learning Results through Development of Driil Problem based Complex Analysis Books Mariamah, Syarifuddin	195-201
P_ICST2018_40	Application of Three Dimensional Media and Computer Simulation to Improve Student Learning Outcomes Hikmawati, Kusmiyati, Sutrio	202-210
P_ICST2018_41	Determination of In Situ Permeability using Characteristics of Stoneley Waves Kosim	211-215
P_ICST2018_43	Tripolyphosphate-Crosslinked Chitosan Beads for Pb(II) Metal Ions Adsorption Made Ganesh Darmayanti, Siti Hana Itqiyah, Dedy Suhendra, Dina Asnawati	216-220
P_ICST2018_44	Growth Rate, Molting Percentage and Ecdyson Titre of Juvenile *Panulirus homarus L.* with Artemia Silage Muhsinul Ihsan, Trijoko, Nastiti Wijayanti, Handa Muliasari	221-226

P_ICST2018_45	Encryption and Decryption of Text using Hill Cipher Modified Deni Hamdani, Sjamsjiar Rachman	227-235
P_ICST2018_46	Design of Sedayu Beach Area as A Beach and Turtle Tourism Destination in North Lombok Regency Rini Srikus Saptaningtyas, Sitti Hilyana, Humairo Saidah	236-245

REVIEW: APPLICATION OF PROBIONTS TO ENHANCE GROWTH RATE AND THE IMMUNE SYSTEMS IN TERRESTRIAL AND AQUATIC SPECIES

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Abstract. Probiont has gained a lot of interest in the last few decades as a way to enhance growth and disease resistance in cultured animals including terrestrial and aquatic organisms. This approach is considered as the best method to replace the indiscriminative use of antibiotics, which are previously criticized due to safety and environmental issues. Unlike antibiotics, probionts is an eco-friendly way to enhance growth rate and improve hosts' immune systems. There are several common mechanisms how probiotic strains are contributing to their hosts, including: (1) the synthesis of digestive enzymes which participates in digestion processes; (2) the modification of intestinal environment and gut epithelial cells which allow better solubility and absorption of nutrients, (3) stimulating the expression of two genes involved in growth (Insulin-Like Growth Factor, IGF-I and Growth Hormone, GH), (4) improving stress tolerance, (5) producing antimicrobial compounds active against pathogens (6) outcompeting pathogens for chemical and adhesion sites, and (7) stimulating or developing the host's immune systems. The application of probionts has a long story, especially in terrestrial organisms. Thereafter, the successfulness of probiotic application in the terrestrials has triggered the use of probionts in aquaculture species. This review aimed at giving an outline about the current status of probiotic applications in cultured animals (poultry and aquaculture industries).

Keywords: Aquaculture, growth, immune system, probiont, poultry.

1. Background

Probionts can be defined as the supplementation of live microorganisms to enhance either growth or disease resistance of cultured animals [1]. The application of probionts was recorded firstly in the 1970s as feed supplements to improve growth or health status in terrestrial animals [2]. Since then, probionts have been widely applied in not only terrestrial organisms (human, pig, cattle, poultry) but also aquatic organisms such as fish, shellfish and crustacean [3,4,5]. In 1979 for instance, probiont was reported to improve the efficiency of feed utilization in broiler chicken [6], and followed by other researchers including in pig in 1983 [7]. While the application of probiont in aquatic species was slower. The earliest publication about probiotic use in aquaculture was documented in 1980 in Artemia by Yasuda and Taga [8].

The interest in the use of probionts was mainly due to: (1) being considered as environmentally-friendly treatments, (2) the ability to enhance feed digestibility and growth of cultivated species, (3) increased disease resistance of hosts, and (4) suitability for application to animals lacking adaptive immune systems such as invertebrates and early stages of fish (larvae) [5]. For these reasons, probionts have been defined as a major area for further research in aquaculture industries by FAO of the United Nations [9]. Since then, probiont has gained a lot of interest to be applied in cultured animals.

The aim of this review is to give an outline about current applications of probionts in cultured animals mainly in poultry and aquaculture industries.

2. The application of probionts

The interest on probiotic application in cultured animals has increased recently due to being considered as an eco-friendly technology to enhance growth and disease resistance of cultured animals. However, the application of probionts in terrestrial animals such as poultry have been earlier and much more developed compared to aquatic species.

2.1 Application probiont in Poultry

The ban of antibiotic uses in poultry has caused some problems such as wet litter, intestinal bacteria overgrowth, poor growth performance, malabsorption and diseases [10]. The first work of probiotic on broiler was published by Dilworth and Day [11]. Since then, many authors have reported the isolation of diverse probiotic strains as probiotic candidates for poultry, Table 1. Some of these studies showed that probiotics are very effective to enhance growth, immune system, intestinal balance, but also meat and egg quality in broiler and laying hen. The most recent work was published by Wu *et al.* [12], in which *Bacillus coagulans* was demonstrated to increase the growth performance and gut health of broiler chickens with *Clostridium perfringens*-induced necrotic enteritis.

Table 1. Examples of probiotic supplementation and their mechanisms in enhancing the growth of poultry

No	Probiotic strains	Suggested mechanisms	Reference (s)
1	*Enterococcus faecium* M-74	Enhance feed digestibility in laying hens	Koudela *et al.* (1996) [13]
2	*Enterococcus* sp. and *Veillonella* sp.	Inhibit the growth of *Salmonella typhimurium* in chicken	Durant *et al.* (2000) [14]
3	*Lactobacillus fermentum* subsp. *cellobiosus*	Controlling salmonellosis in Chicken intestines	Gusils *et al.* (1999) [15]
4	*Lactobacullus* spp.	Increasing body weight, higher feed intake, and lower feed efficiency	Zulkifli *et al.* (2000) [16]
5	*Bacillus subtilis C-3102*	Enhance feed digestion and feed conversion in Broiler chicken	Fritts *et al.* (2000) [17]
6	*Pichia farinosa* SKM-1, *Pichia anomala* SKM-T, and *Galactomyces geotrichum* SJM-59	Improve laying performances, and egg quality in laying hens	Mo *et al.* (2004) [18]
7	*Bacilius subtilis*	Inhibiting the growth of *Clostridium perfringens* in chicken	Teo and Tan (2005) [19]
8	*Lactobacillus* sp.	Stimulating immune system in broiler chicken	Dalloul *et al.* (2005) [20]
		Improve egg quality in Brown layers	Xu *et al.* (2006) [21]

9	*Lactobacillus fermentum* and *Enterococcus faecium*	increased serum calcium and iron level, decreased triglycerides content in blood and slightly increased body weight of broiler chicken.	Capcarova *et al.* (2010) [22]
10	*Bacillus licheniformis*	Enhancing Growth Performance and Meat Quality of Broiler Chickens	Liu *et al.* (2012) [23]
11	*Lactobacillus Pentosus* Ita23 and *L. Acidipiscis* Ita44	Enhancing Feed Conversion Efficiency and Beneficial Gut Microbiota in Broiler Chickens	Altaher *et al.* (2015) [24]
12	*Bacillus subtilis* CGMCC 1.1086	Improving growth performance and intestinal microbiota of broilers	Li *et al.* (2016) [25]
13	*Pediococcus acidilactici*	Producing antimicrobial activity against *Salmonella gallinarum* in broiler chicken	Han *et al.* (2017) [26]
		Poultry: improving feed efficiency and performance, immunity, and meat and egg quality	(Alagawany, et al., 2018 [27]; Mahfuz *et al.*, 2017 [28])
14	*Bacillus coagulans*	Increasing the growth performance and gut health of broiler chickens with *Clostridium perfringens*-induced necrotic enteritis	Wu *et al.* (2018) [12]

2.2 Application probiont in aquatic species

The first study which reported the application of probiotic in aquaculture species was in the late of 1980s, and since then the research effort has continually increased [29]. Tabel 2 represents several publications about probiotic application in aquaculture commodities. In general, those probionts contribute in the same ways as those applied in terrestrials (enhance growth rate and disease resistance). However, additional mechanisms were converting toxic chemicals from waste (ammonia and nitrite) to less toxic chemical s such as nitrate.

This approach is very important in the aquaculture field as a way to boost the immune system of invertebrate aquatic organisms with less developed immune systems. Many invertebrate aquatic species have very expensive such as abalone, shrimp, and lobster but very vulnerable to diseases. The practical use of vaccination in these animals has been questioned due to their weak immune systems [30,31].

Table 2. Examples of probiotic supplementation and their mechanisms in enhancing the growth and resistance to pathogens of aquatic animals.

No	Probiotic strains	Suggested mechanism	Reference (s)
1	*Lactobacillus delbrueckii delbrueckii*	Enhanced the expression of gene involved in muscular growth (IGF-I), while lowering and MSTN mRNA transcription in juvenile sea bass	Carnevali *et al.* [32]
2	*Bacillus lincheniformis*	Increased amylase activity in the intestinal tract of white shrimp	Hu *et al.* [33]; Ziaei-Nejad *et al.* [34]
3	*Bacillus* spp.	Enhanced protease, amylase, and lipase in the GITs of Indian shrimp	Ziaei-Nejad *et al.* [34]; ten Doeschate and Coyne [35]
		Enhancing protease, amylase, and lipase in the GIT of common carp	Wang and Xu [36]; Iehata *et al.* [37]
4	*Lactobacillus curvatus* and *Leuconostoc mesenteroides*	Increased amylase, lipase, and protease activity in the intestinal tract of Belunga and Persian sturgeon	Askarian *et al.* [38]; Suzer *et al.* [39]

5	*B. subtilis*, *Bacillus pumilis*, *B. amyloliqueficiens* and *B. licheniformis*	Up-regulated the expression of 2 genes involved in growth: IGF-I and GH	Shaheen *et al.* [40]
6	*Vibrio midae* SY9	Produce protease	Huddy and Coyne [41]; Huddy & Coyne [42]
7	*Vibrio* spp.	Increased agarase activity in the GIT of abalone	Faturrahman *et al.* [43]
8	*Rhodotorula benthica* D30	Increased amylase, cellulase and alginase activity in the GIT of juvenile sea cucumber	Wang *et al.* [44]
9	*B. subtilis*, *B. licheniformis* and *B. pumilus*	Improved intestinal morphology: higher perimeter ratio, larger diameter and denser microvilli count which lead to the increasing of enterocyte absorptive area in Tilapia	Faturrahman *et al.* [43]; Adeoye *et al.* [45];
10	*Lactobacillus plantarum*	Increased protease activity and improved FCR and PER of giant freshwater prawn	Dash *et al.* [46]
11	*Virgibacillus proomi* *Bacillus mojavensis*	Enhanced digestive enzyme activity in the intestinal tract of sea bass	Hamza *et al.* [47]
12	*Bacillus* sp. *Pediococcus* sp. *Enterococcus* sp. and *Lactobacillus* sp.	Increased the height of intestinal villi in Senegalese sole (*Solea senegalensis*)	Barroso *et al.* [48]
13	*B. pumilus* SE5	Produced digestive enzymes	Yan *et al.* [49]; Ozorio *et al.* [50]; Wang *et al.* [44]
14	*Bacillus coagulans*	Increased the activity of protease, amylase, and lipase in the GIT of freshwater prawn	Gupta *et al.* [51]; Yan *et al.* [49]; Ozorio *et al.* [50]
15	*Weissella cibaria*	Produced protease in Siberian sturgeon	Hashemimofrad *et al.* [52]; Gupta *et al.* [51]
16	*Carnobacterium divergens*	Producing antimicrobial compounds which protected cod fry from *V. anguillarum*	Gildberg and Mikkelsen [53]
17	*Lactobacillus rhamnosus*	Protecting rainbow trout against *A. hydrophila* through antimicrobial compound production, competitive exclusion, and immune modulations.	Nikoskelainen *et al.* [54]; Panigrahi *et al.* [55]
18	*Lb. plantarum*	Increasing resistance of white shrimp against *V. alginolyticus* by enhancing haemocyte counts, phenol oxidase and phagocytic activities.	Chiu *et al.* [56]
19	*Leuconostoc mesenteroides CLFP 196* and *Lactobacillus plantarum CLFP 238*	Protecting rainbow trout against lactoccosis	Vendrell *et al.* [57]
20	*Pediococcus acidilactici*	Protecting shrimps from *V. nigripulchritudo* by immune stimulation	Castex *et al.* [58]
21	*Enterococcus faecium* and *Streptococcus phocae*	Protecting shrimp from vibriosis by competitive exclusion and antimicrobial compound production	Swain *et al.* [59]
22	*Pediococcus acidilactici*	Enhanced total antioxidant status in shrimp	Castex *et al.* [60]

GIT: gastrointestinal tract, FCR: feed conversion ratio, PER: protein efficiency ratio

Working Mechanisms of probionts
Improving growth
The production of digestive enzymes

Many bacterial strains contribute by excreting digestive enzymes such as amylase, lipase, cellulase, alginate lyase, phytase and protease to enhance feed digestibility [3,61,4]. The addition of *Lactobacillus* spp., to larvae of sea bream (*Sparus aurata*, L.) via live feed (Artemia and rotifers) was reported to have higher enzyme activity in the intestinal tract, and had 2-9 % higher specific growth rate than the control [39]. The addition of *Metschnikowia* sp. C14 was reported also to increase protease and lipase activity in the intestinal tract of sea cucumber (*Apostichopus japonicus*) [62]. Furthermore, the mixture of *Bacillus subtilis* and *Enterococcus* sp. Was reported by Nimrat *et al.* [63] to improve protease in black tiger shrimp (*Penaeus monodon*). These studies suggest that quantity of digestive enzyme could be increase through probiotic supplementations.

The modification of intestinal conditions

Several probiotic strains have been documented to modify intestinal conditions and gut epithelial cells, which allowed better solubility and absorption of nutrients by cultured animals [64]. Lactic acid bacteria (LAB) for example produced organic acids which lowered pH of intestinal juice [65]. Then, the acidification process has been described to increase nutrients' solubility and absorption [66]. The same result was confirmed by Hadi *et al.* [67] in which the supplementation of acid-producing *Enterococcus* sp. through diets decreased intestinal pH and increased nutrient absorption by abalone.

Stimulating the expression of growth and stress-related genes

Carnevali *et al.* [32] described that *Lactobacillus delbrueckii delbrueckii* in juvenile of seabass enhanced the expression of gene involved in muscular growth (IGF-I), while lowering and MSTN mRNA transcription, which was in agreement with the increase of the fish growth In addition, the administration of *Bacillus* mixture upregulated the expression of two genes involved in growth: IGF-I and GH [40]. The administration of *Lactobacillus delbrueckii delbrueckii* in juvenile of seabass lowered cortisol level which is an indicator for stress condition [32]. Similarly, Olmos *et al.* [68] also documented the administration of *Bacillus* sp. lower cortisol level in shrimp. The mechanisms behind this action are still not clear. A study by Neuman *et al.* [69] suggests that the bacteria produced hormone, respond to hosts hormones and regulate the expression levels of host hormones.

Enhancement of disease resistances

Probionts could enhance disease resistances of cultured animals through several mechanisms (Table 2), including: Among the suggested mechanisms are: (1) producing antimicrobial compounds active against pathogens; (2) outcompeting pathogens for chemical and adhesion sites, and (3) stimulating or developing the host's immune systems. Due to these capacities, probionts have been considered as a potential way to increase the survival of cultivated animals, especially animals with less-developed immune systems.

Production of antagonistic compounds

LAB and other probiotic genera have been documented to elicit antagonistic compounds to other pathogenic bacteria such as bacteriocins, diacetyl, bacteriolytic enzymes, hydrogen peroxide (H_2O_2) and organic acids which could alter environmental pH values [70,71,72,29,73,74]. Some examples are; (1) nisin which is produced by *L. lactis strains*, effectively suppress growth of *A. hydrophila*, and *Staphhylococus aureus*, (2) carnocin which is produced by *Carnobacterium* sp [70,75] and, (3) Enterocins, durancin and mundaticin produced by *Enterococcus faecum* [76] and (4) plantaricin, AMP secreted by *L. plantarum was reported to* inhibit growth of *A. hidrophyla* that even more powerful than chloramphenicol (30 µg) or gentacimin (15 µg) [77, 75, 74]. Lactobacillus paracasei produce a bacteriocin in term of a phenolic compound, and bacillus produce lipopeptide antibiotics and surfactins [74].

Modulating and stimulating immune functions

Probiotics positively could stimulate host's immune systems number of ways. Firstly, the probiotics cause intestinal lining to be less permeable in which harmful microbes and toxins cannot enter into fish bloodstream [78]. This sort of collectively cell response is done because probiotics could strengthen a tight junction between intestinal cells. Secondly, probiotics could stimulate the development and maturation of gut immune system (GALT; gut-associated lymphoid tissues) which stores various immune functions including eosinophil granular cells, lymphocytes, granulocytes and plasma cells [79]. In addition, probiotics could also stimulate the production of immune compounds such as cytokines which promote good inflammation. According to Farzanfar [73] probiotics improve host immunity by increasing macrophage activity, increasing antibody production such as immunoglobulin and interferon and increasing antibody at mucus surfaces in the hosts' gut wall. Gnotobiotic studies revealed that bacteria colonizing fish GI tract could stimulate the 15- gene expressions which are involved in DNA replication and cell division for epithelial proliferation as well as innate immune systems such as serum amyloid, C-reactive protein, complement component 3, angiogenin, glutathione peroxidase and myeloperoxidase [79,80]. The same idea was confirmed by Panigrahi *et al.* [81] in which *Lactobacillus* sp were able to stimulate B cell proliferation in GALT through a classical antigen-specific immune response and increasing of phagocytic and lysozyme activity in rainbow trout. In addition, Chiu *et al.* [82] also reported that the expression level proPO mRNA and phenoloxidase activity was 6.1 fold higher from white shrimp fed with *L. plantarum* containing diets with dose 10^7 cfu. Kg^{-1}diet after 168 h. Furthermore, *Bacillus subtilis* strain L10 and G1 was reported to increase survival rate of white shrimp after challenged with *V. harveyi* due to its ability to induce the expression of four immune-related genes such as serine protein (SP), peroxinectin (PE) genes, β-1,3-glucan binding protein (LGBP) and later on prophenoloxidase (proPO) genes which later increase prophenoloxidase (PO) activities, enzymes that controls and regulate innate immune system in invertebrate [82,83,84]. Avella *et al*. [85] reported that adding lactobacillus rhamnosus to clownfish increased expression of the growth and development genes such as myostatin, peroxisome-proliferator-activated receptors α and β, insulin-like growth factors I and II, vitamin D receptor α, and retinoic acid receptor ϒ.

Competition for chemical and adhesion sites

Nutrients and space are important factors which bacteria may compete on in gastrointestinal tract. The availability of nutrient as energy, carbon or electron source also plays significant roles in establishing microbial composition in fish GI tracts. For instance, Iron is highly needed by all microorganism for growth [86] especially for diseases-causing bacteria. Therefore, harmless LAB that could produce siderophores (soluble Fe^{3+} binding agents) is one important characteristic for probiotics candidates [29].

In addition, bacteria also compete for adhesion sites [29]. The ability of a bacterial strain to attach and associate with mucosal surface of alimentary tract is the most crucial step for being selected as probiotics, because pathogenic bacteria are also use the same step in do infection [87]. The adhesion mechanisms can be based on host-microbe specific interaction or host-microbe non-specific interaction [88,89]. Specific mechanism means that the adhesion is based on physicochemical factors such as carbohydrate-specific molecule of bacterial cell surface component in lactobacillus [88], and nonspecific is based on the involvement of adhesion molecules on the bacteria surface and receptor molecules on the fish mucosal surfaces such as non-covalent bond and hydrophobic interaction (increasing hydrophobicity means better adhesive activity) [29,88,89]. A study by Perez-Sanchez *et al.* [90] suggested that an adhesion capability of bacterial strains could be displayed by their level of hydrophobicity, the higher its hydrophobicity the stronger adhesion ability. Some examples of these strains are *Lactobacillus plantarum* and *Lactococcus lactis*. Ziaei-Nejad *et al.* [91] observed that *Bacillus subtilis* could replace *Vibrio* spp in intestinal tract of Indian white shrimps.

Another example of space competition between probiotics and pathogens is explained by Tannis [78], in which after harbor in GI tract, probiotics struggle to reach intestinal receptor sites along the epithelial cells. Epithelial cells that lines the intestinal tract appears to be able to cross talk, allowing the cells for a communal exchange. Intestinal cells have junctions between them called "tight junction", and through this junctions, the cells communicate to each other's, which enable them to react as a group. When probiotics tell one cell to change to prevent bad microbes from attaching, the cell can then tell all of its neighbors to do the same. The result is large protective change that protects the body from infection and creates immunity. For instance, cells react together by producing mucus which unable pathogenic microbes to attach to intestinal receptors the lining of the intestinal tract is covered in mucus [92].

Conclusion

Probionts could be applied in both terrestrial and aquaculture animals to enhance feed digestibility and immune systems. This approach is very eco-friendly and should be widely recommended to replace the indiscriminative use of antibiotics in cultured animals.

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