Chapter 10
Lactic Acid Bacteria in Animal Breeding and Aquaculture

Gang Wang and Xing Jin

10.1 Application of Lactobacillus in Animal Husbandry

With the rapid development of social economy, animal husbandry, like agriculture, industry and handicraft industry have become one of the social industries. With the increase of mass consumption, the development of animal husbandry industry has reached a new height. However, a series of problems which restrain the development of animal husbandry industry have also entered people's vision with the continuous expansion of its scale. Animal diseases which always occur in livestock farming can lead to the death of livestock or poultry and result in serious economic losses. The common types of animal diseases can be grouped into the following three types.

1. Common diseases: medical, surgical, and obstetrical diseases of animals. The incidence of common diseases is high, and the clinical symptoms are also diversified.
2. Infectious diseases: this kind of disease is mainly caused by pathogenic microorganisms and has certain incubation period and clinical symptoms, epidemic and infectious. Viruses, bacteria, and fungi can be the pathogenic microorganisms which induce their occurrence. The clinical symptoms and pathological changes of animals have certain particularity, and these diseases are difficult to prevent.
3. Parasitic disease: caused by the invasion of parasites (arthropods, protozoa, and worms) into humans. The poultry and livestock eat soil, drinking water or feed containing worm eggs or larvae will sichen. Besides the detrimental factors of animals' growth or activity environment, a series of human factors may also cause animal diseases including the irrational use of drugs in breeding process, the imperfection of breeding equipment and construction, and the adverse management of breeding farms.

G. Wang (✉) · X. Jin
Jiangnan University, Wuxi, China
e-mail: wanggang@jiangnan.edu.cn

W. Chen (ed.), *Lactic Acid Bacteria*,
https://doi.org/10.1007/978-981-13-7283-4_10

10.1.1 Prevention and Treatment of Lactobacillus in Livestock Diseases

It has been a key issue for livestock farmers to prevent and control animal disease, and it plays an important role in improving the economic efficiency of the livestock industry with the development of farming. At present, the use of lactic acid bacteria preparation added to livestock feed increases year by year. The researches have reported that lactic acid bacteria can adjust the mammal intestinal microflora balance, strengthen the body's immunity and resistance, restrain the growth of pathogenic bacteria, keep animal intestinal flora balance, and adjust gastrointestinal digestion and absorption function. Adding a certain amount of lactic acid bacteria in animal feed formulation can compete ecological sites, inhibit the growth of pathogenic microorganisms, decrease the enterobacteriaceae bacteria in the gastrointestinal tract, and keep the balance of animal intestinal microecological bacteria when feed are eaten by animals.

With the rapid development of China's dairy industry, the dairy consumption market will continue to expand and become mature. Effectively expanding the efficiency of cow breeding, improving milk production, and reducing the economic loss caused by diseases also have become key issues to be urgently solved by farmers. Mastitis is a kind of multiple diseases in mammalian diseases and has the characteristic of wide range, high incidence, difficulty in curing, and easy to relapse. Once the cow is infected with mastitis and cannot be effectively controlled in time, it will bring huge economic loss to the farmers (Persson et al. 2011). At present, most antibiotics are used for the prevention and treatment of mastitis. But, it will bring many problems such as increased bacterial resistance, antibiotic residues, and environmental pollution caused by resistance genes. The use of probiotics as a substitute for antibiotics is attracting more attention from scientists. Yang (Yang et al. 2014) used the compound probiotics including *Lactobacillus casei* HM-09 and *Lactobacillus plantarum* HM-10 as 100–200 g per head daily dosage which had the living bacterium acuity 1.5×10^9 CFU/g of lactobacillus in Holstein cows from different areas of pasture. Meanwhile, CMT and DHI methods were adopted to test the cow mastitis inspection and the number of somatic cell detection to research the controlment of lactobacillus for dairy cow mastitis. The result showed that the average somatic cell number of cow decreased by 39.8–62.8% after 5–10 days. And more importantly, the cure rate of mild and severe mastitis was 71.21% and 33.00–74.19%, respectively, after 10–30 days of lactobacillus microecological preparation. It was showed that lactobacillus had a good therapeutic effect on mastitis.

Neonatal calf diarrhea is very harmful to the growth and development of calves. The symptoms are diarrhea and the feces is watery when the calf is sick. At the same time, dehydration and acidosis will also occur in calf which lead to the death of calf. Diarrhea is spreaded quickly and easily through the digestive tract that acts as the main transmission route and brings huge economic losses to cattle breeding industry. Less than 2 months calves have low resistance and less developed digestive system. So they are susceptible cattle. The categories of diarrhea can be divided into bacterial diarrhea and viral diarrhea. The average incidence of each other is 36.7% and 7.5%, respectively. The inhibitory activity of lactobacillus on *Salmonella*, *Shigella*, and *Escherichia coli* has been extensively studied, and the application of lactobacilli preparations in animal husbandry has great significance for promoting the sustainable and healthy development of the industry (Liao et al. 2012).Yao et al. (2014) used lactobacillus preparation to control the calf diarrhea. After 15 days prevention test, the rate of calf diarrhea in the lactobacillus preparation group was 14.29%, while that in the control group was more than twice as high (29.76%). Diarrhea calves in lactobacillus preparation group did not die, while the death rate of diarrhea calves in control group was 3.57%. The rate of diarrhea decreased from 100% to 3.70%, and the cure rate of diarrhea increased from 0% to 85.19% in lactobacillus preparation group. It was also found that early feeding of lactobacillus biologica to new calves would be more effective in preventing diarrhea.

In addition, cows will also suffer from a disease called cow endometritis after parturition. The incidence of cow endometritis is as high as 20–40% in China and has been a common frequently occurring disease. The extension of calving duration, decrease of milk production, and lesion of reproductive organs will happen when the cows are ill. At the same time, the cost of expensive treatment also brings great economic losses to farmers. Lactobacillus were the main bacteria which isolated from healthy cows, but *Staphylococcus aureus*, *Streptococcus agilis*, and *Escherichia coli* were the three most pathogenic bacteria isolated from the uterus of sick cows. Through staining microscopy, biochemical test, and PCR detection, the lactobacillus strain isolated from healthy cows was identified as *Lactobacillus acidophilus.* Safety animal studies determined that the three strains (*Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, and *Bacillus natto*) did not cause death and other diseases in mice. Three strains of bacteria were cultured under the optimal culture conditions and then were mixed into a certain proportion of microecological preparations to treat the successfully modeled mice and rabbits. From the results of autopsy and pathological section, the treatment effect was certain. Some studies showed that lactobacillus fermentation was used to treat endometritis in cows. The results showed that the effective rate of endometritis in cows was 95.23% and the cure rate was 85.71% which indicated that the microecological preparation could be used as a treatment drug for endometritis in cows. It also provided a new approach to solve the problem of high incidence of endometritis in cows and antibiotic residues in milk.

Many studies had shown that compound probiotics as feed additives could improve intestinal development and promote animal health in young animals and have been widely used in pig breeding. Liu (Liu et al. 2012) studied that the fodder added probiotic preparations could significantly improve daily gain early weaning piglets and reduce the material weight ratio and diarrhea of weaning piglets. Bao (Bao et al. 2015) verified that probiotics could significantly improve conservation pig weight, improve daily gain, and reduce material weight ratio and morbidity rate. It was also reported that two different compound probiotics were added to piglet feed. The results showed that the daily weight gain of the group adding 0.1% probiotics increased by 6.53% and 4.02%, and the rates of diarrhea decreased by 15.55% and 4.44% compared with the control group.

In modern pig production, antibiotics are often used to prevent the occurrence of diseases in order to improve their resistance to diseases and their resistance to stress. Such as lincomycin, long-term use of antibiotics affects the intestinal microorganism, the intestinal microflora of pigs, and the digestion of feed. The use of microecological preparations can alleviate the side effects of antibiotics on intestinal microorganisms by supplementing beneficial microorganisms. The microecological preparation can inhibit the adhesion and growth of harmful microorganisms, enhance the elimination of harmful microorganisms, stimulate the differentiation and development of gastrointestinal tract, and improve the digestibility of nutrients by establishing a dominant microflora to maintain beneficial microorganisms in gastrointestinal tract. Harmful bacteria in the intestinal tract break down food residues and produce toxic compounds (such as ammonia, amines, indoles, skatole, and nitrite) which will be absorbed by host can reduce the animals' immunity and even lead to various diseases. Most *Escherichia coli* are normally harmless bacteria in the gut, but overmuch reproduction can cause digestive problems. It was reported that the addition of *saccharomyces cerevisiae* and lactobacillus lactobacilli into the feed could significantly reduce the amount of *Escherichia coli* in the feces of weaned piglets. And the number of *Escherichia coli* in the feces of weaned piglets decreased significantly from second week of the trial after feeding the related composite bacteria.

10.1.2 Improvement of Growth Performance on Livestock by Lactobacillus

With the acceleration of China's modern animal husbandry development and increasingly mature, small investment, short cycle and high yield of development mode has preliminary forming. It is important to promote the industrialized operation of agriculture and increase the income of farmers and herdsmen. It also has a big gap between our country and world's advanced level on breeding scale and management level of animal husbandry and a great potential development space on our country animal husbandry. The development of modern animal husbandry is based on the development of feed industry, the core of which is to vigorously develop green, safe, and efficient feed additives. As one of the main members of green feed additive, microecological preparation is more and more favored by farmers. Therefore, the application of microecological preparation in animal breeding and management becomes more and more important.

Animal microecological preparation is a kind of feed additive which can stimulate the growth of beneficial microorganism in the animal. Animal microecological preparations are mainly divided into probiotics, prebiotics, and biobiotics. At present, there are more than 40 kinds of microorganisms which can be directly fed in the world and 16 kinds of microorganisms which can be directly added to the feed announced by China's ministry of agriculture no. 658. It is reported that probiotics as growth-promoting feed additive substituting antibiotics had great potential. After adding probiotics to feed, it could improve the palatability of feed and improve livestock feed intake. At the same time, probiotics could not only degrade the complicated structure and large molecular weight protein to small molecular peptides and free amino acid, but also compound a variety of animal vitamins. What's more, the probiotics could promote the absorption of trace elements, benefit to animal gastrointestinal digestion and absorption, and improve feed conversion rate.

Through the breeding process on selecting probiotics, the healthy animals are used. The selected probiotics can produce acid and digestive enzymes, inhibit the pathogenic bacteria, and colonize the animal digestive tract. It also can tolerate animal gastric acid environment, bile salt, feed processing, and drug sensitivity at the same time. Probiotics can produce a certain number of bacteriostatic substances (such as antimicrobial peptides, bacteriocin) which can selectively inhibit the growth of some pathogenic bacteria. Meanwhile, a large number of probiotics which colonize in the animal's intestinal tract can form the advantages of bacterial flora and play a protective role for the animal's digestive tract (especially the intestinal tract). At the same time, the colonization of probiotics can maintain the facultative anaerobic environment of the animal digestive tract which can be conducive to the establishment of normal flora of animals and the restoration of animal health. Probiotics can selectively stimulate the rapid reproduction of native probiotics in animals' intestines; it plays an important role in the establishment and recovery of normal flora balance in animals. Therefore, in the process of animal breeding, the effect of promoting animal health and improving animal growth and production performance can be achieved by adding microecological preparations.

Probiotics are widely used in pig breeding, and there are different purposes at pig different stages (Chaucheyras-Durand and Durand 2010). In pregnant sows, probiotics are mainly used to improve the digestibility of feed, reduce constipation, and reduce stress. In lactating sows, the main purpose of adding lactobacillus is to improve the quality and yield of milk and increase the survival rate of piglets. For piglets, *Lactobacillus* is added mainly to increase body weight and reduce diarrhea. In the fattening stage, probiotics are mainly used to reduce diarrhea and improve feed utilization rate and meat quality. Weaning for piglets is a very complicated period which piglets need to be strongly separated from gilts, milk and delivery room to transited, plant feed and nursery. In addition, the digestive system of piglet is not well developed which is likely to cause stress, intestinal diseases, and economic losses.

Some studies have shown that probiotics could balance the intestinal flora, reduce the colonization of pathogenic bacteria, inhibit the proliferation of pathogenic bacteria, and improve the digestibility of nutrients and growth performance. Yang et al. (2009) added 350 mg/kg of compound probiotics containing lactobacillus, yeast, and bacillus subtilis to the feed of weaned pigs. The result showed that probiotics could improve the activity of protease, lipase, and amylase in the intestinal tract. Zeyner and Boldt (2006) showed that giving piglets oral fecal enterobacteria every day from birth to weaning could improve growth performance and reduce the incidence of diarrhea. Adding BioPlus 2B (*Bacillus subtilis*) to pig feed could reduce ammonia emissions, but it had no significant effect on growth performance. It is reported that adding 0.05% condensation bacillus on the basis of weaned piglet diet made weight significantly higher than the blank control group. In the experiment, feed conversion rate was observably enhanced, and diarrhea rate was decreased significantly. At the same time, lactobacillus and bifidobacterium increased, and *E. coli* decreased significantly in excrement and urine. Condensation bacillus preparation added in the growing pig feed can significantly increase the average daily gain of pigs and reduce feed costs. There was no significant difference between the bacillus and antibacterial dysentery bacteria (Zhou et al. 2012). Scientists believed that the addition of bacillus subtilis could promote the growth performance of the body, prevent the occurrence of diarrhea, increase the daily intake and daily weight gain of piglets, and decrease the rate of diarrhea and feed conversion. After feeding suckling pigs with *Bacillus coagulum* CNCMI-1061, Adami and Cavazzoni (1999) found that *Bacillus coagulum* could colonize the intestinal tract and inhibit the growth of strains such as *E. coli*. The results showed that adding probiotics into the feed could significantly improve its productive growth performance and promote the balance and stability of its intestinal flora. It also could improve the body immunity and prevent or treat diarrhea effectively.

In young ruminants, probiotics added into fodder mainly promote the perfection of rumen microflora, reduce the stress of weaning, and reduce the perniciousness of pathogenic microorganism. In milk production, probiotics are used to increase the milk quality and yield. In beef cattle production, the main purpose of probiotics is to increase body weight and improve feed conversion. Studies have shown that adding probiotics can regulate intestinal pH and reduce the risk of acidosis. Adams et al. (2008) found that *Propionibacterium jensenii* added into the diet could increase the body weight of calves and promote rumen development. The growth performance of beef cattle could be improved at the later stage, but the experimental results were often inconsistent. Qiao (Qiao and Shan 2007) found that the addition of bacillus licheniformis to the diets of Holstein cows significantly increased milk production. It is also reported that adding probiotics could improve the survival rate and weaning weight of lamb.

Although the digestive system of ruminants is complex, probiotics can improve the intestinal flora and inhibit pathogenic bacteria. Luan et al. (2008) found that *Bacillus natto* could significantly improve milk fat rate and increase milk protein rate and milk production. Zhang et al. (2008) believed that *Bacillus natto* could increase the daily weight gain and daily food intake of calves, reduce the feed weight ratio, and improve the ketone body rate. In addition, probiotics could promote the degradation metabolism of feed and the generation of volatile fatty acids in rumen. Other research results showed that feeding bacillus subtilis could significantly reduce the pH of chyme in duodenum and jejunum of calves and improve the quality of weaning early calves.

10.1.3 Preparation and Application of Lactobacillus Silage

According to the law of world agriculture development, the traditional agriculture which mainly produces grain has been transformed into modern agriculture which mainly produces animal husbandry. Since the beginning of twenty-first century, with the rapid development of China's economy and the gradual improvement of people's living standards, animal husbandry has maintained a rapid growth. But, conventional feed shortage has already turned into the main factors restricting the development of China's animal husbandry. Mining the potential of existing feed resources and adjusting measures to local conditions of land development and agricultural by-products (rice straw and green feed) have become the main ways to solve this challenge. It is also one of the world today the trend of the development of the feed at the same time.

With the development of science and technology, the fermentation method of pure seed inoculation is used to simplify the silage production process. This fermentation method guarantees the ecological security, is conducive to maintaining the stability of the product, and improves the nutritional quality of forage after fermentation. The simple and convenient use of microbiological preparations is conducive to realizing industrialized production, shortening the mature period of finished products, playing a normative role in the standardization and safety of product features, and greatly improving the market competitiveness of products. At present, more and more studies have been reported on the addition of biological agents to improve the quality of fermentation. Many countries have developed many additives specially used for silage and put them into the market as commodities.

By fermentation of lactobacillus, forage can improve quality essentially and solve food safety problems caused by food chain from the source. From the perspective of economy, lactobacillus can make use of almost all common plants and reduce the cost of farming. From the perspective of resources, the nutrients of the feed after fermentation by lactobacillus are more abundant than before fermentation, which improves the utilization rate of raw materials by animals and saves natural resources. The experience of the rapid development of animal husbandry at home and abroad proves that the development of silage is an important way of modern animal husbandry. With the development of modern biotechnology, silage processing technology has become more and more mature. New biological additives can significantly improve the quality of silage and make a qualitative leap in the feeding value of forage. Not only that, the safety of silage products with biological starter added has been greatly guaranteed. Therefore, the development of high-quality lactobacillus starter has become a key part of silage technology.

The mainly metabolites produced by lactobacillus are lactic acid, which can resultfully reduce the growth of plants' own pathogenic bacteria in the fermentation process and give the materials good flavor and texture. The material becomes soft and has the favorable fragrant sour taste and palatability after fermentation. It can stimulate the appetite of livestock, promote the secretion of digestive fluid, and increase the frequency of intestinal peristalsis. So, the fermented feed can enhance the digestive function of animals and prevent constipation. After fermentation, the nutritional characteristics of plant raw materials were improved. Generally, higher water and protein content will lead to lower fiber content. This will improved the digestibility of animal feed. These fodders are rich in vitamin and appropriate proportion of nitrogen and phosphorus. The artificial control of fermentation process makes it free from natural factors such as weather and reduces the probability of pollution and corruption. The metabolic products such as lactic acid can prevent the deterioration of the material, so as to achieve long-term preservation and meet the need of winter forage shortage.

Some researches showed that the content of pH, amino acid nitrogen, and cellulose of lactobacillus silage alfalfa was significantly reduced, and the number of living bacteria of lactobacillus was significantly increased. The digestibility of nutrients of fermentative alfalfa increased significantly with the extension of time, and the loss rate of dry matter did not exceed 12%. They also found that the contents of soluble carbohydrates, the digestibility of organics, the recovery rate of dry matter, the content of lactic acid, and the ratio of lactic acid or acetic acid were increased in the silage with lactobacillus addition. The content of neutral washing fiber, acidic washing fiber, pH, ammonia nitrogen/total nitrogen, and volatile fatty acid decreased.

In recent years, some studies have carried on the preliminary fermentation juice (previously fermented juices, PFJ). PFJ is similar to lactobacillus preparation. It is prepared by fermenting the inherent lactobacillus on the plant to achieve the goal of rapid proliferation of lactobacillus. The addition of PFJ can rapidly increase the content of lactic acid in the stock, reduce pH, inhibit the activity of *Clostridium difficile*, and protect the decomposition loss of protein. Its addition effect is not affected by the growth period of silage material, DM content, and silage conditions. It is more stable than lactobacillus in improving silage fermentation quality. PFJ enables the proliferation of lactobacillus on the surface of forage grass as a promoter of lactic acid fermentation. What's more, it is also a good substitute for lactobacillus preparation.

A large number of experimental studies have confirmed that probiotics can improve production efficiency and improve animal health in animal husbandry, but there are still some results that show that probiotics have no promoting effect on animal husbandry production and even have negative side effects. The main factors influencing the effect of probiotics include the species and physiological state of target animals, the preparation and storage conditions of feed, the adding mode of probiotics, and the characteristics and dosage of probiotics.

10.1.3.1 Characteristics and Dosage of Probiotics

Probiotics (such as bifidobacterium, lactobacillus, and bacillus) have benefits in gastrointestinal tract function mainly in the form of living bacteria. For forming the stomach competitive exclusion engraftment on harmful bacteria and producing antibacterial substances to inhibit the growth of harmful bacteria, the probiotic bacteria must be able to pass the acidic environment of the stomach and intestinal bile secretion. According to statistical analysis by Jadamus et al. (2002), one of the important reasons for the inefficacy of probiotics in production was that the probiotics used were difficult to survive in the animal intestines. Therefore, the intestinal normal bacteria should be selected as much as possible to improve the tolerance of probiotics to the intestinal environment. The synergism and antagonism between different strains should be fully considered. The effects of probiotics are closely related to the number of live bacteria. If the number of live bacteria ingested by animals is too small, it will be difficult to colonize and grow in the intestinal tract. When the number of live bacteria is too large, it is easy to cause waste and even produce side effects. Because the proliferation of microorganisms requires energy consumption.

10.1.3.2 The Adding Mode of Probiotics

Timmerman et al. (2005) emphasized that the feeding mode and adding time of probiotics were important factors influencing the effect of probiotics. The main ways to add probiotics are through feed, water, spray, or irrigation. In production practice, it is often used in feed and drinking water. Studies had shown that probiotics were more effective when added to water than when added to feed. Because of drinking water sanitation, interference, and the need for special quantitative adding equipment, probiotics are mainly added to feed in actual production (Karimi Torshizi et al. 2010). Because probiotics cannot be permanently colonized in the gastrointestinal tract, the addition of probiotics needs to be sustained to ensure its effectiveness.

10.1.3.3 The Preparation and Storage Conditions of Fodder

Some ingredients in feed can also affect the use of probiotics, such as antibiotics and pesticides added to feed that reduce the effect of probiotics. Wheat and barley would increase the viscosity of intestinal chyme and also had adverse effects on probiotics (Choct et al. 1996). High temperature of machine may lead to inactivation of probiotics during the production of feed. In addition, direct friction and extrusion of feed ingredients also affect the activity of probiotics during the mixing of feed. When the storage temperature exceeds 30 °C,, chemical substance and enzyme reaction in majority of probiotic bacteria (such as lactobacillus and bifidobacterium) will be quickened. It may cause the probiotic inactivation. For fear of influencing the activity of bacteria, probiotic preparations should generally be refrigerated in 2–8 °C environment temperature and prevented oxidation reaction.

10.1.3.4 The Species and Physiological Stage of Target Animals

Probiotics play a role on the gastrointestinal tract through colonization. Different probiotics have different colonization abilities in the intestinal tracts of different animals. Therefore, it is better for the probiotics to come from the normal intestinal flora of target animals to improve the colonization and growth capacity. Early addition of probiotics is important for animals because probiotics can regulate the expression of genes in intestinal epithelial cells and create a better environment for the colonization of probiotics. In addition, some special physiological states (such as weaning, lactation, and stress) may affect the effect of probiotics.

To sum up, probiotics must remain active at the action site to realize function. Therefore, the tolerance of strains to temperature, gastric acid, and bile in gastrointestinal tract should be fully considered in the selection of strains. Probiotics can be embedded in microcapsules to maintain sufficient activity when reaching the action site. In addition, probiotics can be combined with additives (such as oligosaccharides, enzyme preparations, and acidifier) to maximize the effect of probiotics.

10.1.4 The Other Application of Lactobacillus

10.1.4.1 The Microcapsules Technology of Probiotics

Microencapsulation technology refers to cover solid, gas, or liquid with natural or artificial polymers continuous thin film. The embedded object is isolated from the external environment. When embedded objects encounter certain external stimuli or can be gradually released in a specific environment. PH, mechanical extrusion, temperature, enzyme activity, osmotic pressure, infiltration of water molecules, the presence of certain chemicals, and changes in storage time are all possible factors which stimulate the release of membrane objects. Many tests had shown that microencapsulation could keep probiotics alive in acidic, alkaline, or bile environments. Microencapsulation can improve the survival rate of probiotics in low-temperature drying, high-temperature processing, and the presence of antibiotics. Both lactobacillus and bifidobacterium are highly sensitive to pH and oxygen in the environment. Sufficient number of live bacteria can be guaranteed when it reaches the action site through microcapsule embedding technology.

10.1.4.2 The Combination Between Probiotics and Oligosaccharides

Fructo-Oligosaccharides, also known as oligosaccharides, are compounds formed by two to ten glycosidic bond polymerizations. Due to the lack of enzymes capable of degrading oligosaccharides in animals, oligosaccharides are difficult to be digested and utilized by passive objects. So it enters the animal's posterior intestine directly. The animal's hindgut is teeming with microbes which can use oligosaccharides. Oligosaccharides can be used as nutrients for beneficial bacteria such as lactobacillus and bifidobacterium, but cannot be used by pathogenic bacteria such as *Escherichia coli*, *Salmonella*, and *Clostridium perfringens*. Oligosaccharides can be the energy source of probiotics to improve the competitive advantage of probiotics. In addition, the digestive enzymes secreted by probiotics can degrade oligosaccharides into monosaccharides which can be used by the body. The effect of probiotics can be improved by adding oligosaccharides. The results showed that the growth performance of weaned piglets was improved by the combination of probiotics and oligosaccharides. It is also reported that the addition of both oligosaccharides and probiotics can significantly improve the growth performance of broilers and increase the amount of lactobacillus and bifidobacterium in the intestinal tract.

10.1.4.3 The Combination Between Probiotics and Enzyme Preparations

Enzyme preparation is a biochemical product with high efficiency and specific catalysis. Enzyme preparations are mainly used to supplement the endogenous enzymes of animals which are not enough to improve the utilization rate of nutrients in animals so as to improve the production efficiency. At present, the enzymatic preparation used in animal production mainly includes phytase, protease, lipase, xylanase, and various complex enzymes. There is a synergistic relationship between enzymatic preparations and probiotics. The catalyzed nutrients are not only used for animals but also provide nutrients for probiotics. In addition, probiotics can produce digestive enzymes such as amylase and protease and improve the activity of enzyme preparations. Lin qian et al. (2012) found that combined use of probiotics and complex enzyme preparations could yield better daily weight gain and metabolic energy than using singly.

10.1.4.4 The Combination Between Probiotics and Acidulant

Acidifier can be divided into organic acid, inorganic acid, and compound acidifier. Acidifier can improve the environment of the intestinal tract by reducing the pH of the gastrointestinal tract and inhibiting the growth of harmful bacteria such as *Escherichia coli* and *Salmonella.* The digestive tract function of young livestock and poultry is not sound; the secretion of gastric acid and digestive enzymes cannot meet the demand. Acidifier can activate the secretion of digestive enzymes in the gastrointestinal tract. In addition, acidifier can mask some bad odors in the feed, improve the palatability of the feed, stimulate taste bud cells, increase the secretion of digestive enzymes in the mouth, and enhance animal appetite. 0.1% fumaric acid, lactic acid, and probiotics were added into the feed of 12-day-old yellow-feathered broiler chickens. The results showed that the combination of organic acids and probiotics increased the daily weight gain of broiler chickens and improved the feed conversion rate.

10.2 Application of Lactobacillus in Poultry Production

10.2.1 Prevention and Treatment of Lactobacillus in Poultry Disease

How to maintain the balance of the intestinal flora of animals, promote the development of their immune organs, inhibit the production of harmful bacteria, and improve the body immunity and speed up the production capacity of poultry has become the common goal of every farmer in the poultry farming industry. With the expansion of the scale of farming, it is urgent to reduce the poultry disease probability and control the spread of disease. At present, antibiotics added in the feed are the main way to solve the poultry disease from the source. But now, the use of antibiotics has been more and more excluded by people, and its residues in food have caused wide public concern. A growing number of antibiotics substitutes are researched and developed by the people.

Lactobacillus is reported to be able to effectively antagonize pathogenic bacteria in poultry. The mechanisms involved can be divided into the following categories:

1. Theory of dominant flora: excluding pathogenic bacteria through competitive inhibition. There are tens of thousands of microorganisms in poultry intestinal tract. Beneficial bacteria in the intestinal tract can form competitive adhesion to intestinal epithelial cells and compete for the adsorption sites and nutrients of pathogenic bacteria in the intestinal tract.
2. Biological oxygen capture theory: the pathogenic bacteria are mostly aerobic bacteria. The anaerobic bacteria are predominant in the intestinal tract of poultry. Some probiotics which belong to aerobic bacteria grow and colonized in the animal body to consume oxygen and create an anaerobic environment. It inhibits the reproduction and growth of aerobic bacteria and facultative anaerobic pathogenic bacteria.
3. Bacteria barrier theory: metabolic products of lactic acid bacteria, such as organic acids and bacteriocins, can kill pathogenic bacteria to a certain extent.

Aiba et al. (Yuji Aiba and Nobuyuki Suzuki 1998) found that lactobacilli could produce 15–156 mmol/L of lactic acid with it growing. It could significantly inhibit the urease activity of *Helicobacter pylori*, reduce the activity of *H. pylori*, and antagonize the growth of *H. pylori* co-cultured with *H. pylori*. For some Gram-negative bacteria, bacteriocin contains bioactive protein components and has antibacterial or bactericidal effects. It is reported that coccus bacteriocins had a significant effect on inhibiting *H. pylori*. A variety of pathogenic bacteria will colonize in poultry intestinal tract including *Salmonella.* When people eat poultry with undevitalized *Salmonella*, it will cause a series of complications such as nausea, vomiting, diarrhea, fever, and abdominal cramps. Infants, the elderly or immunocompromised symptoms are usually more serious. *Salmonella* infection also causes other serious complications by bacteremia. Therefore, it is imminent to avoid poultry infected with *Salmonella* strictly. Several types of lactobacillus have been found to be effective in preventing or reducing salmonella infection in broiler chickens. Jin et al. (1996) found that lactobacillus inhibited the growth of salmonella enteritis in chickens and different species had different mechanisms. Pascual et al. (1999) thoroughly cleared the 1.0×10^6 CFU *Salmonella* typhimurium through intragastric administration of both 1.0×10^8 CFU *Lactobacillus salivarius* CTC219 in 21 days chicken experiment. Inoculation of *Lactobacillus reuteri* on hatching eggs could reduce the colonization of newly hatched chicks of *Salmonella* and *Escherichia coli* to reduce the poultry death. *Lactobacillus plantarum* is a sensitive receptor of mannose, which can compete with pathogenic bacteria for the binding site of mannose in chicken intestines to inhibit the colonization of pathogenic bacteria in chicken intestines. Scientists also isolated *Enterococcus faecium* J96 from fecal. It could colonize in poultry intestinal tract and secrete lactic acid and bacteriocin to inhibit *Salmonella* in chickens. Some probiotics compound feed have been gradually developed and become a commercial product. Mountzouris (Mountzouris et al. 2009) studied the treatment result in *Salmonella*-infected chicken of probiotics and antibiotics added into chicks feed and drinking water. They used 6.0×10^5 CFU of *Salmonella* to infect 5 days of age chicks then fed chicken with probiotics and antibiotics added feed, respectively. The probiotic group was 2.0×10^9 CFU/kg of probiotics BP5S feed additives. Probiotics BP5S feed additives included *Lactobacillus reuteri* isolated from healthy adult chicken crop, *Enterococcus faecium* isolated from jejunum, bifidobacterium isolated from ileum, and *Lactobacillus salivarius* isolated from cecum and lactobacillus salivary. The tests found that probiotics significantly reduced the prevalence of salmonella in broilers infected with enteritis and the amount of *salmonella* in broilers with typhoid caecum. The therapeutic effect was essentially effective in adding antibiotics to feed.

Campylobacter jejuni is also a kind of pathogenic bacteria colonized in the poultry intestinal. The poultry itself have no obvious clinical symptoms when they carry germs. However, people will have diarrhea, fever, and acute enteritis by *Campylobacter jejuni* infection when they eat undercooked chicken or duck. Therefore, controlment of *C. jejuni* colonization in poultry is one of the necessary problems for farmers. Chaveerach et al. found that organic acids could be effectively saved and inhibited *C. jejuni* growth effectively in low pH. Lactobacillus P30 isolated from poultry had good tolerance in the artificial simulation of the gastrointestinal tract. It also could effectively reduce the *Campylobacter jejuni* in chickens' in vivo engraftment. Another key factor that lactobacillus could effectively inhibit the growth of *C. jejuni* was its secretion of bacteriocin OR-7 from *Lactobacillus salivarius* NRULB-30514. Bacteriocin OR-7 could effectively antagonize *C. jejuni* and inhibit pathogenic bacteria colonization in chickens (Stern et al. 2006).

10.2.2 Improvement of Growth Performance on Poultry by Lactobacillus

In a normal, healthy, and stress-free poultry gut, the microecological environment is balanced, and the poultry can maximize their growth. Beneficial bacteria, especially lactic acid bacteria, will decrease, and harmful bacteria begin to grow in the case of external stimuli. Poultries in subhealth state are prone to diarrhea, and production efficiency or feed utilization will decline. The balance of gut flora will be broken by factors such as feed and environment. Under natural conditions, chicks can get a complete microbial system from the hen's feces to avoid bacterial infection. However, under commercial incubation conditions, chicks are usually incubated in an incubator without normal gut microbes which is completely isolated from hens or other adult chickens. So, the use of probiotics in the pre-growth phase of poultry is more important and more useful than in other animals.

In broiler production, probiotics can increase body weight and feed conversion, inhibit the colonization and growth of harmful bacteria, and improve meat quality and immune function. Molnar et al. (2012) added *Bacillus subtilis* to the feed of broilers; the results showed that probiotics significantly improved body weight gain, feed conversion rate, and immune response. Adding 1000 mg/kg of lactobacilli into the feed of broiler chickens increased body weight, improved feed conversion rate, and decreased the deposition of belly fat on 8 days chicken (Kalavathy et al. 2003). Mountzouris et al. (2007) added probiotics (*Lactobacillus reuteri*, *Enterococcus faecium*, *Bifidobacterium*, and *Lactobacillus salivarius*) to drinking water of chicken to improve the body weight gain, food intake, feed conversion rate, and digestive enzyme activity of broilers. It is reported that lactobacillus increased the pectoral muscle rate of broilers, the content of flavoring amino acids (such as glycine and alanine), water, and intermuscular fat.

In the production of laying hens, the main purpose of adding probiotics is to improve the utilization rate of feed, the amount of eggs, and the quality of eggs. Mikulski (Mikulski et al. 2012) pointed out that the addition of 100 mg/kg *Lactococcus lactis* into the diet of laying hens increased the weight of eggs, the thickness of shells, the relative mass of shells, and feed conversion rate. At the same time, it could also reduce the rate of broken eggs, unshelled eggs, and the cholesterol content of the yolk. Two thousand layers were used to carry out the fermentation feed feeding test of multiple strains. The result showed that the material egg ratio of the experimental group was significantly lower than the control group, and the egg production rate and weight of the experimental group were higher. At the same time, the feed cost was declined by fermentation. Wang et al. (Wang and Zhang 2013) showed that the addition of 150 g/kg bacillus coagulum preparation (8.0×10^9CFU/g) in the feed for laying ducks could improve the egg production rate, daily output, average weight of eggs, and feed conversion rate. The microbial preparation also could reduce the intake of eggs and improve the color of egg yolk, protein content, and quality of eggs.

10.3 Application of Lactobacillus in Aquaculture

With the gradual development of China's aquaculture industry, large-scale intensive farming mode gradually replaced the traditional extensive cultivation mode. But, the competition of feed and survival space and the accumulation of waste residue lead to the breeding pollution and deterioration of water quality and the growth of harmful microbes. It causes frequent disease of aquatic animal and product quality decline, which hinders the rapid development of aquaculture. In the face of all kinds of disease problem, it is main to use a variety of broad-spectrum antibiotics and chemical drugs for controlling the occurrence and spread of the disease. But, it causes new problems such as bacteria resistant, water quality pollution, microbial flora, and ecological balance destruction with long-term use. Meanwhile, the chemical drugs and antibiotics left in aquatic products will seriously threaten human health and do not meet the basic requirements of food safety production. Therefore, it is especially important to find a safe, nontoxic, non-residue, and nonresistant alternative to achieve sustainable development of aquaculture.

As an important probiotics, lactobacillus has been widely used in the fields of medicine, light industry, and food and animal husbandry. Lactobacillus plays a very important role in aquaculture as a microecological preparation. It is reported that lactobacillus applied in aquaculture could purify water body, improve water environment, colonize in host body, inhibit the growth of harmful bacteria, provide nutrients, and maintain ecological balance. Moreover, lactic acid bacteria in the fermentation process would produce a series of metabolites such as organic acids, special enzymes and bacteria on the surface of the active ingredients to stimulate tissue growth, body's physiological function, immune response, nutritional status, drug effect, and stress reaction. It could also be applied in fish, crustaceans, and trepang farming.

10.3.1 The Probiotic Function of Lactobacillus on Aquatic Animals

Lactobacillus preparation or feed used in aquaculture has the basic function of regulating host intestinal flora, maintaining microecological balance, and supplementing nutrients. The role of lactobacillus as probiotics in aquaculture is mainly reflected in two aspects: (1) the effects on water microecological balance and water environment and (2) the effects on host gastrointestinal microecological balance. Therefore, it can give full play to the probiotics of lactic acid bacteria, such as regulating the balance of intestinal flora, purifying water, improving water quality, pretreating diseases, promoting growth, and improving host body immunity.

10.3.1.1 Regulating the Balance of Intestinal Flora

Lactic acid bacteria can produce a variety of metabolites such as organic acids, hydrogen peroxide, and bacteriocins during fermentation. Hydrogen peroxide secreted by probiotics could activate the catalase system which had antibacterial effect and could inhibit the growth and reproduction of pathogenic bacteria such as *Escherichia coli* and *Salmonella* (Zheng et al. 2005). Acidic substances such as lactic acid reduced the pH of the environment and antagonized harmful microorganisms with weak acid tolerance. This environment could inhibit the growth of harmful bacteria and regulate the microecological balance of intestinal flora (Lin 2012). The secreted bacteriocins such as nisin have a good germicidal effect. Through the destruction of the cell membrane of harmful microorganisms and membrane potential changes, bacteriocins improve the permeability of the cell membrane to lose a large amount of ATP and ions in the cell membrane and lead to the death of pathogenic bacteria at last. At the same time, the bacteriocin has a broad spectrum of antibacterial activity and a good inhibitory effect on the closely related strains. Some studies have found that the metabolites produced by lactobacillus after fermentation have an inhibitory effect on harmful microorganisms which played a key role in the inhibitory effect.

Lactobacillus is a kind of facultative anaerobic microorganism. After entering the intestinal tract of aquatic animals, it conducts aerobic metabolism, consumes the oxygen in the intestinal tract, and reduces its concentration to inhibit the growth of harmful aerobic bacteria. By competing for oxygen with pathogenic bacteria, it inhibits the pathogenic bacteria and maintains the microecological balance of the intestinal flora.

Lactic acid bacteria are a kind of normal flora in fish intestinal tract and have a good colonization ability to become the advantage bacterium group. It can contend the nutrients from living space and living environment with pathogenic bacteria and make the pathogens rarely get adequate nutrition and space to grow. This domino effect can inhibit the growth of harmful bacteria and maintain the balance of intestinal flora. Therefore, lactobacillus mainly inhibits the growth of harmful bacteria through the effect of metabolites and the competition with harmful bacteria for oxygen, space, and nutrients.

10.3.1.2 Improving the Water Quality of Aquaculture

With the continuous development of aquaculture, intensive aquaculture mode is continuously improved. Overstocking and concentrated feeding will lead to a large number of residual bait, excretion waste of aquaculture animals, and accumulation of animal or plant at the bottom of the pond. Especially under the condition of insufficient dissolved oxygen in water, the decaying and decomposition of organics will produce ammonia nitrogen, nitrite, hydrogen sulfide, and other toxic substances. These harmful substances will pollute the water environment and seriously damage the entire ecological environment of aquaculture to bring huge economic losses to the aquaculture industry (Hall and Holby 1992). Studies have shown that lactobacillus could effectively remove nitrites in culture water, such as the application of *Lactobacillus brevis* A1.558, which had a good removal effect in complex water environment (Wei 2007). The removal of nitrite by lactobacillus mainly has two stages (enzyme removal and acid removal). When the environmental pH is >4.5, enzyme removal is the main part, and nitrite reductase is generated during the fermentation process to degrade harmful nitrites into nontoxic ammonia. When environmental pH <4.0, acid removal is the main part. In this process, through oxidation, ammonification, nitrification, denitrification, and nitrogen fixation effect, the metabolism organic acid decomposed the accumulated residual bait, waste, and other harmful substances into carbon dioxide, nitrate, phosphate, and nontoxic harmless beneficial substances. These effects achieved the purpose of purifying aquaculture water and improving water quality (Gong et al. 2011; Qifang 2002). Li (Chang and Wang 2010) studied the removal capability of several lactic acid bacteria to nitrite and found that acid may be the main factor to remove nitrite. The lower the pH, the faster the removal speed. Lactobacillus could also inhibit the growth and reproduction of pathogenic bacteria and corrupt bacteria in aquaculture water to decompose harmful substances in water and avoid eutrophication in water. Some special enzymes could also be produced to reduce ammonia nitrogen, chemical oxygen consumption, and nitrite in the water to improve the water quality (Feng and Chen 2005). Zhang et al. (Zhang and Li 1999) studied the impact of bacillus lactobacilli on the water environment. The results showed that the content of ammonia nitrogen and nitrite in the water environment of the feeding bacteria were relatively low compared with that of the water body of tilapia lactobacillus.

10.3.1.3 Preventing and Treating the Disease

Lactobacillus is the most widely used type of probiotics at present. After entering the host intestinal colonization, lactobacillus regulates the microecological structure in the host body through the growth, reproduction, and metabolites of the bacteria. So it can show the optimal physiological state and growth rate under the balance of the microecosystem. In the process of aquaculture, the most serious diseases are infectious diseases. The pathogenic microorganisms mainly include viruses, bacteria, and fungi such as intestinal point-like aeromonas and mild aeromonas which belong to opportunistic pathogenic bacteria. A large number of harmful substances (such as ammonia and nitrite) were the main reason for aquatic animal disease outbreak. The harmful substance destroyed the normal microbiota on animal skin mucous membrane which causes pathogenic bacteria to enter the body. At the same time, these substances increased secretion of bacteria endotoxin activity and broke microbial ecological balance. It is reported that lactic acid bacteria could prevent and cure diarrhea or enteritis caused by intestinal bacteria. Lactic acid bacteria produced strong adhesion force through the proteins and fat teichoic acids secreted by bacteria on intestinal mucosa cells. This adhesion ability could increase the lactic acid bacteria colonization and make them become the advantage bacterium group. The pH was low through bacteriocin, organic acid, and hydrogen peroxide to inhibit the growth of harmful bacteria and make lactic acid bacteria become the preponderant bacterium group.

10.3.1.4 Improving the Host Immunity

Disease hazards and antibiotic residues have been the main problems in aquaculture industry. The farmers not only restrain the growth of harmful pathogenic bacteria and reduce the probability of the occurrence of diseases but also improve the immunity ability of aquatic animals. Fish and crustaceans are lower vertebrates, and their immune systems are relatively weak compared with mammals. Immune organs and tissues mainly include the spleen, thymus, and anterior kidney; blood lymphoid and alimentary tract lymphoid tissues rely on the non-specific immune system to improve the body's resistance to external antigens. Lactobacillus can stimulate the development of aquatic animals' immune organs and enhance their activity against interferon, macrophages, complement, and lysozyme. Fish are more dependent on non-specific immune regulation than higher vertebrates. Crustaceans are mainly non-specific and have no specific immune response. Probiotics improve the immune capacity of the body mainly by enhancing the activity of various immune enzymes such as lysozyme, catalase, superoxide dismutase, and phosphatase in animal body fluids or walls.

In addition, lactobacillus can improve the immune function of aquaculture animals. Lactobacilli and their metabolites are immune to intestinal mucosa. In the aspect of humoral immunity, the production of cytokines such as IL-1, IL-5, and IL-6 can be increased by activating macrophages, NK cells, and B lymphocytes. In terms of cellular immunity, lactobacillus can enable antigens to enter ileal junction through M cells, activate Th2 cells, produce factor IgA, and enhance the secretion of SIgA antibody. SIgA can effectively prevent virus adhesion, neutralize bacterial toxins, inhibit bacterial growth, and maintain the ecological balance of normal intestinal flora. It plays an important role in local anti-infection process.

10.3.1.5 Promoting Growth

Lactobacillus produces growth-promoting factors such as folic acid, biotin, vitamin B6, vitamin K, and amino acids. It provides the nutrients needed by animals directly and participates in their metabolism to promote their development and growth. In addition, the metabolism of lactobacillus can produce a large number of acidic substances to improve the speed of enzymatic reaction which is conducive to the digestion and utilization of nutrients by enzymes. The acidic substances can accelerate the intestinal peristalsis and improve the digestibility of intestinal feed. Lactobacillus also can produce a variety of digestive enzymes or secretory enzymes (such as protease, amylase) to help the body to digest protein, sugar, and other nutrients. It also helps to digest substances which are not available to the animals themselves and promote the dissociation of certain mineral elements. Lactic acid bacteria fermentation, such as amino acids and vitamins, is also beneficial to the health of animals.

10.3.2 Application of Lactobacillus in Fish Farming

China is a big country in aquaculture, but there is a certain gap between its overall farming efficiency and that of developed countries. The problems of poor benefit and high incidence of aquaculture generally exist which seriously affect the development of aquaculture. In the intensive and high-density breeding process, a large amount of residual bait and fecal excreta enter the water environment. Because of the limit for automatic purification function of water, the waste will lead to the increase of harmful microorganisms and the growth of pathogenic bacteria. It brings great economic losses of the breeding industry. Therefore, people are constantly looking for safe, nontoxic, and effective prevention and treatment methods. Green, healthy, and safe aquaculture has become a new trend in the future. Since the 1980s, lactobacillus as an ideal probiotics in aquaculture has been applied in aquaculture as a biological additive. The primary condition for screening probiotics is whether the lactobacillus strain can survive in the water environment and give play to the effect of superior flora, whether it can have a certain tolerance to the gastrointestinal environment, and whether it can promote the growth of aquaculture animals and the body immunity. At present, lactic acid bacteria strains with good physiological and biochemical functions have been isolated from aquaculture animals and their water environment. Various microbial preparations have been developed which have been fully utilized in fish, crustaceans, and shellfish breeding and can play its beneficial effects.

In the process of fish breeding, the pathogenic bacteria, caused by disease hazards that mainly include salmon biocidium and vibrio, can respectively induce fish furuncle disease and vibrio disease. It brings huge economic losses to the aquaculture industry. Pathogenic bacteria enter the body mainly through the skin, gills, and gastrointestinal system of fish. Therefore, the key to fish disease is whether the pathogenic bacteria successfully colonize and grow in these tissues. The mucosa of the gastrointestinal tract of fish is composed of epithelial cells, and different locations have different structure and function. Pathogenic bacteria can enter the body through epithelial cells and cause diseases. The probiotic lactobacillus isolated from healthy fish intestinal tract or water environment has significant bacteriostatic effect on its metabolites such as competing with pathogenic bacteria for adhesion sites, preventing pathogenic bacteria from colonizing in fish sensitive locations and reducing the release of pathogenic toxin. As a pathogenic bacteria in the intestinal tract, *Escherichia coli* could adhere to the intestinal epithelial cells through the mannose receptor. *Lactobacillus plantarum* could metabolize the specific adhesion of mannose to inhibit the colonization of the intestinal epithelial cells of *E. coli*. Salina et al. (2008) found that *Carnobacterium divergens* which isolated from salmon had a certain inhibitory effect on colonization and reproduction of pathogenic bacteria in the host.

As a natural active microecological preparation, lactobacillus preparation could colonize slickly in the fish intestinal tract without obvious host specificity. It also could effectively regulate the intestinal flora to maintain the microecological balance and enhance the immune capacity of fish or the barrier layer of epithelial cells (Villamil and Tafalla 2002). Liu found that the superoxide dismutase, alkaline phosphatase, and acidic phosphatase activity in serum were significantly increased when *Lactobacillus acidophilus* was added to the fish fodder. At the same time, the weight gain rate, protein efficiency, and growth rate were also increased. Suzer et al. (2008) found that the total number of bacteria in the intestinal and water bodies of gingival bream was significantly different from that of the control group when lactobacillus was fed on *Sparus aurata*. The total number of bacteria in the water bodies of the control group was significantly higher than that of the experimental group. In addition, the activity, survival rate, and specific growth rate of digestive enzymes in larva were also significantly improved.

However, lactobacillus is not dominant in fish gut flora. Lactobacillus usually is regarded as the normal intestinal flora in fish and has good ability to colonize in intestinal tract without apparent host specificity. It is a certain relationship between lactobacillus quantity and environmental factors or nutrition. But, the number of lactic acid bacteria can maintain at a high level through artificial feeding (Ring 1998). Grouper is one of the most important commercial farmed fish in Southeast Asia. However, the pollution and deterioration of water environment increase the incidence of these diseases, and the pathogenic virus can induce nerve necrosis and other diseases of post-ovulation grouper (Fukuda et al. 1996). Some pathogenic bacteria (such as pseudomonas and vibrio) can also make grouper pathogenic, which brings certain difficulties to grouper breeding. Some scientists added different concentrations of *Lactobacillus plantarum* into fish fodder and found the average weight of grouper, feed conversion rate, and survival rate were significantly improved than the control group. Especially in the 1.0×10^{10} CFU/kg lactobacillus group, the average weight and feed conversion rate increased by 404.6% and 1.26 times, and the fish survival rate increased by 36.7% compared with the control group. In addition, the grouper hind gut lysozyme activity and glutathione peroxidase activity compared with the control group increased by 136.6% and 113.3%, respectively. The phagocyte cells in the immune system activity index, index and head kidney leukocytes breath also increased at the same time. The results confirmed that the plant lactobacillus could promote the growth of grouper and improve immune function and resistance to disease.

Grass carp enteritis is a common infectious disease caused by pathogenic bacteria. In order to control the occurrence of diseases, antibiotics, synthetic drugs, and pesticides are used in large quantities. It also results in the emergence of drug-resistant strains, water environment pollution, and drug residue problems of aquatic products which pose a threat to the safety of aquatic products. In order to find antibiotics substitute, Xia et al. (Xia and Fang 2013) adopted modern biological fermentation technology and unique freeze-drying process to make composite living bacteria preparations. The results of clinical trials showed that compound lactobacillus could improve the water transparency, dissolved oxygen, nitrite, ammonia nitrogen, and other water quality indexes of aquaculture water, promote the growth of cultured grass carp, reduce the feed coefficient, and increase the output of fish. Lactic acid bacteria can inhibit the reproduction of spoilage organisms and pathogenic bacteria in water, decompose harmful material to avoid eutrophication of water body, produce some special enzyme system, and reduce the ammonia nitrogen in water, chemical oxygen consumption, and nitrite content.

In grass carp aquaculture, Xu (2007) studied the influence between *Lactobacillus pentosus* and fish breeding water or intestinal flora. In this experiment, after adding *Lactobacillus pentosaceus* R1 to fish, the number of lactobacillus in the intestinal tract increased from the original 1.0×10^2 to 1.0×10^6 CFU/g. When the juvenile grass carps were fed, part of R1 is colonized in the gastrointestinal tract and a large part is excreted into the water through feces. Only part of the water in the tank was exchanged every day, so the amount of R1 in the water was increased. While the number of *Lactobacillus pentosaceus* R1 increased, the number of vibrio presented a downward trend. This suggest that *Lactobacillus pentosaceus* R1 could colonize in the intestinal tract of juvenile grass carps and control the amount of vibrio in the intestinal tract. In the first 30 days of the experiment, *Lactobacillus pentosaceus* R1 colonized in the intestinal tract of grass carps larvae and antagonized the proliferation of vibrio. This domino effect was achieved through the adjustment of intestinal flora by R1. But it had no effect on the aerobic heterotrophic bacteria in the intestinal tract. Although R1 was not the dominant microbiome in the intestinal tract and its composition in the intestinal microbiome was very small even after a large amount of feeding, the stable colonization of R1 in the intestinal tract inhibited the increase of the number of vibrio in the intestinal tract. At the same time, vibrio in water was also controlled to some extent by lactobacillus in the excretion of feces. According to the test results of each indicator, it was found that the effect of feeding R1 was similar to that of antibiotics.

In the application of fish feed, lactobacillus preparation is also gradually affecting the change of feed situation. Scientists conducted the application test and analysis of lactobacillus preparation in pond mud fish culture. They found that the use of lactobacillus preparations reduced the levels of ammonia nitrogen, nitrite, and hydrogen sulfide in aquaculture water. This effect improved the water aquaculture environment and the bottom of ponds, inhibited pathogenic microorganisms, enhanced the immune defense ability of cultured objects, reduced the use of fishing drugs, provided a good growth environment for the mud carp breeding, and promoted the healthy growth of fish ecology. The water amount in the breeding production of dace fish had been reduced by 50–60%. At the same time, the discharge of waste water had reached the qualified standard which played an ecological protection role to the surrounding environment. The use of lactobacillus preparation not only improved the breeding environment but also reduced the occurrence of blight. The feed coefficient of breeding production was reduced by 0.08–0.15. Although the use cost of lactobacillus preparation in the test ponds in 2011 and 2012 was 24 yuan/667 m^2, the production cost of dace fish per kg decreased by 0.71–0.78 yuan/kg. At the same time, the production increased by 93.5–125 kg/667 m^2.

10.3.3 Application of Lactobacillus in Crustaciculture

A large number of studies have shown that probiotics have been widely used in the cultivation of crustaceans. The probiotics can promote the growth of shrimp and crabs, improve the activity of digestive enzymes, and enhance immunity. Lactic acid bacteria can carry out metabolic activities in shrimp and crab aquatic animals and provide them with necessary nutrients such as amino acids, multivitamins, and growth-promoting factors. It even increases the biological activity of mineral elements to enhance the nutrient metabolism and promote the reproduction and growth of shrimp and crab. Adding lactobacillus into the feed can improve the activity of protease and amylase in the liver, pancreas, and intestinal tract. The immune system of crustacean breeding animals is not perfect, which mainly relies on non-specific immunity to improve disease resistance. An important indicator of immune ability is the level of lysozyme and peroxidase in serum. Including lysozyme can destroy and clean up into the host for the foreign body, and peroxidase has the ability of foreign body recognition and defense capabilities. Lactic acid bacteria can activate crustaceans peroxidase in the original system, increase the activity of peroxidase, improve the recognition of foreign bodies and host defense function, and reduce free radical damage to normal cells and removal of reactive oxygen species in the process of cell metabolism.

The giant prawns, also known as Malaysian prawns and freshwater longarm prawns, are the largest freshwater prawns in the world. It is widely distributed in the tropical and subtropical freshwater waters of the west Pacific region of India. It has the advantages of fast growth; large individual size; wide edible, delicious taste; rich nutrition; easy domestication; strong adaptability; and short production cycle. It is a kind of breeding variety with high economic efficiency and one of the main shrimp species in freshwater aquaculture in China. When the feeding density of *Macrobrachium rosenbergii* is relatively high, the overfeeding feed will enter the water body in the form of residual feed. The ammonium nitrogen, nitrite, hydrogen sulfide, and total nitrogen produced by the decomposition of residual feed and shrimp dung will exceed the standard which leads to deterioration of water quality and higher incidence of *Macrobrachium rosenbergii*. Especially in the mid and late stage of cultivation, the amount of organic matter such as baits, excreta, and animal carcasses in the breeding pond increased. This matter will result in the decrease of dissolved oxygen in the pond and the sharp increase of ammonium nitrogen and nitrite nitrogen which lead to the overgrowth of cyanobacteria. Therefore, low-density cultivation of *Macrobrachium rosenbergii* shrimp has become a major problem to restrain its reproduction and sale to adapt to the market. Tan et al. (Yang and Zhang 2013) added lactobacilli preparation in the feed through high-density macrobrachium rosenbloom shrimp cultivation. In the breeding cycle of *Macrobrachium rosenbergii*, the overall change of water quality in the breeding pond was as follows: (1) The pH was relatively stable in the tolerance range of *Macrobrachium rosenbergii*. (2) The concentration of ammonia nitrogen showed a trend of accumulation over time, which was higher than the environmental quality of surface water. (3) Total phosphorus concentration exceeded the environmental quality of surface water in five categories. (4) The concentration of total nitrogen increased with time and exceeded five standards of environmental quality of surface water. (5) The treatment effect of lactobacilli preparation on ammonia nitrogen and total nitrogen was obvious. Cyanobacteria and cyanobacteria also proliferated in the high-density aquaculture ponds in the late stage of aquaculture, but there was no "water bloom." Under the treatment of lactobacillus, the high-density *Macrobrachium rosenbergii* pond showed a similar pattern to the low-density pond which had a significant effect of increasing yield. It is reported that complex microbial preparations including yeast, bacillus, and lactobacillus had been used into the feed of *Macrobrachium rosenbergii*. The results showed that this kind of compound microbial preparation not only promoted growth but also significantly increased the number of beneficial microorganisms in the intestinal flora compared with the control group.

The river crab breeding industry in China has developed rapidly. With the continuous expansion of the breeding scale and the deterioration of the breeding environment, the occurrence of river crab diseases is frequent, which seriously restricts the development of the river crab breeding industry. The prevention and treatment of river crabs has been using antibiotics and other chemical drugs to kill pathogenic microorganisms. This method not only causes the endogenous and secondary infections of animals to develop resistance and reduce the resistance but also directly affects human health and ecological environment. There are a number of research applying probiotic preparations to open duck and conventional breeding through the mechanism of action of probiotics and the application of probiotic preparations for reference in other aquaculture experience. The scientists also explore the probiotic preparations of river crab breeding ecological environment improvement, prevention, and growth-promoting role and influence on breeding efficiency. And it provides a scientific basis for the popularization and application of probiotic preparations in river crab breeding. It is studied the application of probiotics in river crab breeding. They found that probiotics significantly improved the ecological environment of river crab breeding in the monitoring results of water samples and the observation of breeding process. It was mainly reflected in the effective control of chemical oxygen demand, ammonia nitrogen, nitrite nitrogen, and sulfide in the pond. The dissolved oxygen content gradually increased, while the control pond was the opposite. The results of bacterial count monitoring showed that the total number of bacteria decreased significantly after probiotics were applied to the test tank, while the control tank showed an increasing trend. No disinfectant or drug was applied to the test tank, and no deaths were found in river crabs. The average survival rate was 77.9%. However, after using disinfectant and drugs in the control pool, the morbidity and mortality were still very serious with a survival rate of only 60.6%. The results indicated that probiotic preparation had good preventive effect on pond raising river crab. In addition, it could be seen from the observation and record in the process of breeding that the river crabs in the test pool had a greater appetite and growth than those in the control pool. It could be seen that probiotics preparation has a remarkable growth-promoting effect on river crabs.

10.3.4 Application of Lactobacillus in Mollusk Breeding

Apostichopus japonicus is a kind of highly commercial mariculture animal in China which belongs to the family phylum Echinodermata. It is also a marine organism with the same origin of medicine and food. Because of its higher drug value, economic value, and nutritional value, *Apostichopus japonicas* has become the largest mariculture species in North China. However, in the process of *Apostichopus japonicas* culture, the problems of skin diseases such as skin ulcer syndrome and dermatitis caused by vibrio seriously lead to water pollution. It reduces the host immunity and increases the mortality of *Apostichopus japonicas*. It has become a bottleneck problem in the development of the *Apostichopus japonicas* industry (Huan et al. 2009; Xiong and Yao 2006). Lactic acid bacteria can grow under the condition of pH 6.0–8.5, 40 °C. The temperature of trepang feeding is 5–28 °C, and water for aquaculture environment pH ranges 7.5–8.6 (Xiong and Yao 2006). It can be seen that the conditions of the cultivation of ginseng can make the normal growth and reproduction of lactobacillus. In addition, as an anaerobic microorganism, lactobacillus can also survive and grow under the condition of oxygen deficiency and play a probiotic effect. Successful colonization of lactobacillus japonicus in the body will inhibit the reproduction of pathogenic bacteria.

It is reported that two strains of *Lactobacillus acidophilus* RS-1 and RS-2 from the intestinal and aquatic breeding environment of stichopus japonicus by certain screening methods and analyzed their probiotics effect on the radix salviae japonicus. The results showed that lactobacillus could secrete inhibitory substances and significantly inhibit vibrio growth by way of competing nutrients. At the same time, *Bacillus subtilis* YB-1 and *Bacillus cereus* YB-2 were also isolated. The effects of compound bacteria on immunity and growth of *Salvia miltiorrhiza* were investigated. The results showed that the compound microecological preparation could stimulate the activity of coeliac immune cells and intestinal digestive enzymes of the ginseng, promote its growth, inhibit the infection of vibrio, and reduce the mortality rate of the ginseng by more than 70%. In addition, the compound bacillus could also play the role of purifying the water in the cultivation of the ginseng. Wang (2009) study the addition of lactic acid bacteria and bacillus mixed formulation into the trepang water for aquaculture environment. It was found that probiotics mix not only made the trepang's growth rate increase but also reduced the harmful substances in aquaculture water such as ammonia nitrogen, nitrite, and phosphate content to enhance the purification capacity of water body. Zhang et al. (Zhang 2009) isolated *Lactobacillus* L-2 and *Bacillus* K-3 and J-9 from the intestinal tract of healthy *Codonopsis pilosula* and found that the activity of codonopsis protease and amylase could be improved and the immune function could be enhanced. Lactobacillus preparations and their metabolites could also increase the growth rate of ginseng and enhance the activity of alkaline phosphatase, acidic phosphatase, and lysozyme in the body cavity cells. Compared with the control group, the number of intestine vomit in the experimental group after anhydrous stress was lower. At the same time, the number of heterobacteria, vibrio, and *Escherichia coli* in the intestinal flora and aquaculture water was also significantly reduced, while the number of lactic acid bacteria was significantly increased. The results showed that lactobacillus preparation and its metabolites could regulate the intestinal flora of salvia japonicus.

References

Adami A, Cavazzoni V (1999) Occurrence of selected bacterial groups in the feces of piglets fed with *Bacillus coagulans* as probiotic. Basic Microbiol 39(1):3–9

Adams MC, Luo J, Rayward D (2008) Selection of a novel direct-fed microbial to enhance weight gain in intensively reared calves. Anim Feed Sci Technol 145(1–4):41–52

Bao J, Zhang Y, Ping F (2015) Effect of probiotics on production performance and the incidence rate in nursery pigs. Feed Ind 36(2):39–40

Chang Y, Wang L (2010) The effect on the amino butyric acid of black bean in the different cultivation condition. Food Ind 4:7–9

Chaucheyras-Durand F, Durand H (2010) Probiotics in animal nutrition and health. Benefic Microbes 1(1):3–9

Choct M, Hughes RJ, Wang J (1996) Increased small intestinal fermentation is partly responsible for the anti-nutritive activity of non-starch polysaccharides in chickens. Br Poult Sci 37(3):609–621

Feng J, Chen Y (2005) Influence of microecologicson the water quality. Trans Oceanol Limnol 4(15):104–108

Fukuda Y, Nguyen HD, Furuhashi M (1996) Mass mortality of cultured sevenband grouper, *Epinephelus septemfasciatus*, associated with viral nervous necrosis. Fish Pathol 31(3):165–170

Gong G, Lu Y, Guan S (2011) Preparation and properties of nitrite reductase from lactobacillus. China Brew 1:58–60

Huan D, Chongbo H, Zhou Z (2009) Isolation and pathogenicity of pathogens from skin ulceration disease and viscera ejection syndrome of the sea cucumber *Apostichopus japonicus*. Aquaculture 287:18–27

Jadamus A, Vahjen W, Schafer K (2002) Influence of the probiotic strain *Bacillus cereus* var. toyoi on the development of enterobacterial growth and on selected parameters of bacterial metabolism in digesta samples of piglets. J Anim Physiol Anim Nutr 86:42–54

Jin LZ, Ho YW, Ali MA (1996) Effect of adherent Lactobacillus spp. on in vitro adherence of salmonellae to the intestinal epithelial cells of chicken. J Appl Bacteriol 81:201–206

Kalavathy R, Abdullah N, Jalaludin S (2003) Effects of Lactobacillus cultures on growth performance, abdominal fat deposition, serum lipids and weight of organs of broiler chickens. Br Poult Sci 44(1):139–144

Karimi Torshizi MA, Moghaddam AR, Rahimi S (2010) Assessing the effect of administering probiotics in water or as a feed supplement on broiler performance and immune response. Br Poult Sci 51(2):178–184

Liao D-j, Ye Y-g, Wang M (2012) Investigation and measures to solve the prevalence of diarrhea disease in calves. Dairy Health 16:34–35

Lin SU (2012) Lactic acid bacillus mechanism of action and application prospects in aquaculture. Jilin Anim Husb Vet 5:25–29
Lin Q, Dai Q, Bin S (2012) Probiotics and enzyme preparation: effects on growth performance of yellow- feathered broilers and its mechanism. Chin J Anim Nutr 24(10):1955–1965
Liu H, Zhang M, Feng J (2012) Effects of probiotic preparation on growth performance and immune indices of early weaner piglets. Chin J Anim Nutr 24(6):1124–1131
Luan G, Wang J, Bu D (2008) Effects of supplementation of Bacillus subtilis Natto on milk production and quality of lactating cow. J Northeast Agric Univ 39(9):58–61
Mikulski D, Jankowski J, Naczmanski J et al (2012) Effects of dietary probiotic (Pediococcus acidilactici) supplementation on performance, nutrient digestibility, egg traits, egg yolk cholesterol, and fatty acid profile in laying hens. Poult Sci 91(10):2691–2700
Molnár AK, Podmaniczky B, Kürti P (2012) Effect of different concentrations of Bacillus subtilis on growth performance, carcase quality, gut microflora and immune response of broiler chickens. Br Poult Sci 20(43):1–9
Mountzouris KC, Tsirtsikos P, Kalamara E (2007) Evaluation of the efficacy of a probiotic containing lactobacillus, bifidobacterium, enterococcus, and pediococcus strains in promoting broiler performance and modulating cecal microflora composition and metabolic activities. Poult Sci 86:309–317
Mountzouris KC, Balaskas C, Xanthakos I (2009) Effects of a multi-species probiotic on biomarkers of competitive exclusion efficacy in broilers challenged with Salmonella enteritidis. Br Poult Sci 50(4):467–478
Pascual MN, Hugas M, Badiola JI (1999) Lactobacillus salivarius CTC2197 prevents Salmonella enteritidis colonization in chickens. Appl Environ Microbiol 65(11):4981–4986
Per Hall OJ, Holby O (1992) Chemical fluxes and mass balances in a marine fish cage farm. IV. Nitrogen. Mar Ecol Prog Ser 89:81–91
Persson Y, Nyman AK, Gronlund-Andersson U (2011) Etiology and antimicrobial susceptibility of udder pathogens from cases of subclinical mastitis in dairy cows in Sweden. Acta Vet Scand 53(36):1–8
Qiao G, Shan A (2007) Study of the effect of probiotics on performance and rumen fermentation in dairy cattle. Chin Dairy Cattle 3:10–14
Qifang Z (2002) The study on mechanism of nitrite degradation by lactic acid Bacteria. Food Ferment Ind 28(8):28–31
Ring E (1998) Lactic acid bacteria in fish: a review. Aquaculture 160:177–203
Salinas I, Myklebust R, Esteban MA (2008) In vitro studies of Lactobacillus delbrueckii subsp. lactis in Atlantic salmon (*Salmo salar* L.) foregut: tissue responses and evidence of protection against *Aeromonas salmonicida subsp. salmonicida* epithelial damage. Vet Microbiol 128(1–2):167–177
Stern NJ, Svetoch EA, Eruslanov BV (2006) Isolation of a Lactobacillus salivarius strain and purification of its bacteriocin, which is inhibitory to Campylobacter jejuni in the chicken gastrointestinal system. Antimicrob Agents Chemother 50(9):3111–3116
Suzer C, Çoban D, Kamaci HO (2008) Lactobacillus spp. bacteria as probiotics in gilthead sea bream (*Sparus aurata*, L.) larvae: effects on growth performance and digestive enzyme activities. Aquaculture 280(4):140–145
Timmerman HM, Mulder L, Everts H (2005) Health and growth of veal calves fed milk replacers with or without probiotics. J Dairy Sci 88:2154–2165
Villamil L, Tafalla C (2002) Evaluation of immunomodulatory effects of lactic acid bacteria in turbot (*Scophthalmus maximus*). Clin Vaccine Immunol 9(6):1318–1323
Wang L (2009) Effects of microecological preparation on the culture of *Apostichopus japonicus* sclenkain a closed recalculating aquaculture system. Chin J Microecol 21(6):497–504
Wang X-r, Zhang X (2013) Effects of *Bacillus coagulans* on laying performance, egg quality and serum biochemical indices of laying ducks. Acta Ecol Anim Domastici 34(2):69–74
Wei W (2007) Removal of nitrite from aquacultural water with lactobacillus brevis. J Ecol Rural Environ 23(4):37–40

Xia L, Fang M (2013) Study on the safety evaluation of grass carp and the effect evaluation of control bacterial enteritis from multiple-lactobacillus. Fish Inf Strategy 28(1):44–49
Xiong S, Yao X (2006) Effects of different temperatures and pH on the growth of lactic acid bacteria. Xinjiang Agric Sci 43(6):533–538
Xu T (2007) Effects of Lactobacillus pentosaceus R1 on the microflora in water and larvae guts of grass carp (*Ctenopharyngodon idella*). J Anhui Agri Sci 35(34):11112–11114
Yang M, Zhang Y (2013) Application test of EM bacteria in *Macrobrachium Rosenbergii* aquaculture. Pop Sci Technol 15(165):152–154
Yang H-y, Yang Z-b, Yang W-r (2009) Effect of probiotics and xylo -oligosaccharide on performance, digestive enzyme activities, blood index and intestinal microflora of early weaned piglets. Chin J Vet Sci 29(7):914–919
Yang H-j, Zhang S-t, Li J, Cui J-l (2014) The application of lactobacillus probiotics in prevention and treatment of cow subclinical mastitis. Dairy Health 17(2):51–54
Yao G-q, Zhao S-p, Gao P-f (2014) Applied research of lactobacillus probiotics in the prevention and treatment of calf diarrhea. Dairy Health 17:55–58
Yuji Aiba MT, Suzuki N (1998) Lactic acid-mediated suppression of helicobacter pylori by the oral administration of lactobacillus salivarius as a probiotic in a gnotobiotic murine model. Am J Gastroenterol 93(11):2098–2101
Zeyner A, Boldt E (2006) Effects of a probiotic *Enterococcus faecium* strain supplemented from birth to weaning on diarrhoea patterns and performance of piglets. J Anim Physiol Anim Nutr (Berl) 90(1–2):25–31
Zhang T (2009) Effect of different combinations of probiotics on digestibility and immunity index in sea cucumber *apostichopus japonicus*. J DaLian Fish Univ 24:64–68
Zhang Q, Li Z (1999) The effects of microbiological compound on ecological factors in culture waters. J Shanghai Fish Univ 8(1):43–47
Zhang H-t, Wang J-q, Bu D-p (2008) Effect of Bacillus subtilis Natto on growth performance of weaned calves. Chin J Anim Nutr 20(2):158–162
Zheng H, Shi Q-q, Shi B-h (2005) Comparison of Bacillus sp. on depuration of aquaculture waterbody. J Microbiol 25(6):41–44
Zhou Y, Zhou X, Wu S (2012) Effect of different level of Bacillus subtilis on the growth performance and diarrhea in weaned piglets. Feed Rev 4:29–31