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Probiotics, with a spesial focus in Animals

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1. Abstract

This study is conducted to understand probiotics in a better way; how it can be used since the ancient times and up till now. Through scrutinizing and reviewing the literature, it is clear that probiotics were used in many areas since the early years of human civilization. The study attempts to clarify that the types of probiotics included bacteria, yeast, and how effective these types can be on animals, birds, and aquatic animals. The benefits of probiotics will be surveyed, such as improving food quality, reducing cholesterol, the activity of the lactase enzyme, enhancing the immune system in animals and humans, and how it can be used in allergy and minimizing the colon cancer as well as bowel diseases, diarrhea, constipation, and other gastrointestinal maladies. The uses of probiotics are highlighted in the areas of water environment, the aquatic fish farms and the method of making probiotics operate effectively to minimize infection with disease and lessen the stress. In addition to improving the water environment, probiotics can play a big role in maximizing the fish reproduction. By reviewing and examining the literature, it is obvious that probiotics can be used as an alternative to many types of antibiotics as well as vaccine in raising the fish in aquatic fish farms.

1. **Keywords:** Probiotics, gastrointestinal maladies, aquatic environment, antibiotics.

Sammendrag

Denne studien ble utført for å bedre forstå probiotika; hvordan det har blitt brukt fra gamle tider til dags dato. Gjennom gransking og gjennomgang av litteratur er det klart at probiotika ble brukt på mange områder siden de tidlige årene av sivilisasjonen. Studien forsøker å avklare at typene probiotika inkluderte bakterier, gjær og hvor effektive disse typene kan være på dyr, fugler og vannmiljø. Fordelene med probiotika vil bli undersøkt, for eksempel det å forbedre matkvaliteten, redusere kolesterol, aktiviteten til laktasenzymet, forbedre immunsystemet til dyr og mennesker, og hvordan det kan brukes i allergier og minimere tykktarmskreft så vel som tarmsykdommer , diaré, forstoppelse og andre gastrointestinale sykdommer. Bruken av probiotika i vannmiljøet, akvatisk fisk, og metoden for å gjøre probiotika effektive for å minimere sykdomsinfeksjon og redusere stress, er sentrale i denne studien. I tillegg til forbedring av vannmiljøet kan probiotika spille en viktig rolle i maksimering av reproduksjon av fisk. Fra litteratur er det åpenbart at probiotika kan brukes som et alternativ til mange typer antibiotika, samt som vaksine i akvakultur.

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2. Introduction

2.1. The history of probiotics in humans

This word is derived from the Latin words Pro (which means for) and the Greek word Bios (which means life). The message of these lexical items would be (for betterment of life).

The earliest link between microorganisms, health, and fermentation was casually detected in 1680 by Leeuwenhoek's microscope observing yeast cells in fermenting beer (46). In the late eighteenth century, Lavoisier gave a description of alcoholic fermentation, even though it was inaccurate. In 1840, Theodor Schwann and Charles Cagniard-Latour proposed a link between yeast and fermentation. Later on, Louis Pasteur concluded that microorganisms initiated the lactic acid fermentation (92). In 1899, Henry Tissier recommended the administration of Bifidobacteria to infants with diarrhea (127). This can be considered as the first usage of probiotics in the field of medicine and human health.

Later, Ilya Illyich Metchnikoff suggested that the Bulgarian peasants inclusion of yoghurt in their diet in large quantities prolonged their lives, because it contained Lactobacillus. In 1907, his book had come out: The Prolongation of Life describing the improvement of health through diet that benefits the gastrointestinal micro-flora, which is now regarded as a sound probiotics principle. In 1908, Metchnikoff was awarded the Nobel Prize in medicine for his studies in immunology which investigated the ingestion of living microorganisms.

In 1907, Edward Buchner from Germany received the Nobel Prize for proving that fermentation was caused by enzymes in yeast cells. In 1929, Arthur Harden and Hans Euler-Chelpin received the Nobel Prize for clarifying how the enzymes in yeast cells are responsible for fermentation.

In 1930, the famous Japanese Minoru Shirota discovered the Bacteria Flora. He, consequently, isolated and cultivated the Lactobacillus casei strain which came to be known as Shirota to produce Yakult in 1935, which is a dairy product sold worldwide, containing fermented-bacteria milk (60).

In 1954, Ferdinand Vergin from Germany defined Probiotika as "active substances…essential for a healthy development of life." In 1962, Lily and Stillwell added these words to the definition of probiotics: include “the anaerobic bacteria that are able to produce lactic acid and stimulate the growth of other organisms." In 1974, Parker suggested that the term Probiotics should include microbial organisms as well as additional substances that contributed to intestinal microbial balance (64, 91).

The word in use nowadays was suggested by Roy Fuller who re-wrote the definition as "live microbial feed supplements which beneficially affects the host animal by improving its intestinal microbial balance." The most recent definition was drafted by FAO/WHO with guidelines in 2002 regarding symbiotic for the survival of ingested microorganisms. From 1930 onwards, the interest in probiotics dwindled in consequence of the Great Depression, World War II, and the discovery of different types of antibiotics (35, 24).

But, in 1980s, the interest renewed to treat many infections instead of using antibiotics. A few sentences in the Dietary Supplement Health and Education Act in 1994 caused a rise in the sales of probiotics, which allowed the marketing of the products without the rigorous approval of drugstore regulations (1).

2.2. The silent story of probiotics

The use of probiotics, according to molecular archeology, may be traced back to nearly 10,000 years ago (39). So, cultures around the globe used yeast in producing beverages, in ancient Egypt as well as elsewhere. The fermentation process was perfected by trial and error experiments.

In 1856, Pasteur's help was sought to see why a distillery failed to deliver good wine. It was found out that smaller cells than yeast were formed. Pasteur discovered that there were two kinds of fermentation: alcoholic and lactic acid. The first occurred because of yeast. The second happened due to bacteria factor (75).

In the ancient history, there was much revealed about fermentation, particularly in Jihau, in the Republic of China from 7000 BC, prepared from rice, fruits, honey, and yeast (137). Also, Prof. John Darnell, Yale University, discovered in Egypt during 1995 a recipe of wine mixed with herbs as a medical beverage. But, Prof. Patrick

McGovern states in 2007 that fermented wine was first made in Georgia 6000 BC. The earliest wine jars come from 5500 BC in Zagros, Western Iran (96).

Moreover, a cave in Armenia found in 2007 was reported to be the oldest winery in existence, presumed to be 6000 years old, as mentioned in (King James Bible, Genesis, 9:20-21).

The Holy Scriptures of various religions document the food as functional. The oldest belief is Hinduism, called ‘Sanatana Dharma’‘Sanatana Dharma’ meaning the everlasting religion, in which the Holy Veda refers to the importance of fermented products for a healthy life.

The Sumerian tablets talk about the fermented milk. There is a Sumerian Poem on how to produce Beer, from 3000 BC, which is a Hymn to the Goddess of beverages, called the Goddess Ninkasi. Likewise, the ancient Jewish prophets and the Jewish Talmud stressed the importance of wine as an antiseptic and as a sedative after circumcision (139).

Hippocrates the ancient Greek physician (460-370 BC) recommended wine provided that it was consumed with good sense. The medicinal uses of wine were: anti-anemic,antipyretic, anti diarrheal, purgative, anticonvulsive, stimulant for appetite, hypnotic, sedative, diuretic, anesthetic, and antiseptic (106).

In the middle Ages, beer saved the lives of the sailors because this fermented beverage resisted contamination. It was also important to drink it during the plague instead of water. Some of the saints advised that wine be used for medical purposes: they are Saint Nicholas of Myra, Saint Augustine of Hippo, Saint Wenceslas, the founder of the Czech state, and the first Saint was Luke the Apostle, Author of the Gospel of Luke, New Testament.

In the Roman History, Pliny the Elder refers to the soured milk of the barbarians for its healing quality. That was sour milk used for the treatment of diarrhea and vaginal diseases (133).

In the Mongolian History, the sour milk became the provision for the armies of Genghis Khan, who occupied 22% of the earth's total area (73). The great scholar Mahmud al- Kashgari (1008-1105) and Yusuf Has Hacip (1017-1077), who wrote about the Turkish communities from Asia to the Middle East, emphasized the importance of the Turkish Yoghurt in the diet of the Turks. The Ottoman doctor of Sultan Suleiman the magnificent treated the gastrointestinal diseases of King Francis I of France by goat yoghurt (127).

In the 14th Century, Arnaldus de Villa Nova wrote a textbook on the medical uses of wine (134). When Robert Hook in the 17th Century improved Anton van Leeuwenhoek's microscope, there came about a drastic change in the discovery of bacteria, yeasts, and blood samples (31).

The Pasteur Institute also played a major role in microbiology. Henry Tissier discovered Bifidobacterium in 1889. He reported that gastroenteritis could be treated by balance in favor of useful bacteria, called friendly bacteria (127, 48). This was presented in Paris University in 1906. In 1908, Metchnikoff suggested that phagocytosis in intestinal flora was responsible for toxic substances that caused ageing (124). He suggested also that lactic acid bacteria in dairy products could reduce in the colon, because hillside peasants in Bulgaria, who consumed fermented milk in large quantities, live long and healthy lives. It was suggested to him by the young Bulgarian doctor Stamen Grigorov in 1905, which lived between(1878-1945). He published his work in Étude sur une lait, reporting a lactic acid bacillus in yoghurt called Kisselo Milako. He made a presentation in the Pasteur Institute Great Hall, which impressed the audiences. The bacteria was named *Lactobacillus bulgaricum* in the memory of Dr. Stamen Grigorov, even though people may think that the reason may have been Metchnikoff's statement that "the Bulgarians live longer."(119).

The German Physician Alfred Nissle isolated a nonpathogenic strain of *E. coli* Nissle strain 1917. The discovery had results proving that probiotics can also be used in the diseases of the gastrointestinal tract in addition to the infectious diseases (118).

The rise of Yoghurt owes much to Emmanuel Karasu, a Jewish native of Thessaloniki, who produced yoghurt in his native city, then in Paris, then in USA, known as Dannon Milk Products. Also, the Armenian Brothers Sarkis and Rose Colombosian sold their yoghurt product called ‘madzoon’ in USA, which they later renamed Colombo and Sons Creamery in 1929. Later on, it was incorporated into General Mills in 1993.

In 1935, Dr. Shirota developed Yakult, which is the word Yoghurt in Esperanto, the proposed universal language. Dr. Shirota suggested that the daily intake of this drink would prolong the lifespan of the consumer (135).

As seen above humans throughout history exploited the bacteria and especially probiotics to improve their life quality in different ways. The use of probiotics has developed from bacteria to yeast and finally to fungi. This development is used in food production and health improvement such as disease control, strengthening the immunity system and water quality improvement. Nowadays probiotics play a vital role in the improvement of human life and health and in the future most probably it can be used instead of medicine.

3. Aim and Scope

The intensification of fish in aquacultures, leads to problems in their sector like disease increase and water pollution, more exhaustion, more bacterial, parasite and virus ailments, which calls for the use of antibiotics widely in animal farms including fish farming to control the hindrances and improve the growth for nutrition transformation. But, the large quantities in using them took a high toll on the useful biotics of the microflora of the guts. This negative effect indicates an accumulation of impact in the meat consumed by the humans. The risks maximized by the likelihood of transferring the bacteria to humans in two directions: either by the humans consuming the meat or by direct infections contacts (FAO 2005).

For these reasons, there was an advertisement by the European Union in January 2006 by which antibiotics were banned to promote the increase in the fish sector growth. This encouraged the people in charge of this sector to study the contents of the bowels of the warm water fish and the cold water fish in the seas to identify the useful elements among the microscopic creatures (138). The damage caused by antibiotics overuse for the intestines sent an alarm to the specialists in this field because this badly affects human health and animal health altogether equally, which inspired the researchers to the use of the minute living organisms as nutrition addenda or food supplement. Thus, some of the trademarks appeared which commercial marketing of probiotics.

The use of these supplements and probiotics was a contribution to improve the appetite and the living organism's use of nourishment better by producing vitamins by the added probiotics. This also reduced the poison of some composites in the nourishment and maximized the digestibility of indigestible items. Various studies indicated the positive impact of the probiotics against ailments, sickness, and the morbid bacteria (87). This called for the widespread use of intestinal microflora to cure many diseases in the early 1980s.

The USA Food & Drug Administration (FDA) asked the specialists in microscopic biology to publish GRAS (General Recognition as Safe) including dozens of types and species which are biologically useful: they are permissible by the international health organization to be added as supplement to the food, which encouraged the researchers to abide by the list. There rules and regulations were the preface to the flourished role of the microflora in the intestines to make the microbial balance in the digestive system discovered by Elie Mechnikoff (1845-1916) defined as the animal getting some living organisms which add positive health effects on the host.

The topic of probiotics is chosen because it has a big role in the improvement of the life quality of man. It is going to be used as an alternative or a replacement of antibiotics in the future medicine. It is significant to see the miraculous role of probiotics play in the increase and the improvement of man's health and food quality for man or animals. The focus this study will be on the use of probiotics in aquaculture and fish industry.

4. Definition and types of probiotics

4.1. Definition

Probiotics form living microbes in various foodstuffs, beverages, and nourishment as well as some drugs, the species called *Lactobacillus* and *Bifidobacterium* are the most commonly used for probiotics. For thousands of years, men used the lactic acid bacteria of the *Lactobacillus species* for food fermentation as well as for health improvement. The word probiotics must be correctly used to indicate the live microbes that according to academic studies, in part health benefits. The fermentation of the food stuff improves the taste and lowers, preventing the probable contamination by pathogens. Fermentation is used worldwide to preserve agricultural products like fruits, vegetables, roots, cereals, tubers, milk, meat, fish, and other stuffs.

The microorganisms involved are numerous when it comes to probiotics, including bacteria, moulds, and yeasts. One probiotics class is for humans, another one is for animal use. The animal probiotics are an alternative to antibiotics and thus promote growth. Therefore, the progress in coming up with substitutes for animal antibiotics is getting underway to be licensed and it is of great interest (141).

As the humans using probiotics, there are very few restrictions because the foods are already supplements in various forms like dairy products containing live bacteria on capsules with lyophilized preparations of bacteria improving health and cleanliness-online, there are products purchased to treat gastro intestinal disorders like diarrhea.

At present, the most available types of probiotics are, universally, *Lactobacillus spp*. which is lactic acid bacteria. They are found in gastro intestinal tract in both animals and humans. It is generally thought that the use of commensal microorganisms can restore the natural microflora to the gut. The second class is unavailable in gastro intestinal tract. Examples include *Saccharomyces boulardii*, which are able to prevent the recurrence of *Clostridium difficile* induced pseudo-membranous colitis, as well as antagonistic action of *E coli*. Recently, the products of the *S. boulardii* are marketed for human use. Within this group of allochthonous, the spore-forming bacteria are the genus bacillus that forms the probiotics microbes. The spore-format products can be stored for very long times (140).

4.2. Common Types

The microorganisms used as probiotics are various. It is useful to know that genus is the first name of a bacterium. It is general and stands for the group of organisms sharing similar qualities, like physical features, metabolic and end products.

The second part of the bacterium name refers to the species. It is more specific, and focuses on what is distinctive from other species. Strain, also, further divides members of the same species into smaller subgroups in accordance with the distinctions shared (45).

4.2.1. *Lactobacillus sp.*

This is a lactic acid producing Gram-positive species in the form of rods that are both obligate and facultative anaerobes in the gastro intestinal and genitourinary tracts (70).

Lactobacillus belongs to the group lactic acid bacteria which are known to produce lactic acid during fermentation (129). Lactobacilli are used therapeutically as probiotics, the opposite of antibiotics. They are friendly bacteria that can recognize areas of the body to provide nutritional benefits and decrease intestinal permeability (114). There is a theory that the intake of *Lactobacillus* probiotics during antibiotic treatment can prevent normal flora depletion and bacterial colonization (120). Lactic acid in the vagina prevents pathogen growth by decrease vaginal pH (71).Lactobacilli, might help against cancer by decreasing the tumor development in the colon. *L. plantarum* reduces the severe pain of chemo therapy (72).

L. bulgaricus and *L. sporogenes* can have hypolipidemic and anti-atherosclerotic effects. It can reduce low-density lipoprotein cholesterol with no relation with high-density lipoprotein (27). Dairy products have a positive effect on cholesterol. They also increase fatty acid in the intestine and decreasing hepatic cholesterol synthesis or retransfer cholesterol to the liver from plasma. Lactobacilli and other probiotics are effective as long as they can colonize an area of the tissue. Storage plays an important role in keeping the probiotics viable. *L. bulgaricus* and *L. sporogenes* have effect on hormone level (77).

4.2.2. *Bifidobacterium sp.*

The *Bifidobacterium* is an anaerobic bacterium. It is gram-positive, non-spore forming, pleomorphic rod. *B. longum* BB536 is a type isolated first from the intestinal tract of healthy infants. They seem to reduce the unfavorable effects of *Heliobacter* therapy. *Bifidobacterium* combined with *L. acidophilus* reduce NEC morality in critically ill newborns (47).

4.2.3. *Bacillus sp.*

This is *B. coagulans* which is a gram-positive rod. It can be misclassified as a lactic acid bacterium because it can produce lactic acid. It can form spores. It is used just like other probiotics like *Lactobacillus* and *Bifidobacterium*, but it is not normally a component of the human flora. It is undisclosed if the bacillus spore can terminate in the intestinal tract.

B. coagulans can reduce pathogenic bacteria colonization as it produces lactic acid. Ingesting bacteria spores strengthens the immune response (29). This probiotics species offers advantages over *Lactobacillus* because it can be indefinitely kept in store in desiccated forms resistant to high temperatures and acid (29).

4.2.4. *Saccharomyces spp.*

S. boulardii is a non-pathogenic yeast strain used for the treatment and prevention of diarrhea. This is isolated from tropical Indochina fruit skins. The locals there used the fruit skins therefore the treatment of their diarrhea (12).

It is prepared by freeze-drying of live yeasts organisms, using lactose in the process the identification of the strains requires molecular typing. This *S. boulardii* is genetically almost identical with *S. cerevisiae* (33).

5. Mechanisms of probiotics action

5.1. How the probiotics act in aquaculture

The mechanism acts as follows:

Probiotics create an unfriendly environment for the pathogens by producing inhibitors such as (bacteriocins, lysozymes, proteases, hydrogen peroxide). All these inhibitory compounds compete with those pathogens for nutrients and they strengthen the immune responses of the host plus essential nutrient supplementation, vitamins, enzymes, straight uptake of diluted organic stuff mediated through beneficial bacteria modulating with the environment.

Probiotics mostly play a great role in biological control agents that belong to lactic acid bacteria (*Lactobacillus and Carnobacterium*) and genera Vibrio (*V. alginolyticus and V. parahaemolyticus)*, *Bacillus, Pseudomonas*, *Aeromonas*, and *Flavobacterium* (6).Multiple screening is needed for the identification of probiotics bacteria to confirm how feasible it is for field application. Molecular identification has a number of steps to verify the beneficial bacteria, such as randomly amplified polymorphic DNA, and amplified ribosomal DNA restriction analysis. The DNA and peptidoglycan can be important in effectiveness, determining the specific probiotics action and application for the treatment of a disease.

Probiotics can bind with colonic cells and mucin to aid the colonization of the gut system in animals (55).

Microflora in the gastrointestinal tract (GIT) in aquatic animals is modifiable by ingesting additional microorganisms. But, the major bacteria in fish intestine are unlike those observed in mammals (37).

Microbial communities as communities are under the impact of environment conditions and practices of husbandry. For example, water, culture water, biotic, abiotic, and additional factors stimulate to the increase of chosen bacterial species. Thus, to add beneficial bacteria to the culture water or to the feed supplementation during the egg fertilization in pre-larval phases may be advantageous for the competitive exclusion for attachment sites on egg surface or in the (GIT) according to

(52). Competitive exclusion by potential probiotics bacteria can be evaluated by in vitro antagonistic screening against multiple strains of pathogenic bacteria.

One of the common phenomena in nature is called Bacterial Antagonism. Probiotics may stop opportunistic pathogens from colonizing eggs surface or the (GIT) may be of antimicrobial compounds or by outcompeting them for the mucosal room or for the nutrients or mucosal space Some Pathogens make proteolytic enzymes to dissolve and digest the bacteria approaching them (8).

Lactic acid bacteria, notably *Lactobacillus sp*. *Bifidobacterium sp*. and *Streptococcus sp*. are effective against diseases caused by *Vibrio sp*. (36, 89). *Bacillus subtilis* BT23 has inhibitory effects against vibriosis in tiger shrimp *Penaeus monodon* (130). Application of a *Bacillus* strain to the common snook *Centropomus undecimalis* increased larval survival, promoted better growth and decreased the number of suspected pathogenic bacteria in the fish gut (57). *Alteromonas sp*. can reduce the infection of *Vibrio sp*. in Pacific oyster *Crassostrea gigas* larviculture (28). Some *Pseudomonas fluorescens* strains reduce the mortality of rainbow trout Oncorhynchus mykiss fingerlings which are infected with a pathogenic *V. anguillarum* (40) (figure1).

5.2. The contributions of nutrients and enzymes to digestion

It is well established that probiotics are beneficial to the intestinal health of host animals, among the beneficial bacteria, *Agrobacterium sp. Brevibacterium sp*.

Clostridium sp. *Microbacterium sp*. *Pseudomonas sp*. and *Staphylococcus sp*. can contribute to the host's nutrition, especially in supplying fatty acids and vitamins to host cells (108, 105). Some fish gut probiotics may participate directly in the digestion processes of fish. Enzyme-producing probiotics such as Bacillus and Enterobacteriacea (*Acinetobacter sp. Aeromonas sp. Flavobacterium sp. Photobacterium sp. Pseudomonas sp. Vibrio sp. Microbacterium sp. Micrococcus sp. Staphylococcus sp*) and some unidentified anaerobes and yeasts are potential contributors (101).

The metabolic and physiological roles of fish gut probiotics have been the subject of several studies. These probiotics are able to stimulate gut epithelial differentiation and proliferation, gut motility, protein uptake, nutrient metabolism, and innate immunity (99, 100, 10). However, these functional roles are mostly limited to the initial stage of fish. In bivalves and crustaceans, probiotics in the gut facilitate digestion by producing extracellular enzymes such as proteases and lipases, as well as providing necessary growth factors (97, 132).

5.3. Enhancement of immune response

The close association of probiotics and the intestinal mucosa as well as intestinal cellular components seems to improve immunological functions of the GIT (59). The response is specific to (GALT) it is short for Gut – associated lymphoid tissues. Some infections are combated by serum, called an effect of humoral immunity which reacts by circulating antibodies of (Ig) meaning: Immunoglobulin. Five of these have roles in the response system. They are (IgA, IgD, IgE, IgG, IgM). They were observed in the aquatic animals when the protection mechanism was achieved by cellular and humoral defenses of immunity (103).

Monospecies or multispecies mixtures of supplementation probiotics can make the fish immunity response up-regulated. Phagocytic, lysozyme, complement, respiratory burst activity and the expression of various cytokines in fish can be stimulated by different probiotics (85).

Phagocytic activity of leucocytes is increased after oral administration of *Clostridium butyricum* bacteria to rainbow trout, which subsequently enhanced the resistance of fish to vibriosis (107). Similarly, administration of a mixture of bacterial strains (*Bacillus sp. and Vibrio sp*.) positively influenced the protective effect of white shrimp against the pathogens *V. harveyi* and white spot syndrome virus (WSSV) (7). This protection is largely attributed to the stimulation of the immune system by phagocytosis and antibacterial activity. Administration of a lactic acid bacterium *L. rhamnosus* (strain ATCC 53103) stimulates respiratory burst activity in rainbow trout (87). Some bacteriocin-like inhibitory substances such as antimicrobial peptides, proteins, or protein complexes excreted/synthesized by probiotics are also effective to control several fish disease, including *V. parahaemolyticus*, *Flavobacterium sp*. and *Aeromonas hydrophila* (111,13).

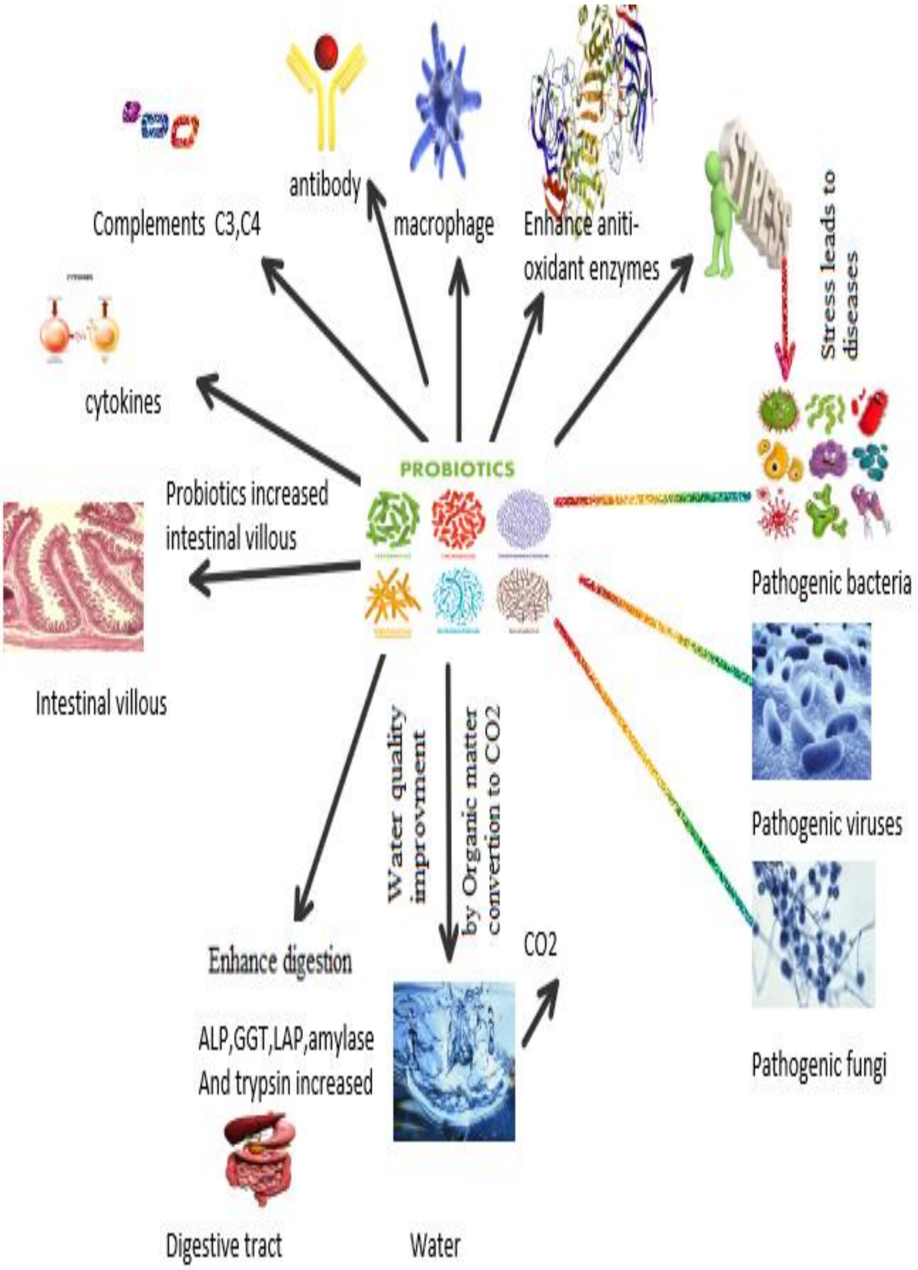

Figure1. Probiotics have several mechanism to provide the benefits to the fish(144).

5.4. How the probiotics act in animals

The probiotics in animals have a chemotactic effect on epithelial cells and the cells are connected with the changes in the intestinal epithelium of the host. The chemotactic effect is accomplished by mediators such as cytokines, metaloproteins (elastase and cathepsin), prostaglandins, oxygen and nitrogen reactive metabolites, elevating the production of IgA, IgM and IgG immunoglobulin, activating differentiation and proliferation of NK (Natural Killer), CD3, CD4 and CD8 lymphocytes, increasing the migration of lymphocyte T and the production of interferon (86). Probiotics induce changes in the intestinal epithelium and theses changes are accentuated by the decrease in luminal, antimicrobial activity and secretion of antimicrobial peptides inhibiting bacterial invasion and blocking the adhesion to epithelial cells. Thus, they enhance the intestinal layer or barrier elevating the production of cytokines (TNF-α, IFN-γ, IL-10 and IL-12), which in turn, induce the secretion of IgA in the intestinal mucosa, causing the release of mucins (42, 3). Mucins, the layer of glycoprotein that when in contact with water, form a film that lubricates and protects the intestinal epithelium against pathogens by creating a physical layer between the epithelium and the content from the intestinal lumen keeping the bacteria in check in the intestinal lumen (74).

It is implied by studies that the inhibiting effect of bacterial translocation by *Lactobacillus casei* GG in vivo and in vitro could be related with the regulation of the MUC- 2 gene, which increases the expression of mucin by goblet cells (74). In the intestine, probiotics interact with enterocytes, goblet cells, M cells from Peyer´s patches, isolated follicles that are extended through the mucosa and sub mucosa in the small intestine, forming GALT (Gut Associated Lymphoid Tissue) and immune cells among them, intraepithelial lymphocytes.

These interactions increase the number of IgA producing cells accompanied by the production of secretory IgM and IgA that are particularly important to the immunity of the mucosa, contributing to the barrier against pathogenic micro-organisms (121, 19). Thus, in the modulation of the immune response, the suppression of potential pathogens has been observed through the increase of intestinal motility, increase in the population of intraepithelial lymphocytes in the intestinal epithelium, removal of pathogens, modification of intestinal probiotics, and an increase in the height of intestinal villi (94,113,110). Besides these effects, there is the capacity of bacterial groups to develop a fimbria network that blocks the linking location of some enteric pathogens. An additional aspect is connected with various bacterial genera, which colonize and which are grown to produce a nearly competitive exclusion mechanism, representing the contest for adhesion locations to the membrane of the goblet cells, enteroendocrine cells and enterocytes in the intestinal mucosa, which promote a status of physical barrier to the mucosa by creating a special integrity system, preventing intestinal pathogens from becoming established (109).

5.5. How the probiotics act in poultry.

The probiotics mechanism action on the immune system of broiler mucosa is partly unclear. Nevertheless, the probiotics really have immune modulating effects admitted that probiotics have immune-modulating effects (86). The bacterium genera present in probiotics that are directly related to the increase in immunity of poultry are *Lactobacillus and Bifidobacterium*, mainly when related to diseases affecting the gastrointestinal tract. (46). The insusceptible balancing sway in poultry happens in these two ways: (a) from the probiotics, in which the probiotics moves along the wall of the digestive tract and is increased to a restricted expansion, or (b) the antigen discharged by the dead organisms are retained and hence animate the immune system (44). Antigens (lipopolysaccharides and peptidoglycans) are always discharged in intestinal lumen. Then again, this discharge is expanded during infectious procedures, when these segments play a vital role in the improvement and support of nearby immune response (66).

6. Current use of probiotics in humans and animals

6.1. Probiotics in current use in humans

For thousands of years, the humans used microbial cultures for food and fermentation of beverages. In the past hundred years, they were also used for the prevention of numerous ailments and diseases. Probiotics at present are available in nutritious products, in food and dietary supplements. The probiotics have wide applications to combat cancer, cholesterol, and allergies and so on.

6.2. What are the features of probiotics

The useful probiotics agents must be non-toxic, non-pathogenic, and resistant to gastric acid, adherent to gut epithelial tissue. They produce antibacterial substances. The probiotics resist for brief periods the gastro-intestinal tract affecting the metabolic activities like cholesterol, lactose and vitamin production.

The probiotics survive in the gut depending on the colonization factors in possession or whether there are antibacterial mechanisms in the gut. Furthermore, the antibacterial mechanisms need to avoid the bad effects of the peristalsis, which flush out both bacteria and food together. It can be done by either immobilizing themselves or by growth at a faster rate than that of removal by peristalsis.

The probiotics strain has to resist to the gastric acid, which is bifidobacteria, the strains proved to be less capable of acid resistance than the *Lactobacillus* strains, especially when exposed to the juice produced by the human gastric enzyme (126).

6.2.1. Cholesterol lowering

Lactic acid bacteria among probiotics strains have a big part to play in the mechanism of lowering cholesterol. When the level of cholesterol goes upward, it can be brought down by taking probiotics (35). The mechanism can be both direct and indirect. The direct one happens by inhibiting the de novo synthesis, and it can also happen by decreasing the intestinal absorption of the dietary cholesterol. The de novo inhibition can be done by hypocholesterolemic factors like uric acid, calcium hydroxyl methyl glutarate, lactose, orotic acid and whey proteins. The absorption of dietary cholesterol is reduced by three methods - assimilating, binding and degradation. The probiotics strains can assimilate the cholesterol for their own inner metabolism. The probiotics strains get linked with the cholesterol molecule so they become able to degrade cholesterol down to its catabolic products.

The cholesterol level will be indirectly lowered by deconjugating the cholesterol to bile acid, meaning the reduction of the entire body pool. The bile acids commonly formed as bile salts with glycine and taurine. The deconjugations of lactic acid bacterial cultures were approached by two kinds of bile salts. Namely, they are taurocholates and glycocholate. But most of the strains are able to deconjugate glycocholates. For instance, among the thirteen strains of *L.casei* which were tested, nine of them could deconjugate glycol-cholate but none of them deconjugated taurocholate.

The free bile acid maximum quantity was present in reduced medium by addition of thioglycolate but it was minimum in the aerobic incubation of optimum of pH6.0.

The potential hypocholestromic effect of probiotics triggered keen interest on evidence regarding animal work and from human studies stating that 0.5-51 yoghurt/day was used and the ingestion of realistic quantities of it, meaning the probiotics in yoghurt for man to show the reduction of cholesterol levels significantly (23).Conducted researches about the removal of cholesterol by *B. longum, B. infantis, B. breve, B. animalis* and *B. thermos-phyllum* with the presence of bile salts. He observed that the removal from the growth medium by bifidobacteria as well as the precipitation of cholesterol (123). Studied cholesterol reduction abilities in six strains of *L. acidophilus* and discovered in vivo hypocholesterolemic the ability likely to be the result of assimilating cholesterol by *L. acidophilus* cells, or it could be due to the attachment of cholesterol to the surface of *L. acidophilus* cells(65) .

6.2.2. Anticancer effects

There are anticancer benefits in the fermented foods. These benefits were regarded as folklore or the traditional way of thinking that is baseless in scientific terms. But at present there is a strong testimony that lactobacilli are important in the human diet and has a good effect on the human health because there is an interrelationship between the nutrition factors and the cancer as diets (83). Food stuff high in animal protein and rich with fat may increase colon cancer, through conversion of pro-carcinogens to carcinogens by the intestinal microflora. Fats and fried food can be responsible for breast cancer, prostate cancer and pancreas cancer. Drinking milk has been negative in relation to the gastric cancer. To consume milk prevents human stomach cancer, the kind caused by alkylating agents. But milk consumption is regarded as positive in relation to colon prostate and breast cancer .when it is full of fat which causes the modification of the intestinal flora by milk components. It has been attributed to the milk hormones and the presence of an oncogenic virus or other contaminations in the milk consumed.

There are studies on the impact of probiotics consumption on cancer which are hopeful and encouraging .There are indications in animal and in vitro studies that probiotics bacteria are likely to reduce colon cancer risk by lowering the number of tumors. It was shown in a clinical study that there was a period of recurrence – free time in patients of bladder cancer (4). But, it is also too early to jump into hasty conclusions or to develop specific recommendations on the intake of probiotics as cancer preventions in humans (102).

L. acidophilus remarkably lowered the number and size of colon tumors caused by another carcinogen. Oberreuther-Moschner, D.L., et al (2004) made a study to show the existence of proof directly concluding that the intake of probiotics has a beneficial influence on the diseases of the colon cancer in the humans (88). There are positive and encouraging results from current researches, but there are gaps in our knowledge; the main gap is linked with the mechanism of how the probiotics can generate anticancer impacts. There are changes in the bacterial enzymes of the gut, generating carcinogens and tumor promoters such as NH3 and secondary bile acids, stimulating the immune surveillance. The suppression of inflammatory processes, the binding of carcinogens in the gut must have levels of scientific support higher than the present one (11).

6.2.3. Lactose intolerance

The lactose intolerance problem can be solved by probiotics strains. The lactose intolerance is a physiological feature in some humans, which is to lack the ability to produce an enzyme called lactose or beta-galactosidase.

B-galactosidase is very important to assimilate the disaccharide in milk and it needs to be separated into two parts, glucose and galactose.

The people deficient in b-galactosidase will be unable to digest milk, which is a real problem in new born infants. Humans who suffer from lactose intolerance feel abdominal discomfort, diarrhea, cramps, flatulence, nausea and actual vomiting. An additional problem is linked with lactose intolerance is calcium deficiency. Anyone suffering from lactose intolerance had better take non-milk diet.

The bacteria in the colon ferment undigested lactose to produce acid and gas which causes symptoms ranging from stomach pain, bloating, diarrhea but

yoghurt contains less lactose than milk has and it delays gastric emptying which explains somehow the reason why lactose intolerant people can eat yoghurt. But yoghurt intake tolerance is due to the supply of lactose activity in the lactic acid bacteria of the yoghurt itself. The bacteria must be live and present in good numbers to be useful (95).

The richest source of calcium is milk. The calcium requirement of the human body is met only by the intake of milk. Therefore, anyone eating non milk food will eventually suffer from calcium deficiency which will cause osteoporosis. proved that lactose deficiency caused calcium malabsorption which in itself resulted in osteoporosis the calcium malabsorption can be caused by skipping the nutrients that has milk in it to avoid lactose intolerance lactose intolerance complications. The absorption of calcium is better achieved in acidic conditions. Therefore, if lactose is changed into lactic acid, pH of the gut will decrease so it will become acidic and favorable for the enhanced.

Absorption of calcium consequently, if probiotics are given to intolerance patients, then milk lactose is hydrolyzed by the probiotics strains and in this case lactose is assimilated and CA absorption is unhindered.

6.2.4. Allergy

Allergy can present itself as a change in the proper function of the immunity system. The indications of the studies point out that an alternation in the microflora in the gut, like a decrease in lactobacilli precedes the appearance of allergy. Probiotics reduce the occurrence of childhood eczema by half, compared to placebo, when administered during pregnancy up to six months postnatally (56).

A further study proved a two-fold increase in transforming the development of growth factor B2, which is an anti-inflammatory cytokine in the mother’s breast milk. These mothers received probiotics and were compared to the mothers who were recipients of placebo. There was a reduction in the atopic eczema risk among children whose mothers received probiotics rather than placebo.

Evident even at two years perhaps, probiotics do have beneficial effects on allergic reactions by improving the function of the mucosal barrier (98). Moreover, young children's intake of probiotics can have benefits for the immune system and its growth. *Lactobacillus* GC can be helpful in alleviating some food allergies associated with milk protein. The intake of probiotics can, perhaps, prevent allergy in susceptible individuals, which can be a key-role in reducing allergic cases in the west after its dramatic rise about forty years back from the present day.

6.2.5. Additional useful benefits

Constipation can be relaxed by probiotics intake. 50% of the studies prove that there is a benefit to remove constipation. There were researches demonstrating the improvement in the transit time. Most researches state that probiotics have a more pronounced effect than pure yoghurt (93). The power attributed to the lactic acid bacteria, like anti-microbial agent production and the rivalry against likely pathogens in the gut provide the impetus for examining the role of probiotics in diarrhea. Infantile acute gastroenteritis diarrhea is caused by the rotavirus but it showed a minimization in a number of times (18). So, the therapeutic and preventive impacts of probiotics were proven. The exact mechanism is unknown even though the evidence indicates it stimulates the immunity system. The broad-spectrum of antibiotics is prescribed to treat infections, but diarrhea is going to be a side-effect. Eight medical researches demonstrated that diarrhea can be lowered in response to probiotics against placebo (18). This is a convincing proof for the prevention of antibiotic-related diarrhea by using probiotics. But, the evidence is lacking for the protective effect of probiotics intraveller's diarrhea, which can be ascribed to a large number of viruses and bacteria. Diseases such as Chron's disease, ulcerative colitis/pouchitis, and other inflammatory bowel diseases are painful and long-lasting. Combinations of concentrated probiotics were shown to

Minimize the number of patients with pouchitis better than placebo over 12-18 month period.

6.3. Current use of probiotics in animals

There is little support for the efficiency of probiotics in farm animals. But, the potential benefits can be these points, according to (34):

a) Better resistance to infectious diseases
b) Better growth rate
c) Better food conversion
d) Better digestion.
e) Improved nutrients absorption
f) essential nutrients can be provide by probiotics
g) Better milk quality
h) More eggs laid
i) Better quality of eggs
j) Better carcass
k) less contamination Since

Probiotics substitute antimicrobial growth promoters. Thus, they are important to farm animals. Growth promoters improve animal performance (115). Penicillin and tetraclines used to improve the swine, cattle and bird performance. Antibiotics used as feed additives benefited animals in weight gain, and feeding. Now, they tend to be banned because they are risky for the human health in the long run. So, probiotics are the replacement, through diets that keep the intestinal microbial balance in the animals to prevent digestive tract diseases and improve feed digestibility by greater intake of nutrients.

Probiotics fill the gut together with billions of live and useful microbes to enhance the role of the micro-flora in the gut. This huge number of billions of microbes has a significant role in the growth, well-being and the strength of the animal.

The digestion system in animals and birds is the place where billions of microbes (bacteria, protozoa and fungi, collectively known as micro-flora) interact. A healthy and balanced micro-flora is a detrimental factor for the health of animals because it:

- Facilitates the process of digestion by breaking down cellulose and other materials which are indigestible.
- Helps the making up and the absorption of vitamins and minerals.
- Activates and enhances the immune system.
- Hiders the spread of potential disease-causing pathogens such as *E. coli* and *Salmonella*.

The Major uses and advantages of animal probiotics are:

- Enhancing the growth of Farm Animals by prevention of the subclinical gut infections.
- Promoting the efficiency of the digestive process by increasing the digestion of indigestible materials.
- Decreasing the intestinal problems such as scouring or diarrhea, loss of appetite and poor digestion of food.
- Improving the health by enhancing resistance to infectious diseases and strengthening the immunity system.
- Establishment/re-establishment of micro-flora in immature animals through the use of probiotics.

6.3.1. Animal probiotics can be used in these ways:

- Directly after the birth and this establishes the gut micro-flora and hinders pathogenic bacteria from boosting.
- Directly after administering antibiotics to prevent re-infection by pathogens.

Treatment or prevention of diarrhea caused by *E.coli* and *salmonella*

- Reduction of stress effects such as fear, transportation, change of habitat, change of diet, training and drill, competition, excessive heat, injury, surgical operation, and inoculation.(115).

7. Use of probiotics in fish and aquaculture

7.1. Why use probiotics in Aquaculture

The use of antibiotics and vaccines to protect the fish from disease have become of utmost necessity. The result of this use in the fish has led gradually to the development of resistance to these vaccines and antibiotics. They reduce the effect of probiotics and limit the diversity of the population. The secondary pathogens will gradually disappear from the growth environment. The survivor bacteria that carry the genes of resistance will re-enter the gastro-intestinal tract, and they will reduce the effectiveness of future doses of antibiotics and will eventually lead to the spread of disease in the fish environment. This is dangerous for fish as the fish probiotics is influenced by the rearing environment, according to the study by Merrifield DL., et al(2010), which is due to the breakdown of host pathogens in consequence of abundant fish farming (104,81). This encouraged the scientists to look for other options to replace the antibiotics to promote health. Alternative additives include probiotics (35, 67, and 32). The alternative is the manipulation of gut flora with added probiotics in the diet, nutrition water, and also in the immune-stimulants (38,128). Its use in feed as a growth and health promoter is to lessen diarrhea during weaning period for fish and similar animals.

The European Union (EU) prohibited antibiotics in 2006. So, the probiotics came to be used to promote a balanced therapeutic gut environment. The consumers demand the product without the sediments because such materials are very dangerous for human health. Antibiotics are a double edge sword for piglets and similar animals and it is important to have an approach to eliminate

the Salmonella and Escherichia which may resist antibiotics (25). Probiotics can prevent Gastro-intestinal tract colonization. The best use of probiotics can be done during the first stage of fish growth in time of injecting the vaccination, which can be impractical as a vaccine, so the probiotics replace the antibiotics in performance improvement and disease control by immunity enhancement and pathogenic exclusion (104).

7.2. Role of probiotics in aquaculture

The scientists recommend the use of bacterial amendments in aquaculture pools to get these benefits:

1- Feed efficiency.
2- Immunity enhancement and resistance against disease.
3- Nutrient absorption.
4- Change bacterial composition by removing undesirable forms in the gut.
5- Reduce mortality.
6- Increase production.
7- Decrease antibiotic use (41, 43, 2, 82, 53).

7.3. Selection of probiotics

The main objective of using probiotics is to associate a good link between beneficial and harmful microorganisms which make up the microbiota of the fish intestine. A good probiotics must have special features to make a positive impact as following:

- It should be capable of beneficial effect on the host animal.
- It must avoid side effects, pathogenic or toxic to the host or to the human customer.
- It should live in storage conditions and survive the industrial process.
- It should be able to outlive and metabolise the gut environment by being resistant to bile and low pH.
- It must possess the ability to multiply in the intestine.
- It should be adhesive with the gut of the fish.
- It should have a high antagonistic ability against the pathogenic microorganisms (35).

7.4. Modes of application in aquaculture

It can be done by feeding, injecting or directly adding in the water (52).

- **Feeding application**

Normally probiotics are directly added to the feed or sprayed on the ready feed. The probiotics in the use are *lactobacillus*, *bacillus*, and *saccharomyces cerevisiae*. (FAO) and (WHO) state that probiotics must survive while passing through the gut, resisting the gastric juices and bile. Also they must flourish and settle in the gut to be effective for the host animal (112).

- **Through immersion or injection**

Probiotics can be applied by injection immersion (5).

- **Direct Application to culture water**

Applying probiotics directly in pond or to tank water exhibit a good effect on fish health by improving the microbial composition and qualities of water and sediments.

The probiotics bacteria, particularly *Bacillus spp*. and some other species including *Aerobacter sp. Nitrobacter sp. and Saccharomyces cerevisiae* (yeast), played a remarkable part to change the organic matter to CO_2. Some probiotics strains provide algicidal activity against red tide plankton. Nitrifying bacteria effectively removed ammonia and nitrite from the water, which is the foremost water problem (63).

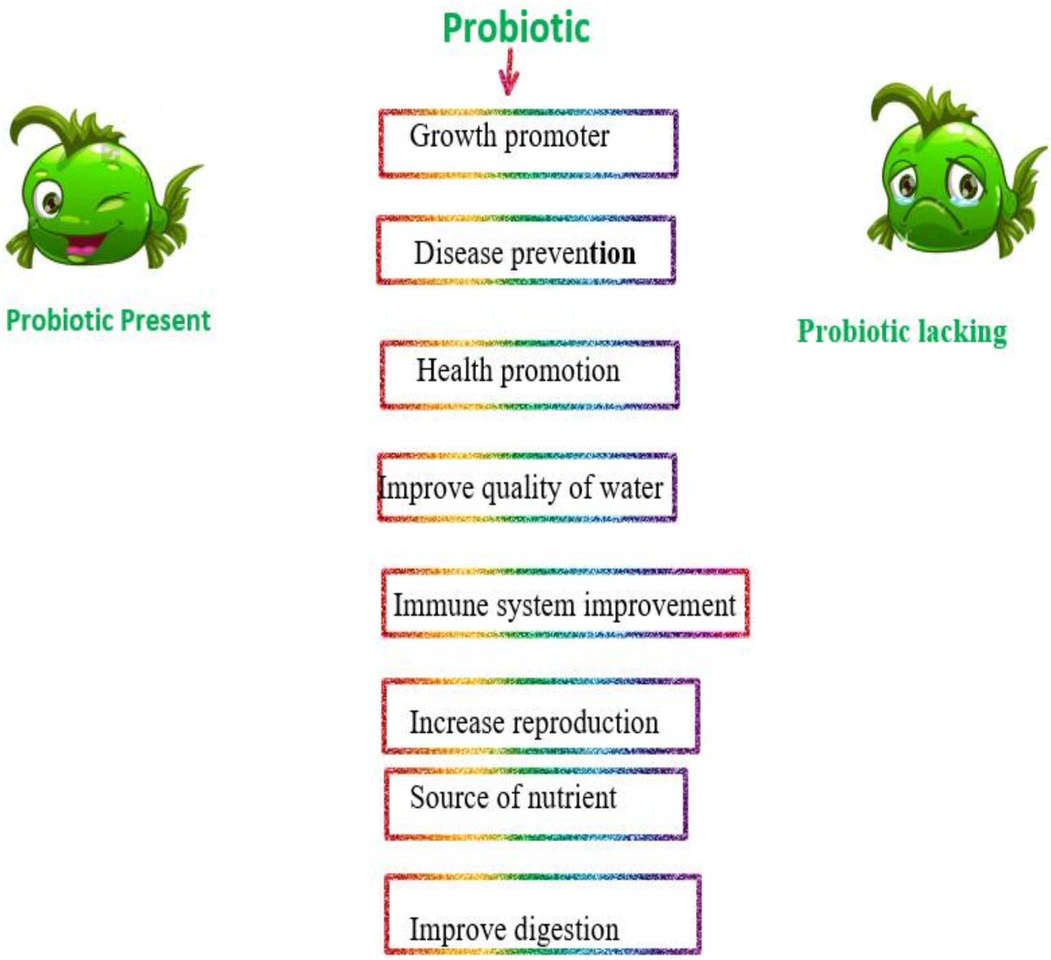

Figure2 . The benefits of probiotics in aquaculture(143).

7.5Application in Aquaculture

The initial interest focused on probiotics to promote and improve the animal's health. Now they have their effect on reproduction or stress tolerance, but this needs more scientific examination (figure2).

7.5.1. Growth promoter

Probiotics increase the growth of cultivated species, may be by improving the appetite, or digestibility or both factors. Perhaps probiotics taste good for the fish. The probiotics creatures colonize the gastrointestinal tract when administrated for long times because they multiply abundantly, so the probiotics adhere to the intestinal mucosa of the gastrointestinal tract in the fish, developing multiple benefits. Also, hydrobionts (any organism that lives in water) temperature, enzyme levels, genetic resistance, and water quality can be important factors (52).

7.5.2. Inhibition of pathogens

Antibiotics prevented diseases in the past but various problems came along the antibiotic residues in tissues, such as resistance mechanism and imbalance in the gastro intestinal microbiotic. The EU regulated antibiotics in the products that the humans will consume. Today, natural products free of antibiotics are much in demand. Today, people want to prevent the disease rather that to use medicine to treat it. So, probiotics is the good choice to inhibit pathogens and disease in fish. Probiotics can give chemicals with bactericidal effect on pathogenic bacteria in the fish, thus preventing the proliferation of undesirable pathogens. This effect is due to: production of antibiotics, bacteriocins, siderophores, enzymes and hydrogen peroxide as well as change in pH in the intestine. The apply of probiotics due to increase non specific immune response (122).

7.5.3. Improvement in Nutrient Digestion.

Probiotics have a beneficial effect on the digestion of fish because the strains synthesize extracellular enzymes like protease, amylases, lipases and vitamins, fatty acids, and amino acids (6). Nutrients are absorbed efficiently when the feed has probiotics (32).

Thus, probiotics are used in fish and in larvae of European bass.

Probiotics yeast *Debaryomyces hansenii* HF1 can produce spermine and spermidine involved in differentiation and maturation of the gastro-tract in mammals. This yeast secrets amylase and trypsin enzymes aiding digestion in bass larvae (17).

7.5.4. Improvement of water quality.

Water quality is related to fish disease in the farming ponds. It is necessary to keep water criteria high. Nitrogen, ammonia, nitrite waste, and organic filth must stay away from fish ponds, as these wastes cause mass fish loss (21). These materials are toxic. The toxic can be changed by the oxidizing bacteria of ammonia to nitrite and from nitrite to nitrate. Probiotics benefits include: decreased algae growth, decreased organic load, increased nutrient concentration, increased bacterial population, inhibition of potential pathogens, and increased concentration of dissolved oxygen (50).

According to studies, Bacillus bacteria are regarded as water treatment probiotics because they can convert organic matter into Co_2 (20). The effects of *B.subtilis, B.cereus, and B.licheniformis* are attributed to bioaccumulation, bio assimilation, and nitrification. In addition, it has been proven that the addition of probiotics bacteria reduces the load of pollutants such as heavy metals (Pb, Cd, Hg, Ni, etc.) (9). Also, the use of *Bacillus spp*. can reduce the incidence of Vibriosis in water .Other probiotics candidates such as *Nitrosomonas sp*. and *Nitrobacter sp*. have been shown to be beneficial in decreasing the pathogenic load in culture ponds (90). Likewise, the species *Rhodopseudomonas palustris*,

L. plantarum, *L.casei,* and *S.cerevisiae* have been attributed to probiotics potential in the maintenance of water quality.

Probiotics for fish culture requires decisive measures to determine its effectiveness, including (abiotic) or (biotic) factors to stimulate the proliferation and dominance of the probiotics with favorable surroundings for it. The probiotics application can be direct to the fish water or mixed with microalgae highly concentrated for fish food in the larval culture two days after hatching. Also, entry in the fish culture can be done through the feed of fish after 19 days of hatching and adding *Artemia* 25 days after hatching. Another way is also, through the fish skin where probiotics can colonize its surface and penetrate through it. Thus, probiotics can be found in sediment, water, and organisms of culture (79).

7.5.5. Stress Tolerance

Stress is an agent causing reactions resulting in disease and death, changes in water criteria have a side effect on aquatic animals. Different types of stress have negative results on fish, including thermal, nutritional, high density, anoxia, hypoxia, chemicals and toxins (26, 22, 68).

Harmful agents in fish environments such as water, soil, air, and in their own body exist in intensive systems of aquaculture (117). Their high density is an important factor for outbreak of stressful conditions more than in wild fish. But, the application of probiotics bacteria as a supplement and for water can stop these conditions in aquatic fish, improving the immune system and reducing various stressors (125).

Oxidative stress is a situation enhancing ROS concentration which disturbs cellular metabolism and by this damaging cellular constituents (54). Ros production may be linked to antioxidant responses (62).The physiological activities of fish can be really affected by temperature and environmental parameters. Also, contaminants, (xenobiotics), UV radiation, hypoxia may cause oxidative stress in fish (84). Feeding the fish with probiotics ca valid biochemical stress signs that indicate fish stress include: blood glucose, cortisol, and RNA/DNA ratio in tissues (116). Stress, tolerance, growth, and health in fish can be assessed by heat shock (16). The effects of probiotics reflect on growth, stress tolerance, and immune response in fish. Plasma lysozyme in the probiotics feed and the water was significantly higher in the diet group than the control group of the experiment on the immune response.

In heat shock stress test, the flounder group showed greater tolerance. Koninkx and Malago (2008) showed that under stress, the normal intestinal micro flora taken for probiotics could improve the defense system to increase the heat shock protein (HSP) (61). Some probiotics bacteria decrease biochemical stress in cortisol level on supplementation of *L. delbruckii ssp* in the feed of European sea bass compared to control group in temperature stress (125). ToakaY, et al (2006) found out that *Bacillus SPP* reduced handling stress by affecting the cortisol level (125). Studied gilt-head bream fish to prove that there was improved tolerance with this treatment under high stock density (14, 15,142).

7.5.6. Reproduction effect of Aquatic Species

Izquierdo proved that aquaculture species have feeding requirements and reproductive capacity depends on the right concentration of carotenoids, Vitamin C and Vitamin E, fatty acid, protein, and lipid. The link of these can affect reproduction, fertility, fertilization, birth, and larvae development.

Now the “brood stock diets” are available for fish species. Many fish hatcheries improve the brood stock by feeding in combination with commercial diet, a number of bi-products. The most common such stuff include squid, cuttlefish, mussels, krill, and small aquatic creatures unprocessed to bridge the gap of the brood stock fish but it may increase the transmission of viruses, bacteria, and parasites, from parent fish to offspring fish.

Consequently, probiotics have to be added to water and to fish feed to prevent infections from pathogenic organisms during production of fish (96, 84).

8. The future of probiotics

Scientific studies in the field of probiotics as preventers of fish infection show that this is a promising approach. Environment friendly diet supplement will be a good option for probiotics in aquaculture. But the results of probiotics are species specific. The tests demonstrate that lactobacilli can stop the disease in fish due to its strong immune system, which is sensitive to the environment and in need of control biologically. The studies need further investigation. The exact mood of actions is still unclear. There are promising effects of probiotics but the research data available is limited in comparison with immune-stimulants other than probiotics. We need more research on antiviral nature of probiotics on the grounds that our comprehension of how it influences the immune system is limited especially in molecular mechanisms of the interactions between the host and the probiotics.

9. Conclusion

To conclude, much use of antibiotics generates antibiotic resistance against the bacteria. Inefficiencies in antibiotics caused losses in economy; however probiotics in fish ponds beneficially affect aquaculture wellbeing, which improves fish cultivation economically. Likewise, probiotics can be useful for the environment by limiting infection and diminishing the requirement for medication.

The use of probiotics in fish nutrient will be an important contribution in aquaculture improvement.

Abbreviation

BC: Before Christ

DNA: Deoxyribonucleic acid

EU: European Union

FAO: Food agriculture organization

FDA: Food and drug administration

GALT: Gut -associated lymphoid tissues

GIT: Gastrointestinal tract

GRAS: General Recognition as safe hypolipidemic

HSP: Heat shock protein

IFN: Inter Freon

NEC: New born ill critically

NK: Natural killing

RNA: Ribonucleic acid

ROS: Reactive oxygen species

TNF: Tumor necrosis factor

WHO: World health organization

WSSV: White spot syndrome virus

List of Figures

10. References

1. Agheyisi R., 2008. http://www.bccresearch.com/report/FOD035B.html
2. Ahilan, B., 2003. Probiotics in aquaculture. Aqua International. 39-40
3. Arvola, T.; Laiho, K.; Torkelli, S.; Mykkanen, H.; Salminen, S.; Maunula, L. & Isolauri, E. ,1999. Prophylactic Lactobacillus GG reduces antibiotic-associated diarrhea in children with respiratory infections: a randomized study. Pediatrics, Vol.104, No.5, (November 1999), pp. 64, 1999, ISSN 1098-4275
4. Aso, Y. and Akazan, H., 1992. Prophylactic effect of a Lactobacillus casei preparation on the recurrence of superficial bladder cancer. Urol. Int., 49, 125–129.
5. Austin B, Stuckey LF, Robertson PAW, Effendi I, Griffith DRW. 1995. A probiotic strain of Vibrio alginolyticus effective in reducing diseases caused by Aeromonas salmonicida, Vibrio anguillarum and Vibrio ordalii. Journal of Fish Disease. 18:93-96
6. Balcázar J.L., I. de Blas, I. Ruiz-Zarzuela, D. Cunningham, D. Vendrell and J.L. Múzquiz. , 2006. The role of probiotic in aquaculture. Veterinary Microbiology 114:173-186.
7. Balcázar, J.L., 2003. Evaluation of probiotic bacterial strains in Litopenaeus vannamei. Final Report, National Center for Marine and Aquaculture Research, Guayaquil, Ecuador.
8. Bandyopadhyay, P. and P.K.D. Mohapatra. , 2009. Effect of a probiotic bacterium Bacillus circulans PB7 in the formulated diets: on growth, nutritional quality and immunity of Catla catla (Ham.) Fish Physiology and Biochemistry 35:467-478
9. Banerjee G, Nandi A, Dan SK, et al.2016. Mode of association, enzyme producing abil- ity and identification of autochthonous bacteria in the gastrointestinal tract of two Indian air-breathing fish, murrel (Channa punctatus) and stinging catfish (Heteropneustes fossilis). Proceedings of the Zoological Society (Calcutta). https://doi.org/10.1007/ s12595-016-0167-x
10. Bates, J.M., E. Mittge, J. Kuhlman, K.N. Baden, S.E. Cheesman and K. Guillemin. , 2006. Distinct signals from the microbiota promote different aspects of zebrafish gut differentiation. Developmental Biology 297:374-386
11. Burns, A. J. and Rowland, I. R., 2000. Ant carcinogenicity of probiotic and prebiotics. Curr. Issues Intest. Microbial. 2, 13–24.

12. ButsJP.,2005.[LyophilizedSaccharomycesboulardii:exampleofaprobioticmedicine].Revista de gastroenterologia del Peru: organo oficial de la Sociedad de Gastroenterologia del Peru.25(2):176–88.
13. Carraturo, A., K. Raieta, D. Ottaviani and G.L. Russo. , 2006. Inhibition of Vibrio parahaemolyticus by a bacteriocin-like inhibitory substance (BLIS) produced by Vibrio mediterranei 1. Journal of Applied Microbiology 101(1):234-241.
14. Castex M, Lemaire P, Wabete N, Chim L., 2009. Effect of dietaryprobiotic Pediococcus acidilacticion antioxidant defences and oxidative stress status of shrimp Litopenaeus stylirost-ris. Aquaculture. 294(3-4):306-313.
15. Chiu CH, Guu YK, Liu CH, Pan TM, Cheng W., 2007. Immune responses and gene expression in white shrimp (Litope-naeus vannamei), induced by Lactobacillus plantarum. Fish Shellfish Immunol. 23:364-377.
16. Cruz PM, Ibanez AL, Monroy Hermosillo OA, Ramirez Saad HC, 2012. Use of probiotic in aquaculture. ISRN Microbiol.2:1-13
17. D. Tovar, J. Zambonino, C. Cahu, F. Gatesoupe, R. Vázquez, and R. Lesel., 2002. “Effect of live yeast incorporation in compound diet on digestive enzyme activity in sea bass (Dicentrarchus labrax) larvae,” Aquaculture, vol. 204, no. 1-2, pp. 113–123.View at Publisher View at Google Scholar · View at Scopus
18. D’Souza, A. L., Rajkumar, C., Cooke, J. and Bulpitt, C. J., 2002.Probiotic in prevention of antibiotic associated diarrhoea: meta-analysis. Br. Med. J.324, 1361–1366.
19. Dalloul, R.A.; Lillehoj, H.S.; Shellem, T.A. & Doerr, J.A., 2003. Enhanced mucosal immunity against Eimeria acervulina in broilers fed a Lactobacillus-based probiotic. Poultry Science, Vol.82, No.1, (January 2003), pp.62-66, ISSN 1525-3171
20. DalminG, KathiresanK, PurushothamanA.2001.Effect of probiotic on bacterialpopulation and health status of shrimp in culture pond ecosystem. Indian Journal of Experimental Biology. 39:939-942
21. Das S, Mondal K, Haque SA.2017. Review on application of probiotic, prebiotics and synbi- otic for sustainable development of aquaculture. Journal of Entomology and Zoology Studies. 5(2):422-429.
22. Das T, Pal AK, Chakraborty SK, Manush SM, Sahu NP,Mukherjee SC. 2005. Thermal tolerance, growth and oxygen consumption of Labeo rohita fry (Hamilton, 1822) accli-mated to four temperatures. J Therm Biol. 30:378-383.

23. De Roos, N. M., Schouten, G. and Katan, M. B., 1999.Yoghurt enriched with Lactobacillus acidophilus does not lower blood lipids in healthy men and women with normal to borderline high serum cholesterol levels. Eur. J. Clin. Nutr. 53, 277–280.
24. De Vrese M, Schrezenmeir J., 2008.Probiotics, prebiotics, and synbiotics. Adv Biochem Eng Biotechnol 111: 1-66.
25. Delforge J., 2004. Monogastric nutrition gets the probiotic treatment: An article. Feed mix 12: No. 5:13-14
26. DeMicco A, Cooper KR, Richardson JR, White LA. 2010. Devel-opmental neurotoxicity of pyrethroid insecticides in zebrafish embryos. Toxicol Sci. 113:177-186.
27. Doncheva NI, Antov GP, Softova EB, Nyagolov YP.,2002. Experimental and clinical study on the hypolipidemic and antisclerotic effect of Lactobacillus Bulgaricus strain GB N 1 (48). Nutrition Research. 22(4):393–403.
28. Douillet, P.A. and C.J. Langdon. , 1994. Use of a probiotic for the culture of larvae of the Pacific oyster (Crassostrea gigas Thunberg). Aquaculture 119:25-40.
29. Duc le H, Hong HA, Barbosa TM, Henriques AO, Cutting SM. ,2004.Characterization of Bacillus probiotics available for human use. Applied and Environmental Microbiology.70 (4):2161–71.
30. Duc le H, Hong HA, Barbosa TM, Henriques AO, Cutting SM., 2004. Characterization of Bacillus probiotics available for human use. Applied and Environmental Microbiology.70 (4):2161–71.
31. Egerton, N.F., 2006. A history of the ecological sciences, part 19: Leeuwenhoek's microscopic natural history. Bulletin of the Ecological Society of America 87: 47-58.
32. El-Haroun ER, A-S Goda AM, Kabir Chowdhury MA., 2006. Effect of dietary probiotic Bioen supplementation as a growth promoter on growth performance and feed utilization of Nile tilapia Oreochromis niloticus (L.) Aquacult. Res. 37:1473-1480.
33. FiettoJL,AraujoRS,ValadaoFN,FiettoLG,BrandaoRL,NevesMJ,etal.,2004.Molecular and physiological comparisons between Saccharomyces cerevisiae and Saccharomyces boulardii. Canadian Journal of Microbiology. 50(8):615–21.
34. Fuller R., 1999.Probiotics for farm animals. Probiotics: A critical Review
35. Fuller R. A., 1989.review: probiotics in man and animals. Journal of Applied Bacteriology. 66:365-378.

36. Gatesoupe, F.J., 1994. Lactic acid bacteria increased the resistance of turbot larvae, Scophthalmus maximus. Aquaculture 96:335-342.
37. Gatesoupe, F.J., 2005. Probiotics and prebiotics for fish culture, at the parting of the ways. Aqua Feeds: Formulation & Beyond 2(3):3-5.
38. Gildberg A, Mikkelsen H, Sandaker H, Ringo E .,1997.Probiotic effect of lactic acid bacteria in the feed on growth and survival of fry of Atlantic cod (Gadus Morhua). Hydrobiologia 352:279-285.
39. Godoy, A., Herrera, T. and Ulloa, M., 2003. Más allá del pulque y el tepache: las bebidas alcohólicas no destiladas indígenas de México. Instituto de Investigaciones Antropológicas, Universidad Nacional Autonoma de Mexico, Mexico City, Mexico.
40. Gram, L., J. Melchiorsen, B. Spanggaard, I. Huber and T.F. Nielsen.
a. 1999. Inhibition of Vibrio anguillarum by Pseudomonas fluorescens AH2, a possible probiotic treatment of fish. Applied Environmental Microbiology 65:969-973
41. Green, A. and M. Green. , 2003. Probiotics in Asian shrimp aquaculture. MPEDA Newsletter.
42. Gupta, V. & Garg, R., 2009. Probiotics. Indian Journal of Medical Microbiology, Vol.27, No.3, (July-September 2009), pp. 202-209, ISSN 1998-3646
43. Haung, H. J., 2003. Important tools to the success of shrimp aquaculture-Aeration and the applications of tea seed cake and probiotics. Aqua International 13-16
44. Havenaar, R. & Spanhaak, S., 1994. Probiotics from an immunological point of view. Current Opinion in Biotechnology, Vol.5, No.3, (June 1994) pp. 320-325, 1994, ISSN 0168-1605
45. HawrelakJ,BNat(Hons).Probiotics.In:PizzornoJE,MurrayMT,editors,2013.Textbookof Natural Medicine. 4th ed. St. Louis, Missouri: Churchill Livingstone Elsevier. p. 979–94.
46. Hong, H.A.; Duc, L.H. & Cutting, S.M., 2005.The use of bacterial spore formers as probiotics. (2005). FEMS Microbiology Reviews, Vol.29, No.4, (September 2005), pp. 813- 835, ISSN 1574-6976
47. Hoyos AB., 1999. Reduced incidence of necrotizing enterocolitis associated with enteral administration of Lactobacillus acidophilus and Bifidobacterium infant to neonates in an intensive care unit. International Journal of Infectious Diseases: IJID: official publica-tion of the International Society for infectious Diseases.3 (4):197-202.

48. Hughes, D.B. and Hoover, D.G., 1991. Bifidobacteria: their potential for use in American dairy products. Food Technology 4: 74-83.
49. Huxley A., 1871.Discourses: Biological & Geological (volumeVIII): Yeast. Collected Essays.
50. IbrahemMD.2015.Evolution of probiotics in aquatic world: Potential effects, thecurrentsta- tus in Egypt and recent prospective. Journal of Advanced Research. 6:765-791
51. Inhibition of Vibrio anguillarum by Pseudomonas fluorescens AH2, a possible probiotic treatment of fish. Applied Environmental Microbiology 65:969-973.
52. Irianto A, Austin B., 2002. Probiotics in aquaculture. Journal of Fish Diseases.25:633-642.
53. Jameson, J. D. 2003. Role of probiotics in aquaculture practices. Fishing Chimes 23/9.
54. Jia X, Zhang H, Liu X., 2011. Low levels of cadmium exposureinduce DNA damage and oxidative stress in the liver of Oujiang colored common carp (Cyprinus carpio var color).Fish Physiol Biochem. 37:97-103.
55. Juntunen, M., P.V. Kirjavainen, A.C. Ouwehand, S.J. Salminen and E. Isolauri. , 2001. Adherence of probiotic bacteria to human mucus in healthy infants and during rotavirus infection. Clinical and Diagnostic Laboratory Immunology 8:293-296.
56. Kalliomaki, M., Salminen, S., Arvilommi, H., Kero, P., Koskinen, P. and Isolauri, E., 2001.Probiotics in primary prevention of atopic dis-ease: A randomized placebo-controlled trial. Lancet.357, 1076–1079.
57. Kennedy, S.B., J.W. Tucker, C.L. Neidig, G.K. Vermeer, V.R. Cooper, J.L. Jarrell and D.G. Sennett., 1998. Bacterial management strategies for stock management of warm water marine fish: a case study with common snook (Centropomus undecimalis). Bulletin of Marine Science 62:573-588.
58. KimJK, ParkKJ, ChoKS, NamS, ParkT,BajpaiR.2005.Aerobicnitrication–Denitrificationby heterotrophic bacillus strains. Bioresource Technology. 96:1897-1906
59. Klaenhammer, T.R., 2007. Probiotics and Prebiotics. Pages 891-910 in: D.P. Doyle and L.R. Beuchat (eds.). Food Microbiology – fundamental and frontiers 3rd Ed., ASM Press, Washington D.C.

60. KOLLATH W., 1953.[The increase of the diseases of civilization and their prevention]. Munch Med Wochenschr 95: 1260-1262.
61. Koninkx JFJG, Malago JJ. , 2008. The protective potency of pro-biotic bacteria and their microbial products against enteric infections-review. *Folia Microbiologica*. 53(3)pp.189-194
62. Lesser MP. , 2006. Oxidative stress in marine environments: biochemistry and physiological ecology. Ann Rev Physiol.68:253-278.
63. Lewis JrWM, Morris DP1989. Toxicity of nitrite to fish: a review. Transactions of the American Fisheries Society. 115(2):183-195
64. Lilly, D. M., & Stillwell, R. H. 1965. Probiotics: growth-promoting factors produced by microorganisms. Science, 147(3659), 747-748.
65. Lin, S. Y. and Chen, C. T., 2000.Reduction of cholesterol by Lactobacillus acidophilus in culture broth. J. Food Drug Anal., 8, 97– 102.
66. Loddi, M. M., 2003.Probióticos, prebióticos e acidificantes orgânicos em dietas para frangos de corte. 52f. Tese (Doutorado em Zootecnia) – Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista "Júlio de Mesquita Filho", Jaboticabal.
67. Lodemann U, Lorenz BM, Weyrauch KD, Martens H., 2007. Effects of Bacillus cereus var. toyoi as a probiotic feed supplement on intestinal transport and barrier function in piglets. Arch. Anim. Nutri. 62:2:87- 106.
68. Logan CA, Somero GN. 2011. Effects of thermal acclimationon transcriptional responses to acute heat stress in theeurythermal fish Gillichthys mirabilis (Cooper). Am J PhysiolRegul Integr Comp Physiol. 300(6):1373-1383.
69. M. Izquierdo, H. Palacios, and A. Tacon., 2001."Effect of brood- stock nutrition on reproductive performance of fish," Aqua- culture, vol. 197, no. 1–4, pp. 25–42.
70. Madsen KL, Doyle JS, Jewell LD, Travertine MM, Fedorak RN. , 1999, Lactobacillus species prevents colitis in interleukin 10 gene-deficient mice. Gastroenterology. 116(5): 1107–14.
71. Maggi L, Mastromarino P, Macchia S, Brigidi P, Pirovano F, Matteuzzi D, et al. ,2000.Technological and biological evaluation of tablets containing different strains of lactobacilli for vaginal administration. European Journal of Pharmaceutics and Biopharmaceutics: official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik eV. 50(3):389–95.

72. Mao Y, Nobaek S, Kasravi B, Adawi D, Stenram U, Molin G, et al. ,1996.The effects of Lactobacillus strains and oat fiber on methotrexate-induced enterocolitis in rats. Gastroenterology. 1996; 111(2):334–44.
73. Marshall, R., 1993. Storm from the East: from Ghengis Khan to Khubilai Khan. University of California Press, Berkeley, CA, USA.
74. Mattar, A.; Daniel, H.; Drongawski, R.; Wongyi, F.; Harmon, C. & Coran, A., 2002. Probiotics up-regulate MUC-2 mucin gene expression in a Caco-2 cell-culture model. Pediatric Surgery International, Vol.18, No.7, (October 2002), pp. 586-590, ISSN 0179-0358
75. Mazzarello, P., 2002. Life out of nowhere? Nature 417: 792-793. McGovern, P.E. (ed.), 2007.
76. Ancient wine: the search for the origins of viniculture. Princeton University Press, West Sussex, UK
77. McGroartyJA.,1993.Probioticuseoflactobacilliinthehumanfemaleurogenitaltract.FEMS Immunology and Medical Microbiology.6(4):251–64.
78. McGroartyJA.,1993.Probioticuseoflactobacilliinthehumanfemaleurogenitaltract.FEMS Immunology and Medical Microbiology. 6(4):251–64.
79. MelgarValdesCE,BarbaMacíasE,AlvarezGonzálezCA,TovillaHernándezC,SánchezAJ. , 2013.
80. Microorganisms effect with probiotic potential in water quality and growth of the shrimp Litopenaeus vannamei (Decapods: Penaeidae) in intensive culture. Revista de Biología Tropical. 61:1215-1228.
81. Merrifield DL, Bradley G, Baker RTM, Davies SJ., 2010.Probiotic applications for rainbow trout (Oncorhyncus mykiss Walbaum) II. Effects on growth performance, feed utilization, intestinal microbiota and related health criteria post-antibiotic treatment. Aquacult. Nutr. 16:496-503.
82. Mishra, S., S. Mohanty, P. Pattnaik and S. Ayyappan. , 2001. Probiotics- Possible role in aquaculture. Fishing Chimes 21/1: 31-36.
83. Mitsuoka, T., 1981.Intestinal flora and cancer. In Second Annual National Symposium for Lactic Acid Bacteria and Health, Korea, pp. 16–40.
84. Mohapatra S, Chakraborty T, Kumar V, De Boeck G, MohantaKN. , 2012. Aquaculture and stress management: a review of probiotic intervention. J Anim Physiol Anim Nut.14:1-26.

85. Nayak, S.K., 2010. Probiotics and immunity: a fish perspective. Fish and Shellfish Immunology 29:2-14
86. Ng, S.C.; Hart, A.L.; Kamm, M.A.; Stagg, A.J. & Knight, S.C., 2009. Mechanisms of Action of Probiotics: Recent Advances. Inflammatory Bowel Diseases, Vol.15, No.2, (February 2009), pp.300-310, ISSN 1536-4844
87. Nikoskelainen, S., A. Ouwehand, G. Bylund, S. Salminen and E.M. Lilius. , 2003. Immune enhancement in rainbow trout (Oncorhynchus mykiss) by potential probiotic bacteria (Lactobacillus rhamnosus). Fish and Shellfish Immunology 15:443-452
88. Oberreuther-Moschner, D. L., Jahreis, G., Rechkemner, G. and Pool Zobel, B. L., 2004.Dietary intervention with the probiotics Lactobacil-lus acidophilus 145 and Bifidobacterium longum 913 modulates the potential of human faecal water to induce damage in HT29 clone 19A cells. Br. J. Nutr., 91, 925–932.
89. Olsson, J.C., K. Jöborn, A. Westerdahl, L. Blomberg, S. Kjelleberg and P.L. Conway. 1998., Survival, persistence and proliferation of Vibrio anguillarum in juvenile turbot, Scophthalmus maximus (L.), intestine and faeces. Journal of Fish Diseases 21:1-9.
90. Padmavathi P, Sunitha K, Veeraiah K.2012. Efficacy of probiotics in improving water quality and bacterial flora in fish ponds. African Journal of Microbiology Research. 6:7471-7478
91. Parker RB. 1974. The other half of the antibiotic story. Anim Nut Health 29: 4-8.
92. Pasteur L., 1858.Mèmoire sur la fermentation appeleé lactique. Annales de Chimie et de Physique 3e. 52: 404-418.
93. Pathmakanthan, S., Meance, S. and Edwards, C. A., 2002.Probiotics: A review of human studies to date and methodological approaches. Microb. Ecol. Health Dis, 2, 10–30.
94. Patterson, J. A. & Burkholder, K.M. ,2003. Application of prebiotics and probiotics in poultry production. Poultry Science, Vol.82, No.4, (April 2003), pp. 627-631, ISSN 1525-3171.
95. Pelletier, X., Laure-Bossuet, S. and Donazzolo, Y., 2001. Hydrogen excretion upon ingestion of dairy products in lactose intolerant male subjects: Importance of the live flora. Eur. J. Clin. Nutr., 2001, 55, 509–512
96. PhysOrg, 2011. Earliest known winery found in Armenian cave. Available at: http://phys.org/news/2011-01-earliest-winery- armenian-cave.html.

97. Prieur, G., J.L. Nicolas, A. Plusquellec and M. Vigneulle. , 1990. Interactions between bivalves, molluscs and bacteria in the marine environment. Oceanography and Marine Biology Annual Review 28:227-352.
98. Rautava, S., Kalliomaki, M. and Isolauri, E., 2002.Probiotics during pregnancy and breast-feeding might confer immunomodulatory protection against atopic disease in the infant. J. Allergy Clin. Im-munol., 109, 119–121.
99. Rawls, J.F., B.A. Samuel and J.I. Gordon. , 2004. Gnotobiotic zebrafish reveal evolutionarily conserved responses to the gut microbiota. Proceedings of the National Academy of Sciences 101:4596-4601.
100. Rawls, J.F., M.A. Mahowald, R.E. Ley and J.I. Gordon. , 2006. Reciprocal gut microbiota transplants from zebrafish and mice to germ-free recipients reveal host habitat selection. Cell 127:423-433
101. Ray, A.K., K. Ghosh and E. Ringø. , 2012. Enzyme-producing bacteria isolated from fish gut: a review. Aquaculture Nutrition18:465-492
102. Reddy, B. S. and Rivenson, A., 1993.Inhibitory effect of Bifidobacte-rium longum colon, mammary and liver carcinogenesis induced by 2-amino, 3-methylimidazo (4, 5-f-quinoline), a food mutagen. Can-cer Res., 53, 3914–3918.
103. Rengpipat, S., S. Rukpratanporn, S. Piyatiratitivorakul and P. Menasaveta. ,2000. Immunity enhancement in black tiger shrimp (Penaeus monodon) by a probiont bacterium (Bacillus S11). Aquaculture 191:271-288
104. Ringo E, Brubeck TH., 1999.Intestinal microflora of fish larvae and fry. Aquac. Res. 30:73-93
105. Ringø, E., E. Strøm and J. Tabacheck. , 1995. Intestinal microflora of salmonids: a review. Aquaculture Research 26:773-789
106. Robinson, J., 2006. The Oxford companion to wine. Oxford University Press, New York, NY, USA.
107. Sakai, M., T. Yoshida, S. Astute and M. Kobayashi. , 1995. Enhancement of resistance to vibriosis in rainbow trout, Oncorhynchus mykiss (Walbaum) by oral administration of Clostridium butyricum bacteria. Journal of Fish Disease 18:187-190.
108. Sakata, T., 1990. Microflora in the digestive tract of fish and shellfish. Pages 171-176 in: R. Lesel (ed.), Microbiology in Poecilotherms. Elsevier, Amsterdam.
109. Salminen, S. Isolauri, E., 1996. Clinical uses of probiotics for stabilizing the gut mucosal barrier: successful strains and future challenges. Antoine van Leeuwenhoek, Vol. 70, No.2-4, pp.347–358, ISSN 1572-9699.

110. Salzman, N.H.; Ghosh, D.; Huttner, K.M.; Paterson, Y. & Benvis, C.L., 2003. Protection against enteric salmonellosis in transgenic mice expressing a human intestinal defensin. Nature, Vol. 422, No.3 (April 2003), pp. 522–526, ISSN 0028-0836.
111. Selvendran, M. and M. Michael Babu. , 2013. Studies on novel bacteriocin like inhibitory substance (BLIS) from microalgal symbiotic Vibrio spp MMB2 and its activity against aquatic bacterial pathogens. Journal of Applied Pharmaceutical Science 3(2):169-175.
112. Senok AC, Ismeel AY, Botta GA.2005. Probiotics: facts and myths. Clinical Microbiology and Infection Diseases. 11:958-960.
113. Shane, S. M., 2001. Mananoligossacarídeos em nutrição de aves: mecanismos e benefícios. Proceedings 17º Simpósio Anual da Alltech, Lexington, 2001, pp.65-77.
114. ShornikovaAV,CasasIA,IsolauriE,MykkanenH,VesikariT.,1997.Lactobacillusreuterias a therapeutic agent in acute diarrhea in young children. Journal of Pediatric Gastroen- terology and Nutrition. 24(4):399–404.
115. Simon O, Vahjen W, Scharek L., 2003.Microorganisms as Feed Additive-Probiotics. Proc. 9 th International Symposium on Digestive Physiology in Pigs, Banff, Canada; Vol 1: 295-318
116. Sivaraman GK, Barat A, Ali S, Mahanta PC. , 2012. Prediction offish growth rate and food availability in the Himalayan water bodies by estimation of RNA/DNA ratios. IUP JGenet Evol. 4(3):15-19.
117. Smith KF, Schmidt V, Rosen GE, Amaral-Zettler L. 2012. Micro-bial diversity and potential pathogens in ornamental fish aquarium water. PLoS One. 7(9):e39971.
118. Sonnenborn, U. and Schulze, J., 2009. The non-pathogenic Eschericha coli strain Nissle 1917 – features of a versatile probiotic. Microbial Ecology in Health and Disease 21: 122-158
119. Stamen Grigorov Foundation, undated. Website. Available at: http:// www.stamengrigorov.org.
120. Sullivan A, Barkholt L, Nord CE. 2003. Lactobacillus acidophilus, Bifidobacterium lactis and Lactobacillus F19 prevent antibiotic-associated ecological disturbances of Bacteroides fragilis in the intestine. The Journal of Antimicrobial Chemotherapy. 52(2):308–11.

121. Szajewska, H.; Kotowska, M.; Mrukowicz, J.Z.; Armanska, M. & Mikolajczyk, W.,2001. Efficacy of Lactobacillus GG in prevention of nosocomial diarrhea in infants. The Journal of Pediatrics, Vol.138, No.3, (March 2001), pp.361–365, ISSN 0022-3476
122. T. Nakano, 2007. Microorganism. En dietary supplements for the health and quality of cultured fish, CAB International, Lon-don, UK.
123. Tahri, K., Grill, J. P. and Schneider, F., 1996.Bifidobacteria strain behavior toward cholesterol: Co precipitation with bile salts and assimilation. Curr. Microbiol. 33, 187–193.
124. Tan, SY. And Dee, MK., 2009. Elie Metchnikoff (1845-1916): discoverer of phagocytosis. Singapore Medical Journal 50: 456-457.
125. Taoka Y, Maeda H, Jo JY. ,2006. Growth, stress tolerance andnon-specific immune response of Japanese flounder Para-lichthys olivaceusto probiotics in a closed recirculating sys-tem. Fish Sci. 72(2):310-321.
126. Thornton, G. M., 1996.Probiotic bacteria selection of Lactobacillus and Bifidobacterium strains from the healthy human gastrointestinal tract, characterization of a novel Lactobacillus-derived antibacterial protein (thesis), National Univ., Ireland.
127. Tissier H .,1906.Tratement des infections intestinales par la méthode de la flore bactérienne de l'intestin. Crit Rev Soc Biol 60: 359-361.
128. Tukmechi A, Morshedi A, Delirezh N., 2007. Changes in intestinal microflora and humoral immune response following probiotic administration rainbow trout oncorhyncus mykiss. J. Anim. Vet. Adv. 6(10):1183-1189.
129. .VanderhoofJA,YoungRJ.,2004.Currentandpotentialusesofprobiotics.AnnalsofAllergy, Asthma & Immunology: Official Publication of the American College of Allergy, Asthma, & Immunology.93(5 Suppl 3):S33–7.
130. Vaseeharan, B. and P. Ramasamy. , 2003. Control of pathogenic Vibrio spp. by Bacillus subtilis BT23, a possible probiotic treatment for black tiger shrimp Penaeus monodon. Letters in Applied Microbiology 36:83-87.
131. Wabete N, Chim L, Lemaire P, Massabuau JC. , 2008. Life on theedge: physiological problems in penaeid prawns Litope-naeus stylirostris, living on the low side of their thermo referendum. Mar Biol. 154:403-412
132. Wang, X., H. Li, X. Zhang, Y. Li, W. Ji and H. Xu. ,2000. Microbial flora in the digestive tract of adult penaeid shrimp (Penaeus chine sis). Journal of Ocean University of Qingdao 30:493-498

133. Wethered, H.N., 1937. The mind of the ancient world. A consideration of Pliny's natural history. Longmans Green and Co Press, London, UK.
134. White, J., 1962. Journal of a voyage to New South Wales. Angus & Robertson in Association with the Royal Australian Historical Society, Sydney, NSW, Australia.
135. Yakult, 2014. History. Available at: http://www.yakultusa.com/ Company/History.php.
136. Yun-Zhang Sun, Hong-Ling Yang, Ru-Long Ma, Wen-Yan Lin., 2010. Probiotic applications of two dominant gut Bacillus strains with antagonistic activity improves the growth performance and immune responses of grouper Epinephelus coioides. Fish Shellfish Immunol. 29:803-809
137. Zhang, J., Harbottle, G., Wang, C. and Kong, Z., 1999. Oldest playable musical instruments found at Jiahu early Neolithic site in China. Nature 401: 366-368.
138. Zhou, A.; Y. Liu; P. Shi; S. He; B. Yao and E. Ringø (2009). Molecular characterization of the autochthonous microbiota in the gastrointestinal tract of adult yellow grouper Epinephelu sawoara cultured in cages. Aquaculture, .184-189 :286
139. Patai, R. (2014). The Jewish alchemists: a history and source book (Vol. 236). Princeton University Press
140. Czerucka, D., Dahan, S., Mograbi, B., Rossi, B. and Rampal, P., 2000. Saccharomyces boulardii preserves the barrier function and modulates the signal transduction pathway induced in enteropathogenic Escherichia coli-infected T84 cells. Infection and immunity, 68(10), pp.5998-6004.
141. Cartman, S.T., La Ragione, R.M. and Woodward, M.J., 2008. Bacillus subtilis spores germinate in the chicken gastrointestinal tract. Appl. Environ. Microbiol, 74(16), pp.5254-5258.
142. Varela, J. L., Ruiz-Jarabo, I., Vargas-Chacoff, L., Arijo, S., León-Rubio, J. M., García-Millán, I., ... & Mancera, J. M. 2010. Dietary administration of probiotic Pdp11 promotes growth and improves stress tolerance to high stocking density in gilthead seabream Sparus auratus. Aquaculture, 309(1-4), 265-271.
143. Sayes, C., Leyton, Y. and Riquelme, C., 2017. Probiotic bacteria as an healthy alternative for fish aquaculture. In Antibiotic Use in Animals. Intech open.
144. Zorriehzahra, M.J., Delshad, S.T., Adel, M., Tiwari, R., Karthik, K., Dhama, K. and Lazado, C.C., 2016. Probiotics as beneficial microbes in aquaculture: an update on their multiple modes of action: a review. Veterinary Quarterly, 36(4), pp.228-241.

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