*Review Article*

Biotechnology in Fisheries and Aquaculture: A Holistic Review of Innovations in Health, Nutrition, and Production

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ABSTRACT

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| The fastest-growing agricultural industry in the world is aquaculture, and both wild fish capture and aquaculture production contribute to global fish production. Global fish production has risen rapidly in recent years to over 120 million tons, accounting for 52% of all fish consumed by humans. Aquaculture production accounted for 82 million tons, or USD 250 billion, of the estimated actual money value of USD 401 billion. Aquaculture biotechnology greatly enhances food security and nutrition by increasing the productivity, sustainability, and efficiency of aquaculture systems. Through the application of advanced genetic techniques including transgenesis, genomic selection, and marker-assisted selection, significant advancements have been made in the breeding of fish with superior traits, such as higher feed conversion efficiency, disease resistance, and faster growth rates. The most recent of these is the creation of transgenic fish, or AquAdvantage salmon, an Atlantic salmon that has undergone genetic modification, via the application of biotechnology. Molecular markers enable genetic tagging in breeding operations and offer precise data on the genetic diversity of natural populations. Additionally, the long-term preservation of reproductive cells by cryopreservation technology protects endangered or economically significant species. To stop undesirable gene escapees from harming biodiversity, biotechnology can also produce sterile fish. This review discusses the notable advancements and applications of biotechnology in aquaculture nutrition, commercially prepared feed, disease and health control through vaccines, and water quality management in the aquaculture sector. |

*Keywords: Aquaculture, Biotechnology, Feed biotechnology, Gnotobiotic, Nutraceticals, Probiotics, Recombinant proteins, Single cell protein and Vaccines,*

**1. INTRODUCTION**

Biotechnologyis defined by several authors using various ways but the simplest one is United Nations Convention on Biological Diversity as “any technological application that uses biological systems, living organisms or derivatives or modifies products or process for specific use (Mukherjee, 2015; Sreenivasulu, 2008). Biotechnology as functional biology that comprises the use of living organisms and bioprocess in engineering technology, medicine and other fields requiring bio-products. Biotechnological interventions such as the development of inducing hormone, disease diagnosis kit, probiotics, bio filter and selective breeding etc, (Bentahar *et al*., 2023). To improve production, disease resistance, and feed efficiency, this review will critically look at how contemporary biotechnological tools- such as genetic engineering, molecular diagnostics, vaccine development, probiotics, and nutrigenomics- are used. Additionally, it aims to draw attention to the difficulties, dangers, and prospects for incorporating biotechnology into sustainable aquaculture methods.

**1.1 Biotechnology in Fisheries**

Numerous potential uses of biotechnology exist for the advancement of aquaculture and fisheries. The technology impacted by biotechnological intervention will play a major role in determining the success of next-generation aquaculture. Aquaculture's four key pillars- seed, feed, disease, and environment can be effectively combined with biotechnology discoveries to increase productivity by multiple times (Nius et al., 2024). According to Muhammad *et al*. (2013), biotechnology offers strong instruments for the food industries and aquaculture's sustainable growth.

**1.2 Role of Biotechnology in Seed Production**

A new avenue for the development of genetic resources in aquaculture has been made possible by biotechnology (Dunham, 2023). The most crucial prerequisite for beginning aquaculture is high-quality, pure seed. The growth of the semi-intensive and intensive aquaculture industries is largely dependent on the availability of high-quality seed with enhanced performance in large quantities. The blue revolution has been illuminated by the groundbreaking advancements in aquaculture hatchery technology, such as the introduction of induced breeding methods and selective breeding (Sonesson *et al*., 2023).

**1.2.1 Induced Breeding**

Although hypophysation-based induced breeding was created in the 1950s, the development of synthetic hormones has been essential in bringing the technology to the public. A method known as "induced breeding" uses pituitary hormone to encourage ripe fish to breed in captivity. Another name for this breeding process is hypophysation (De Silva et al., 2008; Davy and Chouinard, 1981). Nowadays, hatcheries utilize a variety of commercially available inducing chemicals, including ovatide, ovaprime, ovapel, and others. The first effort at induced breeding of fish was made by Houssay (1930) of Argentina, who used pituitary extract on a viviparous fish. Dr. Hiralal Choudhuri used this method on little carps later in 1955. Dr. Hiralal Choudhuri conducted the first successful induced breeding on major carps in 1957, using *Cirrhinus mrigala, C. reba*, and *Labeo rohita* (Harvey and Carolsfeld, 1993; Bandyopadhyay, 2022).

**1.2.2 Assisted Reproductive Techniques**

Nowadays, a lot of people use assisted reproductive procedures (ART) to treat infertile couples. Based on the precise light scattering and fluorescence characteristics of each cell, it offers a method for separating a diverse mixture of cells into two or more containers, one cell at a time. Several dead spermatozoa were also found in the milt that was obtained during stripping, which prevented fertilization. The live and motile sperm cells that were isolated may be gathered in a particular container and utilized in subsequent breeding processes. Although ART is still in its infancy, it has enormous promise, particularly for species that are threatened or have lower fecundity (Huang and Rosenwaks, 2014).

**1.2.3 Transgenic fish:**

By employing the genetic engineering technique of gene transfer, certain alterations in the genome are brought about, creating the transgenic or genetically modified creature. The two GMOs that are commercially accessible for decorative and consumption purposes, respectively, are zebra fish and aqua-advantage salmon (Jena et al., 2017; Gillund, 2011). According to the FDA, "genetically engineered (GE) animals" include any animals that have undergone rDNA alteration, including any progeny that bear the mutation. Scientists are working to create fish that are bigger, grow more quickly, convert feed into muscle more effectively, are disease-resistant, tolerant of low water oxygen levels, and can withstand cold temperatures. Aqua Bounty Technologies created the genetically modified Atlantic salmon known as "Ex Aqua-Advantage salmon" (Maclean and Laight, 2000).

**1.2.4 Disease diagnosis:**

The strength of bio-security may be preserved by keeping an eye on pathogens at various stages of aquaculture. It is urgently necessary to create a method for pathogen identification that is easy to use, sensitive, dependable, and quick (Pawar et al., 2025). The science of diagnostics has been transformed by PCR technology. These days, there are other PCR variations, including RPA (recombinase polymerase amplification) and LAMP (loop-mediated isothermal amplification) (Shahrajabian and Sun, 2023).

**2. VACCINES**

Different antigen formulations made from certain pathogenic organisms that have been rendered non-pathogenic are known as vaccines. Immune system stimulation and increased resistance to illness from recurrent infection by the pathogen are the results of vaccination (Ha et al., 2008). By protecting fish from future diseases and the accompanying expenses of sickness, mortality, and therapeutic treatment, vaccination serves as a preventative intervention. The 1940s saw the first reports of fish vaccination as a disease prevention measure. David C. B. Duff is considered the "Father of fish vaccination" since he wrote the first paper on the topic (Vaseeharan and Jesudhasan, 2024). Only certain illnesses are protected against by a vaccination. Fish that get a vaccination against *Streptococcus iniae*, for instance, will be protected from *S. iniae* infection, but not from *Vibrio anguillarum*. A vaccination may be based on oil or water. Since oil has adjuvant properties, injectable vaccinations are usually oil-based.

**2.1 Types of Vaccine**

Aquaculture may be greatly integrated with biotechnology to improve the future and quicken the blue revolution. Aquaculture may benefit greatly from biotechnology in addressing several difficult problems, including balanced feed and seed disease.

**2.1.1 Conventional Fish Vaccines:**

While live attenuated or subunit protein vaccines (formulated with adjuvants) have been sold, conventional fish vaccines have mostly consisted of inactivated entire organisms. Formalin-killed microorganisms, with or without adjuvant, were used in the first fish vaccinations. A few modified live vaccines were created and made available for use in aquaculture in the 1990s (Ma *et al*., 2019).

**2.1.2 Inactivated/Killed Vaccine:**

The virulent disease-causing microorganism used to make inactivated or killed vaccinations usually undergoes a procedure that renders it incapable of replicating or infecting a host. Most early immunization experiments in aquaculture employed dead vaccines. When Duff studied oral vaccination of *Oncorhynchus clarkii*, he used a dead *Aeromonas salmonicida* vaccine, which was the first documented use of a fish vaccine. A dead *Yersinia ruckeri* vaccine against enteric red mouth disease administered by immersion was the first commercially approved vaccination for fish (Kumar *et al*., 2015). Formalin-killed immersion vaccines were created in response to the efficacy of this bacterin to prevent salmon and trout from contracting vibriosis, which is caused by *Vibrio* spp.

**2.1.3 Live vaccines:**

One or more viruses or bacteria that exhibit attenuated virulence or naturally low pathogenicity toward the target fish species are used to make live vaccines. Because live vaccines may multiply, they are often more immunogenic than dead formulations. In the United States, there are now three approved modified live aquaculture vaccines. *E. ictalurii* vaccine against enteric septicemia of catfish (ESC), *Flavobacterium columnare* vaccine against *columnaris* in catfish, and Arthrobacter vaccine against bacterial kidney disease (BKD) for use in salmonids (Mondal and Thomas, 2022).

**2.1.4 DNA Vaccines:**

One kind of vaccination is called a DNA vaccine, which works by introducing a particular antigen-coding DNA sequence into an organism's cells to trigger an immune response. The plasmid used in DNA vaccines has a particular gene that codes for a particular antigenic protein, which is anticipated to elicit a potent immune response (Liu, 2011). Rainbow trout were used to test the first DNA vaccine against IHN that was reported for use in aquaculture. Since the genetic material needs to be sufficiently protected to reach host cells, most fish species get these vaccines by intramuscular (IM) injection (Marsella *et al.,* 2022).

**2.1.5 RNA-Based Vaccines:**

The translational ability of the RNA—(i) non-amplifying mRNA and (ii) self-amplifying mRNA—distinguishes the two main RNA-based vaccines available today (Perenkov et al., 2023). Since RNA is non-infectious and broken down by regular biological functions, there is no chance of infection, making RNA vaccines safe. Many self-replicating RNA vaccines in use today are based on the genomes of alphaviruses, which are members of the Togaviridae family and include the equine encephalitis, Sindbis, and Semliki Forest viruses. The genes generating the structural proteins are substituted with the desired antigen in the alphavirus vector vaccine, but the single RNA gene encoding the RNA replication machinery remains intact (Aleem *et al*., 2024).

**2.1.6 Subunit Vaccines:**

Purified portions of the pathogen that are antigenic, or required to trigger a protective immune response, are included in subunit vaccines (Hansson *et al.,* 2000). A recombinant subunit vaccination is one that is produced via recombinant DNA expression or disassembled virus particles in cell culture. Neither the host nor non-target species are at danger of disease. Although subunit vaccines have many positive aspects, they frequently fall short of whole cell preparations that are dead or living in terms of their capacity to elicit a strong immune response. Norway has successfully used *E. coli*-based expression to create subunit vaccines for aquaculture, such as those for Infectious Pancreatic Necrosis (IPN). Baculovirus and yeast for VHSV or Infectious Hematopoietic Necrosis Virus (IHNV) proteins are included in fish subunit vaccines that are being tested.

**2.2 Biofilms:**

Communities of microorganisms clinging to a surface and often kept together by a polymetric extracellular matrix are known as biofilms. The benefit of fish vaccine research is that it can stop antigens from being broken down in the stomach. The use of biofilms for fish vaccination is not as well-established as it was in the 2000–2004 literature, which focused on *A. hydrophila*. The gram-negative *A. hydrophila*, a secondary disease of fish that causes significant mortality and financial losses in aquaculture, has been shown to produce biofilms, which is why biofilm-based vaccines are being developed for aquaculture species (Zhao *et al*., 2023).

**2.2.1 Bacterial Biofilms as Fish Oral Vaccines:**

As first proposed by Azad *et al*. (1999), Dr. K.M. developed oral fish vaccines to establish immunological memory for the first time against *A. hydrophila*. Immunofluorescence and immunological peroxidase tests based on monoclonal antibodies were used to describe the intestinal stability of the biofilm-based oral vaccinations. All the published research has shown post-oral delivery of biofilm and free cell-based vaccinations and protection, suggesting that fish can be protected from pathogen infection via mucosal vaccination.

**3. FEED BIOTECHNOLOGY**

**3.1 Probiotics**

In aquaculture, antibiotics have long been employed as a conventional method of disease prevention and management for fish, as well as to enhance growth and feed conversion efficiency. Gut cells (1946) identified the potential use of antibiotics, such as sulphanamides, to treat furunculosis, which essentially marked the beginning of the use of antimicrobials in aquaculture (Subedi and Shrestha, 2020). The two primary risks of using antibiotics in aquaculture antimicrobial residues and antimicrobial resistance were concluded at the joint FAO/OIE/WHO expert workshop on antimicrobial use and resistance in aquaculture in 2017 (World Health Organization, 2019). The overuse of antibiotics causes an imbalance in the gut microbiota and the predominance of antibiotic-resistant microbes, which impacts fish health and causes residual deposits in the fish muscle, potentially endangering the health of consumers. Given the risks associated with the use of antibiotics in aquaculture, probiotics-a microbial intervention strategy have been shown to enhance not just fish health but also, frequently, fish growth, making them an alternative method of managing fish health in the aquaculture business. Probiotics are one of the many non-antibiotic agents that aquaculturists have discovered, and they are crucial for maintaining health in increased aquaculture (Chattogram, 2024). In aquaculture, probiotics can be utilized to boost immune response and disease resistance, increase feed consumption, boost growth, and improve water quality.

**3.1.1 History OF Probiotic**

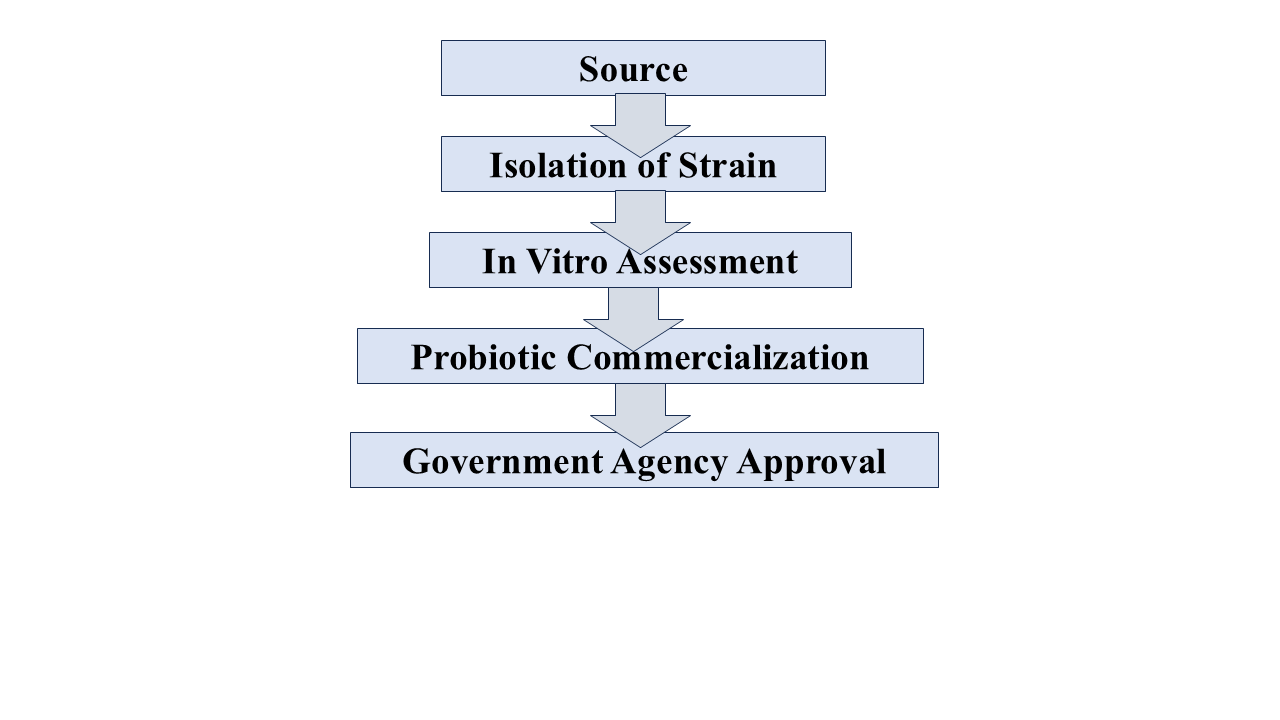
The name "Probiotic" comes from the Greek words "Bios" (life) and "Pro" (favor). Probiotics are "live microorganisms" that, when given in sufficient quantities, have positive health benefits on the host. Liong (2011) Probiotics were first presented by Ellie Metchnikoff in 1908. Lilly and Stillwell were the first to use the word "probiotic" (1965). Probiotics are initially described by Parker (1974) as a microbial feed or dietary supplement. Salam (2014) "Organisms and substances that contribute to intestinal microbial balance" is how probiotics are defined.

**3.1.2 Definition of Probiotic**

Probiotics are "living microorganisms, which once administered in appropriate amounts confer a health profit on the host," according to the Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) (FAO/WHO, 2007). Probiotics can be described as "live or dead, or even a component of the microorganisms that act under different modes of action in conferring beneficial effects to the host or to its environment," according to a 2014 proposal by Lazado and Caipang.

**3.1.3 Selection of Probiotics**

Maintaining or restoring a positive connection between beneficial and harmful microorganisms that make up the flora of fish intestinal or skin mucus is the primary goal of employing probiotics. For a probiotic to be considered effective, it must possess a few key qualities to be certified as useful and some procedures must be considered to develop probiotics for commercialization (Klaenhammer and Kullen, 1999) (Fig. 1). It is necessary to choose a wholesome supply of microorganisms from the digestive tracts of aquatic animals in good condition. Selective culture is used to isolate and identify the microorganisms that will be used for the job. For in vitro assessments, such as pathogen inhibition, pathogenicity to target species, host resistance conditions, and others, a fresh culture containing just the colonies of interest is used. When there are no limitations on the usage of the target species, both small- and large-scale in vivo supplementation tests are conducted to determine whether the host truly benefits. Ultimately, the probiotic that shown a notably positive outcome may be manufactured and used on commercial basis.



**Fig.1** Flow chart for selection of probiotics

**3.1.4 Characteristics of Good probiotics**

To be considered effective and safe, particularly in aquaculture, a probiotic strain must meet several essential criteria. It should clearly provide benefits to the host, such as promoting growth, enhancing feed efficiency, or boosting resistance to infections. The strain must also be non-toxic and non-pathogenic, ensuring it poses no harm to the host organism (Shewale et al., 2014). Additionally, the probiotic must contain enough live, active cells to be biologically effective. It should be able to survive and function within the gastrointestinal environment, which demands resistance to low pH and organic acids (Kailasapathy and Chin, 2000). Finally, an ideal probiotic should demonstrate strong stability, retaining its viability and efficacy over time during storage and settings in real-world application.

**3.1.5 Constraints to probiotics in aquaculture**

According to Michael et al. (2014) (i) The strains’ incapacity to be manufactured in commercial numbers and the ensuing large-scale demonstration. (ii) It is challenging to demonstrate performance at the farm level. (iii) Businesses’ incapacity to carry out in-depth research on how to produce goods especially for aquaculture.

**3.1.6 Probiotics in Aquaculture Management: Administrative Methods**

In aquaculture, probiotics can be given by a variety of methods, including feeding, injection, or direct submersion in water. They can be used alone or in combination (Fig. 2).

***(A)Feed additives, water additives and injection***

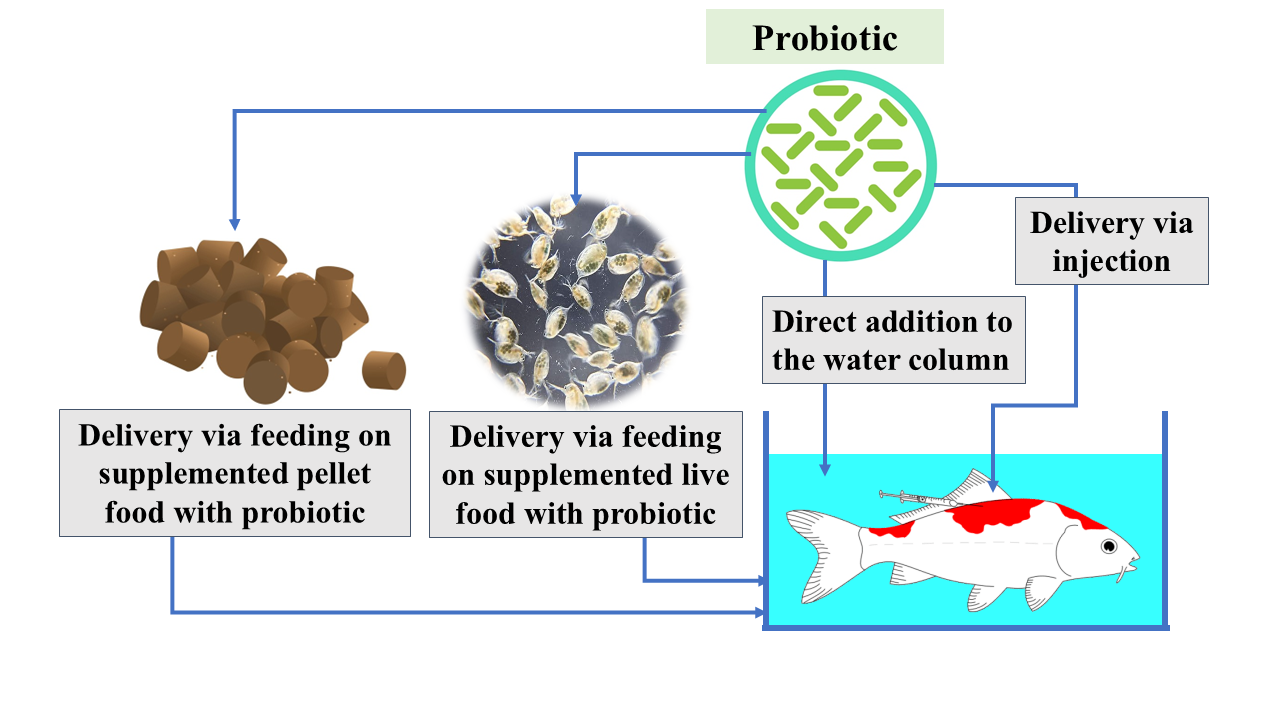
*i) Application in feed*

Most commercial probiotic preparations comprise either *Lactobacillus sp.* or *Saccharomyces cerevisiae*, and they are administered along with the feed and a binder (egg or cod liver oil) (Fenster et al., 2019). Probiotic organisms employed in food must be able to survive travels through the gut, meaning they must be able to withstand exposure to bile and gastric secretions, as per FAO and WHO criteria (Zielińska and Kolożyn-Krajewska, 2018). In addition to being safe, effective, and able to sustain their potency and efficacy during the product's shelf life, they must be able to multiply and colonize the digestive system.

*ii) Direct application to pond water*

Numerous bacterial species, including *Bacillus acidophilus*, *B. subtilis, B. licheniformis, Nitrobacter* sp., *Aerobacter,* and *Sacharomyces cerevisiae*, are present in the water probiotics (Soltani et al., 2025). Since probiotics alter the bacterial makeup of water and sediments, their application in tanks and ponds may also improve other aspects of water quality, which may have an impact on fish health (Padmavathi et al., 2012).

*iii) Application through injection*

It is possible to provide probiotics via injection. According to Newaj‐Fyzul and Austin (2015). the probiotic-like vaccine might be freeze-dried and administered by injection or bathing.

According to Yasir *et al*. (2022) probiotic *Micrococcus luteus* was experimentally administered to *Oreochromis niloticus* via intraperitoneal injection; the death rate was just 25%, compared to 90% for *Pseudomonas* using the same technique. According to Yasir *et al*. (2022) probiotic usage boosts phagocyte activity, complement-mediated bacterial killing, and immunoglobulin synthesis, all of which increase Rainbow trout immunity.

**Fig. 2** Different routes for administration of probiotic

***(B) Single and Combination***

Probiotics come in several forms, including multistrain probiotics, probiotics with plant extract, and probiotics with yeast extract, and can be used alone or in combination (Kwoji et al., 2021). The use of single probiotics was the main emphasis of probiotics in aquaculture; however, a combination of probiotics is more advantageous. Multi-strain probiotics have the benefit of being active against various aquaculture animals and more sensitive to infections. The growth and survival of rohu were positively impacted by the multi-strain probiotics during the hatchling and fry phases, but not at later stages. For catfish *Clarias* sp., the combination of *Bacillus megaterium* PTB 1.4 and *Pediococcus pentosaceus* E2211 administered in feed had better results than probiotics applied alone. In comparison to their individual applications, the probiotic *Bacillus coagulans* and plant extract *Mentha piperita* demonstrated improved growth performance, nutrition retention, and immunity in *Catla catla* (Bhatnagar & Saluja, 2021).

***(C) Dosage***

The right dosage should be administered for the probiotic to have the most effect on the species. Probiotic species, fish species and their physiological states, rearing settings, and the application’s objective all influence the right number of probiotics. Rainbow trout given probiotic Bifidobacterium strains at 1×10⁷ CFU/g showed enhanced growth performance, but not when fed 3×10⁷ CFU/g. When adding functional nutrients to aqua diets, dosage is a crucial factor to consider. When pabda catfish, *Ompok pabda*, were given more than 0.2% of their food in the form of commercial probiotics, their weight increases decreased (Chowdhury *et al*., 2020). The increased concentration may cause disruptions in the metabolism of fat and carbohydrates and may not be able to sustain the fish's entire body physiology.

**3.1.7 Probiotics significance in aquaculture**

Because probiotics enhance growth, reduce disease outbreaks, and promote fish and shrimp health, they are crucial in aquaculture. They enhance digestion, increase immunity, and promote a healthy gut flora, all of which are critical for overall wellness (Table 1).

*i) Improvement in water qualities*

A major worry has been the contamination of fish culture systems and ponds by nitrogenous substances such ammonia, nitrite, and nitrate. Cultured aquatic species are often species-specific in their vulnerability to high concentrations of these chemicals, although in all cases, excessive concentrations of these compounds can be exceedingly toxic and cause mass death. According to Ma *et al*. (2009) *Lactobacillus species* JK-8 and JK-11 may concurrently eliminate infections and nitrogen from tainted shrimp farms. *Bacillus species* have been found to enhance water quality when probiotics are added (Padmavathi et al., 2012). Gram-negative bacteria would convert a larger percentage of organic carbon to bacterial biomass or slime, whereas gram-positive *Bacillus* species are often more effective at turning organic matter back to CO2.

*ii) As growth promoters*

Probiotics have been shown in experiments to potentially improve fish development (Van Hai, 2015). It was a probiotic bacterium because it could outgrow the pathogens in favour of the host or enhance the host's growth without harming the host. In their attempt to employ probiotic bacteria as a growth promoter in *O. niloticus* tilapia, Yasir *et al*. found that the probiotic *M. luteus* produced the best feed conversion ratio and the highest growth performance. In fish aquaculture, *M. luteus* is regarded as a growth booster. The development rate of juvenile carp was influenced by lactic acid bacteria, which function as growth boosters (Kumar et al., 2017).

*iii) For disease prevention*

Aquaculture, terrestrial animals, and human disease control have all found use for probiotics or their products for the host's health. The microbial adjuvant stops pathogens from growing in the digestive tract, on the surface of the organism, and in the culture environment. Beneficial organisms work by strengthening the culture organism's immune system, making them more resistant to illness, or creating substances that stop harmful organisms from infecting the host and causing sickness (Nayak, 2010; FAO/WHO, 2001).

*iv) Source of nutrients and enzymatic contribution to digestion*

Aquatic animals' digestive systems benefit from the presence of microorganisms. Bacteroides and *Clostridium species* help the host eat, particularly by providing vitamins and fatty acids (Ringo et al., 2003). *Salvelinus alpinus* L. may benefit from the nutritional activities of microorganisms including *Agrobacterium* sp., *Pseudomonas* sp., *Brevibacterium* sp., *Microbacterium* sp., and *Staphylococcus* sp. (Ringo et al., 1995).

*v)Enhancement of the immune response*

Among the many positive effects of probiotics, one of the most often touted is immune system regulation. The ability of *Lactobacillus fermentum* LbFF4 isolated from Nigerian fermented food (“fufu”) and L. plantarum LbOGI from a beverage called “Ogi” to induce immunity in *Clarias gariepinus* (Burchell) against certain selected fish bacterial pathogens has been demonstrated (Onilude et al., 2014). *Lactobacillus* larvae, shrimp, and other invertebrates have immune systems that are less developed than adult stages and rely primarily on non-specific immune responses for their resistance to infection (Nayak, 2010).

**Table 1 Different application of probiotics in aquaculture**

| **Application** | **Probiotic** | **Species** | **Reference** |
| --- | --- | --- | --- |
| **Pathogen inhibition** | *Bacillus* sp. | Penaeids | Irianto and Austin (2002) |
| *Enterococcus faecium* SF 68 | *Anguilla Anguilla* |
| *L. rhamnosus* ATCC53103 | *Oncorhynchus mykiss* |
| *Micrococcus luteus* A1-6 | *Oncorhynchus mykiss* |
| *Pseudomonas fluorescens* | *Oncorhynchus mykiss* |
| *P. fluorescens* AH2 | *Oncorhynchus mykiss* |
| *Pseudomonas* spp. | *Oncorhynchus mykiss* |
| *Roseobacter* sp. BS. 107 | Scallop larvae |
| *Saccharomyces cerevisiae*, *S. exiguus*, *Phaffia rhodozyma* | *Litopenaeus vannamei* |
| *Vibrio alginolyticus* | Salmonids |
| *V. fluvialis* | *Oncorhynchus mykiss* |
| *Tetraselmis suecica* | *Salmo salar* |
| *Carnobacterium* sp. Hg4-03 | *Heplalus gonggaensis* larvae |
| *Lactobacillus acidophilus* | *Clarias gariepinus* |
| **Reproduction improvement** | *Bacillus subtilis* | *Poecilia reticulata*, *Xiphophorus maculatus* | Aydın and Çek-Yalnız (2019) |
| *L. rhamnosus* | *Danio rerio* |
| *L. acidophilus*, *L. casei*, *Enterococcus faecium*, *Bifidobacterium thermophilum* | *Xiphophorus helleri* |
| **Nutrient digestibility** | *L. helveticus* | *Scophthalmus maximus* | Zorriehzahra *et al*., 2016 |
| *Bacillus* NL 110, *Vibrio* NE 17 | *Macrobrachium rosenbergii* |
| *Carnobacterium* sp. Hg4-03 | *Heplalus gonggaensis* larvae |
| *Lactobacillus acidophilus* | *Clarias gariepinus* |
| *Shewanella putrefaciens* Pdp11 | *Solea senegalensis* |
| **Water quality** | *Bacillus* sp. 48 | *Penaeus monodon* | Zorriehzahra *et al*., 2016 |
| *Bacillus* NL 110, *Vibrio* sp. NE 17 | *Macrobrachium rosenbergii* |
| *Lactobacillus acidophilus* | *Clarias gariepinus* |
| *B. coagulans* SC8168 | *Pennaeus vannamei* |
| *Bacillus* sp., *Saccharomyces* sp. | *Penaeus monodon* |
| **Stress Tolerance** | *Lactobacillus delbrueckii* | *Dicentrarchus labrax* | Zorriehzahra *et al*., 2016 |
| *Alteromonas* sp. | *Sparus auratus* |
| *B. subtilis*, *L. acidophilus*, *S. cerevisiae* | *Paralichthys olivaceus* |
| *L. casei* | *Poecilopsis gracilis* |
| *Pediococcus acidilactici* | *Litopenaeus stylirostris* |

**4. SINGLE CELL PROTEIN (SCP)**

Algae, yeast, bacteria, and fungus create single cell protein (SCP), which is a bulk of dried cells (biomass). One name for the single-cell protein was "microbial protein." The term "microbial protein" was then replaced by the brand-new term "single cell protein." Additionally, these microorganisms are referred to as "single-cell" proteins because they are often grown and harvested as individual cells rather than as a part of a larger organism (Anupama & Ravindra, 2000). Microbes are used as components or supplements that are high in protein for both humans and animals. Since it does not take a lot of land or water to produce, single-cell protein is an excellent substitute for plant protein sources (Mekonnen and Hoekstra, 2014). Production occurs all year round and is not influenced by seasonal or climatic changes. Selecting inexpensive and appropriate substrates or biodegradable agro-industrial wastes as a source of nutrients for the microorganisms to grow and create tons of protein can lower the cost of producing single-cell proteins. Substrates that are frequently utilized include pineapple waste, papaya waste, citrus pulp, potato peels, apple pomace, and yam peels. Several studies have demonstrated that these low-cost agro-industrial residues serve as effective carbon and energy sources for microbial growth in SCP production (Ukaegbu-Obi et al., 2019; Sadh et al., 2018).

**4.1 History and Definition**

The term "single cell protein" (SCP) describes the dried, dead cells of bacteria, fungus, yeast, and algae. For their protein content, microorganisms thrive on a variety of carbon sources. Carol Wilson coined the phrase "single cell protein" in 1967 to replace the less aesthetically pleasing terms "microbial protein" and "petroleum protein." Since many of the microorganisms utilized are unicellular, their protein is referred to as "single cell protein." About 250 tons of protein may be produced by yeast in a 24-hour period (Taofeek, 2024). Due to their rapid development and increased protein content, fungi and bacteria are the main producers of SCP. There are several types of algae that are grown especially in aquatic medium (Nasseri et al., 2011).

* 1. **Sources for Single Cell Protein**

**4.2.1 Bacterial source**

One of the sources - Bacteria have a very short generation time because their cell masses grow rapidly between 20 and 120 minutes. They have the capacity to grow on a variety of edible substrates, including sugars and starches, as well as raw ingredients and can readily thrive on organic waste and petrochemicals like ethanol, methanol, and nitrogen (Nasseri et al., 2011). They can also grow in natural water that has been supplied with minerals and nutrients to assist meet nutrient deficiencies. Some bacteria, such as *Methylophilus* species, are beneficial components of animal feed and have a relatively quick generation time of two hours (Anupama & Ravindra, 2000). There are also several bacterial species that have long been present in animal feed that can create high amounts of SCP, including *Brevibacterium, Methylophilus methylitropous, Bacillus megaterium, Acinetobacter calcoacenticus, Acromobacter delvaevate, Aeromonas hydrophilla, Cellulomonas spp. Bacillus subtilis, Methylomonas methylotrophus, Thermomonospora fusca, Lactobacillus spp. Rhodopseudomonas capsulate, Flavobacterium species* and *Pseudomonas fluorescens* (Ukaegbu-Obi et al., 2019; Suman et al., 2015).

**4.2.2 Algal sources**

Up to 70% of nutrient-dense protein may be found in certain microalgae that are cultivated for human and animal use. Lipids can be found in abundance in magnesium salts, vitamins, chlorophyll, and omega-3 fatty acids. Nucleic acid concentrations are rather modest, ranging between 3% and 8% (Becker, 2007). In other parts of the world, the biomass of several other species, such *Senedessmus* and *Chlorella*, has also been used as a feed source. Their high protein content, rapid growth, simplicity of cultivation, and effective use of solar energy make them popular feed components across the world (Spolaore et al., 2006). Green algae are thought to have high antioxidant properties. Nutraceuticals and a diet rich in the algae species *Spirulina maxima* can help protect progenitor cells. Fatty liver syndrome can be avoided with it (Belay, 2002).

**4.2.3 Fungal sources**

Because of its chemical makeup and amino acid profile, protein from various fungus species is chosen over protein from other sources. Protein makes up 30% to 50% of fungi. The amino acid profile of fungi also complied with FAO requirements (Anupama and Ravindra, 2000). Since cysteine and methionine are sulfur-containing amino acids that are mostly derived from plants, protein is lacking in these amino acids but there is an abundance in lysine and threonine. When the fungus *Klueromyces fragilis* grows on whey, it can create amino acids that include sulfur (Nasseri et al., 2011). In addition to protein, single cell protein derived from fungus may also offer other nutrients. Vitamins include riboflavin, niacin, thiamine, biotin, pantothenic acid, choline, pyridoxine, glutathione, pamino benzoic acid, streptogenin, and folic acid, which are mostly from the vitamin B-complex (Ukaegbu-Obi et al., 2019). Mycoprotein derived from *Fusarium venenatum* has been shown to lower blood glucose and insulin levels after consumption; fungi have a comparatively higher nucleic acid content than algae (between 7% and 10%) (Finnigan et al., 2019).

**4.3 Substrates for bacteria, algae, and fungi to produce SCP**

Bacteria, algae, and fungi may employ a variety of waste products, agricultural residues, and other materials rich in carbon and nitrogen as substrates to create Single Cell Protein (SCP) table 2. Substrates that are frequently used include agricultural wastes like rice husk and sugarcane bagasse, as well as wastes from fruits and vegetables. Additional potential sources include home sewage, industrial waste, and even odd sources like ethanol or methanol.

**Table 2 Substrates for Microorganisms to Produce SCP**

| **Type** | **Microorganism** | **Substrate** | **Reference** |
| --- | --- | --- | --- |
| Bacteria | Various bacterial species | Waste of fruit processing | (Ravindra, 2000; Suman et al., 2015; Kunasundari and Sudesh, 2011; Dubey and pandey, 2013; Nasseri et al., 2011; Saarela, 2011; Pandey et al., 2000; Roy and Laskar, 2013) |
| *Methylococcaceae* family | C-1 compounds |
| *Bacillus cereus* | Ram horn |
| *Rhodopseudomonas gelatinous* | Wheat bran |
| *Methylomonas* species | Methane broth |
| *Brevibacterium* spp. | C-1 to C-4 compounds |
| *Ralstonia* species | Natural gas |
| *Bacillus licheniformis* | Potato waste |
| *Streptomyces* species | Methanol |
| *Corynebacterium ammoniagenes* | Fructose and Glucose |
| *Escherichia coli* | Ram horn |
| *Cellulomonas* species | Agro-industrial wastes |
| *Corynebacterium glutamicum* | Glucose |
| *Methanomonas methanica* | Methane |
| *Cupriavidus necator* | Synthetic growth media |
| *Methylophilus methanotrophus* | Methanol |
| *Bacillus pumilis* | Potato processing waste |
| *Rhodopseudomonas palustris* | Rubber waste |
| *Bacillus subtilis* | Ram horn |
| Rhizospheric diazotrophs | Brewery wastewater |
| *Pseudomonas fluorescens* | Animal waste & Manure |
| Algae | *Spirulina* species | Carbon dioxide | (Ravindra, 2000; Suman et al., 2015; Nasseri et al., 2011; Becker, 2007; Spolaore et al., 2006) |
| *Chlorella salina* | Alkaline waste effluent |
| *Caulerpa rocemosa* | Carbon dioxide |
| *Spirulina maxima* | Sunlight and carbon dioxide |
| *Chlorella* species | Carbon dioxide |
| *Sargassum* | Carbon dioxide and Sunlight |
| *Dunaliella* | Carbon dioxide and Sunlight |
| *Laminaria* | Carbon dioxide and Sunlight |
| Diatoms and *Chlorella* | Carbon dioxide and Sunlight |
| *Porphyra* | Carbon dioxide and Sunlight |
| Fungi | *Aspergillus flavus* | Rice bran | (Suman et al., 2015; Dubey and pandey, 2013; Nasseri et al., 2011; Saarela, 2011; Pandey et al., 2000) |
| *Aspergillus ochraceus* | Rice bran |
| *Saccharomyces cerevisiae* | Orange pulp, molasses, brewer's spent grain Inulin, crude oil, glycerol waste hydrocarbons |
| *Yarrowia lipolytica* | Apple pomace, Banana waste, Rice bran, Potato |
| *Aspergillus niger* | Starch |
| *Trichoderma virideae* | Citrus pulp |
| *Aspergillus ochraceus* | Rice bran |
| *Trichoderma harzianum* | Cheese whey filtrate |
| *Penicillium citrinum* | Rice bran |
| *Aspergillus oryzae* | Rice bran |
| *Kluyveromyces marxianus* | Orange pulp, molasses, brewer's spent grain, whey, potato pulp |
| *Candida utilis* | Poultry litter; Waste capsicum powder |
| *Cladosporium cladosporioides* | Rice bran |
| *Monascus ruber* | Rice bran |
| *Candida tropicalis* | Molasses |

* 1. **Mechanism of Production of SCP**

To enlarge their cell masses, which are composed of the SCP, microorganisms consume the waste materials that are readily available as growth media. The primary process that produces SCP is fermentation, which can be either solid or submerged. The accessible biomass, which can then be used as a source of protein, is collected once the fermentation process is finished. Purification, cell disruption, washing, and protein extraction are some of the additional processing methods that protein sources go through to provide usually high production rates, improved yields, and simpler production management. According to reports, agricultural waste products make an excellent substrate for the synthesis of SCP. The resulting protein-enriched products from SCP created by wastes and microorganisms are of high quality and cost-effective for application in animal feed (Koukoumaki *et al*., 2024; Sadh et al., 2018).

* 1. **Criteria for the selection of microorganism**

There are some basic criteria in selecting the microorganisms which are the primary producers of the SCP. The criteria mainly based on the ability to utilize carbon and nitrogen sources, moderate growth conditions, tolerance to pH, temperature, and mineral concentrations, resistance against viral infection, non-toxicity, non-pathogenicity, acceptable nutritive value of cell mass (Anupama and Ravindra, 2000; Nasseri et al., 2011).

**4.5 Importance of SCP in Aquaculture**

Microbial biomass or protein extract used as a food or feed ingredient is often referred to as single cell protein. Protein, lipids, carbs, nucleic acids, vitamins, and minerals are all abundant in microorganisms. More study has been focused on using yeast and other microbes in aquafeeds since their significance has been recognized. Up to 50% of fishmeal may be substituted with SCP of microbiological origin when it is used well (Bharti *et al*. 2014). SCP has been produced using a variety of substrates, including whey starch, cellulose hydrocarbon, alcohols, and molasses. SCP not only provides aquafeeds with an alternative protein supply, but it also functions as a probiotic and immunostimulant, significantly enhancing the immune system, growth, and resistance to illness of cultured organisms. Bacterial and yeast single-cell proteins have a comparatively large RNA concentration of nucleic acids. Microorganisms' high RNA content speeds up the production of proteins (Adedayo *et al*. 2011; Nasseri et al., 2011). High protein concentration in single-cell organisms is mostly attributed to rapid protein synthesis and brief multiplication rates. Fish with high nucleotide content in their diets have better hepatic function and lipid metabolism (Nayak, 2010). Because microorganisms can use these materials as nutritional sources, single-cell protein synthesis recycles waste from industry and agriculture. SCP may also be used to recycle ammonia generated by cultured organisms and wastes obtained from feed (Sadh et al., 2018). The success of ornamental fish aquaculture is influenced by the size and colour of the fish. By using SCP made from bacteria and algae that have high concentrations of carotenoid pigments, both can be controlled. The development and colouring of ornamental fish can be enhanced by adding microbial carotenoids to their diet (Matos, 2017).

**5. NUTRACETICALS**

The relationship between the uses of suitable foods for health and therapeutic benefit was first conceptualized by Hippocrates around 2500 years ago. The term “nutraceuticals” came into existence only when Dr. Stephen D. Felice defined it in 1989 as “a food or food product that produces health and medicinal benefits, including prevention and treatment of disease” (Kalra, 2003). Nutrient- rich food like Spirulina, garlic, or specific compounds like vitamins, lycopene or omega-3 fatty acids can be a nutraceutical. Difficult to distinguish the terms food, nutraceuticals, dietary supplements, functional foods and drugs. According to (Zeisel, 1999), nutraceuticals are not dietary supplements but rather than “are consumed as part of a normal diet and deliver one or more active ingredients (that have physiologic effects and may enhance health) within the food matrix.” These are food products of natural origin from terrestrial and marine sources that have healthcare importance. The word nutraceuticals comprise various products derived from terrestrial and marine sources (isolated nutrients, dietary supplements, genetically engineered designer foods, herbal products, processed foods, and Beverages). Nutraceutical is a term used to describe any product derived from food sources with extra health benefits in addition to the essential nutritional value found in foods. Nutraceutical, sometimes known as a “bioceutical,” is a pharmaceutical substitute that asserts to offer physiological advantages for organisms. Nutraceuticals are not identified as essential nutrients but are considered as bioactive substances with one or more health benefits.

**5.1 Types of Nutraceuticals**

Bioactive substances from both terrestrial and marine sources are categorized according to their bio functional characteristics into

1. Dietary Supplements
2. Functional Food
3. Medicinal food

**5.1.1 Dietary Supplements**

In the United States, dietary supplements are described as items made of dietary elements that are taken orally to augment the nutritional requirements of a diet (Food and Administration (FDA), 1995). The term "dietary constituents" primarily refers to bioactive substances that include essential metabolites, vitamins, minerals, fibers, amino acids, and specific enzymes. Extracts that come in pills, capsules, powders, liquids, and any other dosage form are also included in the dietary supplements (Priyadarshani and Rath, 2012).

**5.1.2 Functional Food**

Functional foods are nutrient-dense, naturally occurring foods that have been supplemented with vital nutrients. Common foods that include a component with a particular nutritional value and therapeutic impact (Wildman *et al*., 2016). Functional foods must be sourced naturally and consumed in their natural state rather than processed into various dosage forms like tablets, capsules, or powder. When regularly incorporated into a daily diet, functional foods play a dual role in biological processes and disease prevention and management.

**5.1.3 Medicinal food**

For the dietary treatment of a specific ailment with specific nutritional requirements that cannot be satisfied by a regular di*et al*one, medical foods are specially prepared and are to be taken under a doctor's supervision. Functional foods and dietary supplements do not fit these requirements; hence they are not considered medicinal foods (Radhika *et al*., 2011).

**5.2 Classification of Nutraceuticals**

* **Traditional nutraceuticals:** These are foods or food ingredients that exist naturally and have not been altered from their original form. Salmon's omega-3 fatty acids, soy's saponins, and tomatoes' lycopene are a few examples.
* **Non-traditional nutraceuticals:** This group comprises foods or food items that have been artificially generated, frequently via genetic engineering or biotechnology (Kalra, 2003).

**5.2.1 Based on the chemical nature**

*i)Amino acids and peptides*

Certain peptides are recognized for their antibacterial qualities, whereas amino acids are regarded as necessary nutrients and stress-relieving substances. The capacity of tryptophan, pyridoxine, tyrosine, glycine, and arginine to reduce stress. The production of serotonin (5-HT), which is crucial for reducing fish stress responses, requires the important amino acid tryptophan (Lepage et al., 2002). Fish immunity and antioxidant defense are supported by glycine and arginine (Li et al., 2009; Hoseinifar et al., 2016).

*ii)Fatty acids*

Fish inflammatory responses depend on polyunsaturated fatty acids (PUFA), which are members of the n-3 and n-6 families and are precursors of physiologically active eicosanoids (Chapkin, 2000). In rohu fingerlings, PUFA boosted immunity.

*iii) Nucleotides*

The presence of dietary nucleotides in fish improves immunity. Research conducted on salmon revealed that dietary nucleotide supplementation enhanced the fish's general health (Chapkin, 2000).

*iv) Vitamins*

micronutrients, which are basically needed in lower amounts to carry out the animal's important tasks. Vitamins A, C, and E are essential antioxidants that boost fish immunity. Vitamin E supplementation shown immune-boosting effects in channel catfish and decreased nitrite stress in rohu. Strong antioxidants found in biological systems include vitamin A and beta carotenes, and rainbow trout exhibited immunomodulatory effects when given astaxanthin supplements. Because it can increase serotonin synthesis, pyridoxine, often known as vitamin B6, is said to have an anti-stress impact (Akhtar & Ciji, 2020).

*v)Minerals*

The inorganic nutrients known as minerals have anti-stress properties as well. Fish stress reduction has been demonstrated to be successful when both organic and inorganic mineral supplements are given (Wang et al., 2019).

*vi) Herbal extracts*

Herbal extracts are substances that can improve an animal's immune system and scavenge reactive oxygen species. Numerous plant extracts have been shown to strengthen fish's immune systems and reduce stress. Strong antioxidant and anti-stress bioflavonoids, such as Rutin (Toonasinensis), are among the bioactive chemicals that have been discovered to be beneficial for crustaceans. A study on guava and mango leaf extract revealed that *Labeo rohita* fingerlings' immunity is improved when 0.5% of guava and/or mango leaf extract is added to their food (Fawole *et al*., 2016).

*vii) Polysaccharides*

The non-specific immune system of aquatic animals is the target of polysaccharides. Numerous polysaccharides, including chitin, lactoferrin, inulin, beta glucan, and microbial levan, are employed as immunostimulants. The glucose polymers known as β-glucans are made up of β-(1-3) connected *β-Dglucopyranosyl* units. It can bind to a wide range of cellular receptors, therefore activating them. In fish, β-glucan shown improved resistance to viral and bacterial infections (Zhang *et al*., 2014).

**5.2.2 Based on the functions**

*i) Anti-stress& antioxidant nutraceuticals*

This category includes many vitamins, minerals, and botanical extracts. *Cyprinus carpio* and tilapia have been reported to respond well to the antistress effects of *Astragalus membranaceus* and *Astragalus paniculata* (Wu *et al*., 2007).

*ii) Immunostimulants*

Compared to terrestrial species, fish and shellfish have less developed immune systems, and the non-specific immune system is frequently the main target. In fish and shellfish, many polysaccharides and oligosaccharides strengthen the non-specific immune system (Harikrishnan et al., 2011). Numerous plant substances, including azadirachtin, glycyrrhizin, tannin, and saponin, have also been shown to strengthen fish and shellfish immune systems (Reverter et al., 2014; Hoseinifar et al., 2020).

*iii) Antimicrobial and viral nutraceuticals*

Antimicrobial properties are found in most sulfur-containing nutraceuticals, such as onion, garlic, thioles, and sulphides; terepene-based compounds, such as oregano, turmeric, and ginger; phenolic compounds, such as cloves, nutmeg, cinnamic acid, and tannin; glycosides, such as sugar and aldehyde like citral and citronellol; and esters and alcohols (Rota et al., 2008; Sharma et al., 2019). Antimicrobial and antiviral properties are also present in several plant extracts and immunostimulants, including glucan, chitin, lactoferrin, and levamisole (Harikrishnan et al., 2011; Van Hai, 2015).

*iv) Growth promoters*

Numerous natural substances and micronutrients can promote fish development. Spirulina has been shown to have a growth-promoting impact on tilapia. When added to the diet, spirulina, which is high in carotenoids and phenolic compounds, improved the growth and phosphatase activity of rohu exposed to metals. Extracts from yeast and seaweed have also been shown to help fish develop and strengthen their immune systems. It has been discovered that the transcription rate is supported by the herbal growth promoters to stimulate growth. It was discovered that enriching Artemia nauplii with herbal compounds such as stressol I and stressol II promoted development in Penaeus indicus (Ganesh *et al*., 2022).

*v) Acidifiers*

Often referred to as acidifiers, organic acids, either alone or in combination, can enhance gut microbiota, digestion, and intestinal health. Acidifiers extend the feed's shelf life, aid in digestion by supplying the right pH for enzyme activity, and even serve as TCA cycle intermediaries. Other significant acidifiers that have been researched in animal diets are formic acid and acetic acid (Hernández et al., 2006).

*vi) Probiotics*

"A viable monoculture or mixed culture of microorganisms that, when applied to humans or animals, improve the characteristics of native flora and have a positive effect on the host"(Fuller, 1989). Probiotics decrease bacterial infections and aid in the synthesis of unique compounds that strengthen the immune system. For example, *Streptococcus species*, *Bifidobacterium species,* *Lactobacillus species*, and *Enterococcus species* (Merrifield et al., 2010).

*vii) Prebiotics*

Prebiotics are indigestible dietary components that support the development of gut microbiota colonies. Mannan oligosaccharides (MOS), xylan oligosaccharides (XOS), fructan oligosaccharides (FOS), and other prebiotics are the main ones researched in aquaculture systems. They aid in the development of beneficial microorganisms, aid in nutrient absorption, and modulate the immune system of species (Ringø et al., 2010; Torrecillas et al., 2014).

*viii) Synbiotics*

Symbiotic relationships occur when probiotics and prebiotics are given to the body in tandem and have a synergistic impact (Gibson and Roberfroid, 1995).

*ix) Nutrizymes/exogenous enzymes*

The exogenous enzymes known as nutrizymes, which function as nutraceuticals, aid in improving feed digestion and growth. Exogenous chitinase aids in bettering the digestion of foods that contain chitin. Carnivorous fish use carbohydrates more effectively when exogenous carbohydrase is added (Sinha et al., 2011).

**5.3 Role of nutraceuticals in aquaculture**

The use of nutraceuticals has been heavily advocated for enhancing production performance, growth, immunity, and feed intake. The aqua feed industry and farms use a variety of nutraceuticals, including methyl donors, enzymes, nucleotides, Levans and other immunostimulants, chitin, chitosan, extracts of polychaetes and tunicates, vitamins, antioxidant minerals, amino acids, anabolic growth promoters, organic acids, and carotenoids. There is no need to worry that nutraceuticals in the aquafeed industry will have a greater impact on feed utilization, immunity, meat quality, stress tolerance, reproductive and productive performance, and water quality during the culture because of the decreased feed waste and efficient feed utilization. Aquaculture is under pressure to expand productivity because to the standstill in marine production and the rise in consumer demand. The inland culture system is more intense owing to land and water limits, making it more vulnerable to disease attacks since stress weakens fish immunity. The aquatic environment is full of diseases that are just waiting for a chance to assault fish. Antibiotic resistance, residue buildup in aquatic systems, and several other adverse impacts are caused by the uncontrolled use of these substances. The best way to prevent such issues is to utilize nutraceuticals as a substitute for these medications or chemicals. Nutraceuticals have been shown to improve fish development and strengthen their immune systems. The nutraceuticals promote higher feed intake, improve the fish and shellfish's anti-stress and antibacterial capabilities, and promote maturity without causing any negative side effects. The list of nutraceuticals also includes certain feed additives used in aquaculture. Numerous causes contribute to stress in aquatic systems. Fish can be stressed by a variety of variables, including excessive stocking density, climate fluctuation, pesticide or insecticide exposure, malnutrition, and the usage of plant-based components that include anti-nutritional elements (Chandan *et al*., 2020; Shinde and Sukhdhane, 2023).

**6. GNOTOBIOTIC**

**6.1 Gnotobiotics**

The area of study that focuses on raising animals that are either exclusively connected with recognized species or devoid of all microbes. The phrase covers both the fundamentals of animal husbandry and the description of any biological events that may occur in an animal because of the removal of microorganisms or their connection with microorganisms (Luckey, 1963).

**6.2 Gnotobiote or gnotobiotic animal**

An animal stock or strain that has been created via sterile egg hatching, embryo transfer, or aseptic hysterotomy or hysterectomy and is continually kept using sterile techniques in an isolator where the animal's microbiological condition is completely determined. An animal in which every kind of life has been fully described. Both germfree and specified flora animals are considered gnotobiotics (Gordon & Pesti, 1971).

**6.3 Classification**

**6.3.1 Axenic animals**

These are the germ-free animals which are completely devoid of any detectable microorganisms, including bacteria, viruses, and fungi. These animals are raised in a sterile environment, ensuring that their bodies remain free from microbial colonization. To maintain this germ-free tag, offspring- referred to as pups- are delivered through a sterile hysterectomy procedure and immediately transferred into a sterile isolator. This controlled environment prevents any exposure to external microbes, allowing researchers to study the effects of specific microorganisms under highly regulated conditions (Gordon & Pesti, 1971).

**6.3.2 Associated animals**

* Originated from axenic animals that had one or more microbe species artificially colonized them.
* Monoxenic, dixenic, polyxenic

**6.4 History**

Louis Pasteur (1885) understood the idea of germ-free more than a century ago, yet he concluded that life without microorganisms is impossible. The first GF animal (guinea pigs) was created by Nuttle and Thierfelder at Berlin University ten years later in 1895, and they lived for up to thirteen days. However, it took a further 50 years to generate the first GF rat colonies, which were formed in the late 1940s because to a lack of understanding regarding nutrition (Al-Asmakh and Zadjali, 2015; Al-Asmakh, 2014).

**6.5 Uses of Gnotobiotic animals**

Gnotobiotic animals serve as beneficial models in a variety of scientific and biological investigations because they are kept in a regulated microbial environment. They provide information on how bacteria affect host growth, metabolism, and immunity and are widely employed to study the symbiotic connections between animals and microorganisms. These are also useful for researching the impacts of nutrition, such as how nutrients are absorbed and how certain food ingredients affect health. Gnotobiotic animals are perfect for studying the host's defense systems and offer a consistent supply of sterile organs and tissues for in vitro culture. They are also essential for investigating the causes of infectious illnesses, the physiological aging process, and wound healing processes (Wostmann, 1981).

**6.6 Disadvantages**

Although gnotobiotic animals are useful for carefully monitored research, they have several drawbacks. These animals' normal growth and physiological processes may be impacted by the absence of vital bacteria that naturally exist in conventional animals. Maintaining gnotobiotic animals is a costly and time-consuming procedure that calls for extremely sterile environments and specialist facilities. Additionally, they are extremely vulnerable to nutritional deficiencies; any absence of vital dietary components can cause serious health problems or even death very fast. Animals without germs frequently live shorter lives and have weaker immune systems, which leaves them more susceptible to harmful illnesses. They are also more vulnerable to physiological shock due to their poor cardiac output. The abrupt addition of several microbes might overload their system and cause shock or negative responses, underscoring the need for a careful balance in their care (Wostmann, 1996).

**7. RECOMBINANT PROTEINS**

Recombinant proteins led to significant advances in biomedical biotechnology. They are used not only in biomedical research, but also as medications. In 1982, recombinant human insulin was utilized in therapy for the first time. The recombinant protein sector has quickly expanded. Too far, the US FDA has authorized more than 130 recombinant proteins for clinical use. However, over 170 recombinant proteins are generated and used in medicine across the world (Pham et al., 2018). The occurrence of genetic material being exchanged across distinct areas of two chromosomes or the same chromosome is known as recombination. The physical breaking and rejoining of DNA molecules is another definition of it (Schillberg *et al*., 2019).

* 1. **Types of Recombination**

In living things, recombination occurs spontaneously in at least four different ways (Hardison, 2010). (i)Homologous chromosomes in diploid organisms are an example of **general or homologous recombination**, which happens between DNA molecules with a relatively similar sequence. In diploid species, general recombination can take place utilizing one or a limited number of common enzyme routes across the whole genome. (ii)In areas where there is no discernible large-scale sequence similarity, such as translocations across distinct chromosomes or deletions that eliminate several genes along a chromosome, **illegitimate or nonhomologous recombination** takes place. Short patches of sequence similarity are occasionally discovered, nevertheless, when the DNA sequence at the breakpoints for these events is examined. For example, in somatic cells, recombination between two comparable genes that are separated by millions of base pairs might result in the deletion of the intervening genes. (iii)Certain small regions (about 12 to 24 bp) found on parental molecules that are otherwise distinct undergo **site-specific recombination**. A unique enzymatic apparatus—basically, one enzyme or enzyme system for each specific site—is needed for site-specific recombination. Systems for integrating some bacteriophages, such l, into bacterial chromosomes and the reorganization of immunoglobulin genes in vertebrate animals are both excellent examples. (iv)**Replicative recombination**, the third kind, creates a new copy of a DNA segment. Replicative recombination is a technique used by many transposable elements to create a new copy of the element at a different place.

**7.2 Recombinant DNA vs. Recombinant Protein: Concepts and Clinical Relevance**

Table 3 lists the distinctions between recombinant DNA and recombinant protein. A recombinant protein is a form of modified protein whose code is encoded by recombinant DNA. Recombinant DNA is essentially made up of two DNA segments that have been linked together in a plasmid, which is typically seen in bacteria. When researching biological processes, recombinant proteins are a valuable resource. The market for recombinant proteins has expanded quickly. The US FDA has authorized over 130 recombinant proteins for clinical use. Nonetheless, over 170 recombinant proteins are created and applied in medical settings around the globe (Schillberg *et al*., 2019).

**Table 3 The distinctions between recombinant proteins and recombinant DNA**

| **Aspect** | **Recombinant DNA** | **Recombinant Proteins** | **Reference** |
| --- | --- | --- | --- |
| **1. Definition** | A molecule synthesized by joining DNA fragments from at least two different sources | Proteins expressed because of recombinant DNA | (Alberts et al., 2014) |
| **2. Composition** | Made up of nucleotides | Made up of amino acids | (Lodish et al., 2021) |
| **3. Site of Synthesis** | Synthesized outside the cell (*in vitro*) | Synthesized inside the cell (*in vivo*) | (Alberts et al., 2014) |
| **4. Modifications Required** | Does not require post-translational modifications | Requires post-translational modifications | (Walsh, 2014) |
| **5. Applications** | Used in recombinant DNA technology to produce recombinant proteins | Used commercially to produce antibiotics, enzymes, and protein-based polymer drugs | (Walsh, 2014) |

**7.3 The Background of Recombinant DNA Technology**

Since the beginning of bioengineering and the discovery of genetic material, the concept of creating creatures with desired traits, or simply genetically modified organisms (GMOs), has confounded humanity. Scientists began investigating the idea of altering an organism's DNA in the early seventeenth century. They also wondered if we might introduce desired characteristics into a creature. With the discovery of restriction enzymes in the 1960s, this concept gradually became a reality (Bose and Bose, 2022). Early in the 1970s, restriction enzymes were used to produce the first recombinant DNA molecules.  Using recombinant DNA technology, DNA from many species is joined and then inserted into a host cell, usually a bacterium. Scientists from Stanford and UC San Francisco utilized restriction enzymes to break DNA from several species at locations, then reassembled the cut strands. In 1974, Stanford's Stanley Cohen and UCSF's Herbert Boyer filed for a patent on recombinant DNA technology, which was approved in 1980. In 1976, Boyer would go on to co-found Genentech, Inc. Over 200 biotechnology and pharmaceutical businesses subsequently licensed the Cohen-Boyer patents, and Stanford and UCSF received over $100 million in royalties. Soon after, Paul Berg, a Stanford scientist who was one of the first to create a recombinant DNA molecule in 1972, and 10 other researchers sent a letter to the journal Science (Jackson et al., 1972; Cohen et al., 1973; Berg et al., 1974).

**7.4 Enzymes**

To stabilize the transition state and lower the activation energy required for a chemical reaction to proceed, enzymes-proteins-act on substrate molecules. Reaction rates are accelerated and brought to physiologically meaningful speeds by this stability. At crucial points in their structure known as active sites, enzymes bind substrates. Usually very specialized, they exclusively bind substrates for specific reactions. Without enzymes, most metabolic processes would be substantially slower and insufficiently quick to support life (Lewis and Stone, 2020).

**7.4.1 Enzyme Action**

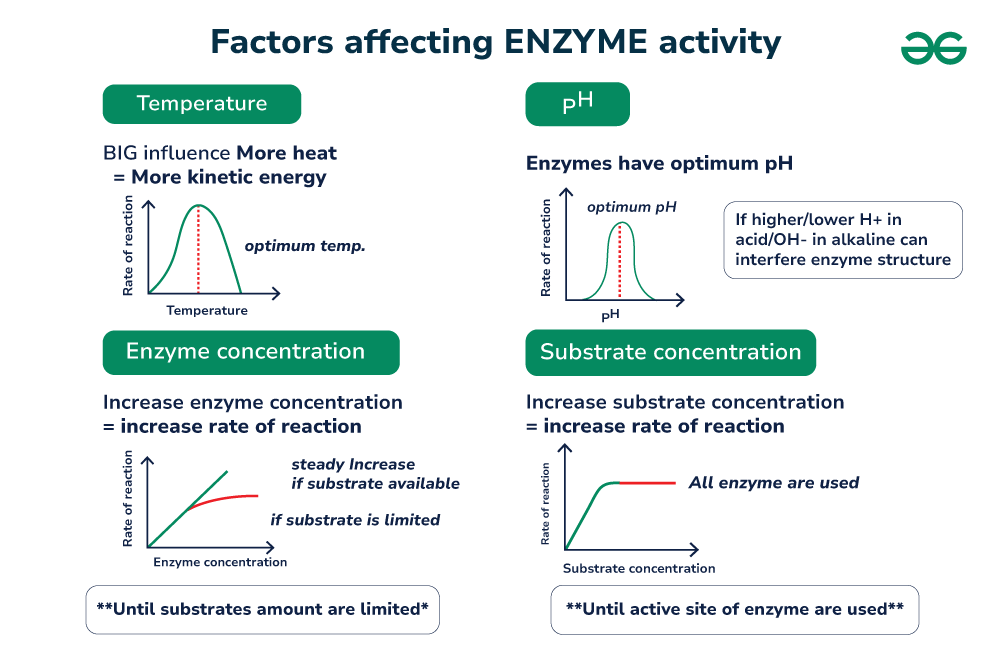
For an enzyme to catalyze a chemical process, it must first bind to its substrate. After attaching to the substrate, the enzyme changes somewhat in shape, which reorients the substrates and lowers the activation energy required for the process to continue. Table 4 shows how enzymes function.

**Table 4 Steps of Enzyme Action**

| **Step** | **Description** |
| --- | --- |
| **1. Substrate Binding** | The active site of the enzyme is penetrated by substrates. The induced fit model describes how the enzyme conforms to fit tightly around them. |
| **2. Formation of Enzyme-Substrate Complex** | Weak bonds, such as ionic interactions and hydrogen bonds, hold substrates in the active site for a short time. |
| **3. Catalysis** | To lower activation energy (Ea), the active site and its amino acid side chains (R groups) do the following: - correctly align the substrate - Apply stress to break existing bonds Establishing an appropriate chemical environment and occasionally taking part in the reaction directly |
| **4. Conversion** | While still attached to the enzyme, substrates undergo chemical transformation to become products. |
| **5. Product Release** | The active site releases the freshly created items. |
| **6. Enzyme Reset** | After reverting to its initial form, the enzyme is prepared to catalyze another reaction. |

**7.4.2 What influences the activity of enzymes**

Among the factors influencing enzyme activity are temperature, pH, substrate and enzyme concentrations, and the presence of activators or inhibitors (Fig 3). These factors can affect the rate of an enzymatic process by changing the structure and binding ability of the enzyme.



**Fig. 3** Factors Affecting Enzyme Activity

(*Adapted from*: <https://www.geeksforgeeks.org/biology/factors-affecting-enzyme-activity>)

**7.5 Recombinant Hormones**

Recombinant hormones are hormones that have been genetically engineered and created artificially in a lab. The process entails merging genetic information from many sources to develop a novel DNA sequence that may be introduced into a host organism, such as bacteria, to generate the desired hormone. The hormones that the human body normally produces are almost the same as these. The following recombinant hormones are either in advanced phases of clinical studies or are now in use: growth hormone (GH), luteinizing hormone, follicular-stimulating hormone, thyroid-stimulating hormone (TSH), parathyroid hormone, and erythropoietin (Sperling, 2020).

Because recombinant hormones improve fish growth, reproduction, health, and disease control, they are crucial to the advancement of aquaculture. These bioengineered hormones are used for several purposes, including growth promotion, illness management, reproductive control, and research support. One of the main applications is growth promotion, which gives aquaculture producers significant financial advantages by using recombinant growth hormone (rGH) to boost fish weight, size, and growth rate (Yoshizaki & Takeuchi, 2015). Recombinant hormones such as gonadotropin-releasing hormone (GnRH) and gonadotropins are employed in reproductive control to increase gamete quality, manage sex determination, and stimulate spawning—all of which are essential components of successful breeding programs (Aizen et al., 2017).

**7.6 Recombinant** **Bioactive Compound.**

Thanks to recombinant DNA technology, a wide range of bioactive compounds have now been introduced into Agriculture and allied. Among the earliest instances were genes that encoded proteins that conferred resistance to insects, viruses, and herbicides. Pharmacologically beneficial substances including immunoglobulin and enkephalin have recently been generated in transgenic plants. One example of modifying already-existing substances to enhance their nutritional value or functional characteristics is the instance of seed storage proteins. In plants, RNAs have recently been shown to be useful as bioactive compounds for suppressing unwanted products and viral infection. Understanding the molecular processes behind the developmentally controlled production of new bioactive chemicals in certain tissues and their localization to subcellular sites is becoming increasingly important from an economic and societal standpoint. According to Patil *et al*. (2009), the bioactive compounds are "food ingredients that affect physiological or cellular activities in the animals or humans that consume them."

**7.6.1 Application of Bioactive Compound in Fisheries and Aquaculture**

Food, medicine, cosmetics, and animal feed are just a few of the many applications for bioactive substances derived from fisheries and aquaculture, such as omega-3 fatty acids, peptides, antioxidants, and polysaccharides (Kim & Wijesekara, 2010; Shahidi & Ambigaipalan, 2015). These substances are frequently extracted from marine algae, crustaceans, and fish byproducts (Rustad et al., 2011). They are then included in nutraceuticals and functional foods to enhance brain and heart health (Kris-Etherton et al., 2002). Due to their antioxidant and antimicrobial qualities, they also aid in preserving the freshness of food (Ngo et al., 2012). They are incorporated into fish feed in aquaculture to enhance the immune system, growth, and general health of the fish (Cerezuela et al., 2011). Fish oil and fishmeal continue to be significant nutrient sources (Tacon & Metian, 2008). Because marine bioactives have anti-inflammatory, antimicrobial, and anticancer qualities, they also aid in drug development and medical applications (Blunt et al., 2018). Additional applications include anti-aging cosmetics and fish-based biodiesel production (Ghaly et al., 2013; Jabeen et al., 2015). By minimizing waste and optimizing resource efficiency in the fisheries and aquaculture sectors, the use of these compounds not only increases economic value but also promotes sustainability and a circular economy (FAO, 2020).

**7.7 Therapeutic proteins**

Therapeutic proteins are proteins that are biologically active and are used to treat, prevent, or diagnose diseases. Their use in fisheries and aquaculture has grown a lot thanks to advances in biotechnology. Recombinant hormones, vaccines, antimicrobial peptides, enzymes, cytokines, and, more recently, monoclonal antibodies are all examples of these proteins. In aquaculture, recombinant hormones like growth hormone (rGH) and gonadotropin-releasing hormone analogs (GnRHa) are used a lot to speed up growth, make feed more efficient, and control reproduction in species that are grown in tanks. Scientists have also made recombinant vaccines and subunit vaccines to protect fish from several pathogens, such as Vibrio and Aeromonas species. These vaccines lower the need for antibiotics and make fish healthier overall (Kim & Austin, 2006). Another class of therapeutic proteins that have broad-spectrum antimicrobial activity and function as natural immune enhancers are antimicrobial peptides (AMPs), which include defensins and Pisidians. These peptides are being added to aquafeeds more frequently as immunostimulants to increase disease resistance because they are essential for innate immunity (Chen & Narayan, 2018). In a similar vein, immune modulators such as interferons and cytokines have demonstrated promise in controlling immune responses, improving fish resistance to infections. In aquaculture, enzymes like lysozymes and proteases are also utilized, especially in feed formulations, to enhance immunity and facilitate digestion. Monoclonal antibodies are also being investigated for their potential as a therapeutic and diagnostic tool in the management of aquatic animal health.

**7.7.1 Application in Fisheries**

The applications of therapeutic proteins in fisheries span several domains. In addition to promoting faster growth and better feed conversion, these proteins help prevent and control diseases, support reproductive management through induced breeding, and contribute to health monitoring and early disease diagnosis. Their use ultimately leads to improved productivity, reduced mortality, and decreased environmental impact through lowered antibiotic use. The integration of therapeutic proteins supports sustainable aquaculture practices and contributes to food security by enhancing the health and welfare of farmed aquatic species (Devlin et al., 1995; FAO, 2020; Reverter et al., 2014).

**7.8 Methods of Producing Recombinant Proteins**

In biotechnology, making recombinant proteins is an important tool that lets you make large amounts of specific proteins for use in medicine, research, and aquaculture. Using a suitable vector, this process puts a gene that codes for the desired protein into a host cell (Rosano

& Ceccarelli, 2014). Plasmids are the most common vectors for yeast and bacteria, and baculoviruses are the most common viral vectors for expressing genes in insect and mammal cells (Walsh, 2018). Understanding the function of vectors is crucial for disease biology in addition to genetic studies since it helps prevent the spread of diseases carried by vectors (Gubler, 2002).

**7.8.1. Vector Types**

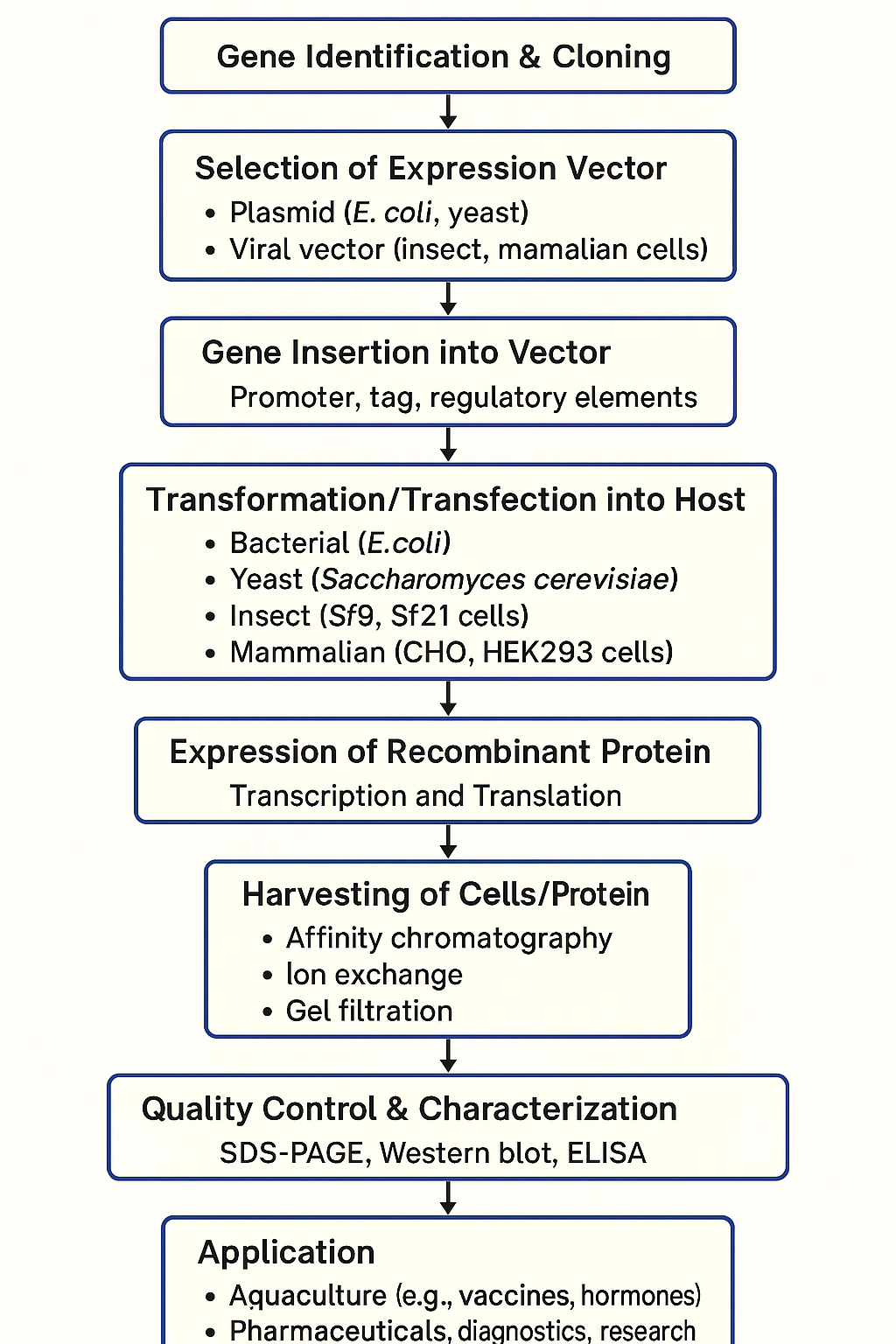
* **Cloning vectors:** They are employed to disseminate DNA inserts and are frequently plasmids.
* **Expression Vectors:** Because these vectors are designed to express inserted DNA, the host cell may produce the encoded protein.
* **Viral vectors:** These employ viruses to directly introduce genetic material, such a therapeutic gene, into a cell.
* **Cosmids:** The cos sequence of a bacteriophage is included in these hybrid vectors, which are commonly used to generate genomic libraries.
* Massive DNA inserts are constructed into artificial chromosomes (BACs, YACs), which are used in genomic research.
* **Shuttle vectors**, which may replicate in a variety of hosts, can transmit genetic material across different species.

***7.8.1.2 Examples of Vectors:***

* **Plasmids:** Circular, extrachromosomal DNA molecules found in bacteria, commonly used for cloning.
* **Bacteriophages:** Viruses that infect bacteria, also used for cloning and gene transfer.
* **Yeast Artificial Chromosomes (YACs):** Designed to carry large DNA inserts and are used for cloning and studying larger genomic regions.
* **Bacterial Artificial Chromosomes (BACs):** Like YACs but designed for cloning in bacteria.
* **Viral Vectors:** Include adeno-associated viruses (AAV), adenoviruses, lentiviruses, and retroviruses, used for gene therapy and other applications.

**7.9 Steps involve to produce Recombinant proteins**

The first step in making the product is to find and clone the gene. This involves amplifying the target gene and putting it into the expression vector with a strong promoter to start making proteins. Then, the recombinant vector is put into a host organism, like *Escherichia coli*, *Saccharomyces cerevisiae*, insect cells (Sf9, Sf21), or mammalian cells (CHO, HEK293), through transformation or transfection (Terpe, 2006). When the recombinant DNA gets inside the host, it is transcribed and translated, which makes proteins. After expression, the recombinant protein is collected and cleaned up using methods like ion exchange, affinity chromatography, or gel filtration (Berlec & Strukelj, 2013). When proteins form inclusion bodies in bacterial systems, they may need extra steps like protein refolding and solubilization. The last steps include quality control and characterization using tests like SDS-PAGE, Western blotting, and ELISA to make sure the protein is pure, has the right structure, and works biologically (Fig. 4) (Tripathi & Shrivastava, 2019). In aquaculture, these proteins are used as hormones, vaccines, and antimicrobial agents to make fish healthier, help them grow, and make them more resistant to disease. This helps make production systems more sustainable and efficient (Sarmasik, 2002).



**Fig. 4** Flowchart of Recombinant Protein Production Process

*The sequential steps required to produce recombinant proteins are depicted in this flowchart. Gene identification and cloning are the first steps, and then an appropriate expression vector is chosen and built. After that, the vector is transformed or transfected into a host organism, such as bacteria, yeast, insects, or mammalian cells. After entering the host, the gene is expressed to create the target protein, which is then extracted, refined, and its quality assessed. The finished recombinant protein finds use in industrial biotechnology, aquaculture, or medicine.*

**7.10 Advantages of recombinant proteins**

Recombinant proteins are extremely helpful in both research and therapy because of their many benefits. Consistent product performance is one of their main advantages as they are made with consistent quality and purity, guaranteeing dependability in both medicinal and experimental applications (Walsh, 2014). Their capacity for large-scale synthesis improves product availability, lowering the cost and increasing the accessibility of these proteins for patients and researchers alike (Kamionka, 2011). Adding to it, the ability to generate recombinant proteins in a variety of host systems enables scalable production, satisfying the needs of diverse applications (Berlec & Štrukelj, 2013). Recombinant proteins are less likely to include contaminated proteins than conventional protein sources, which might affect the findings of experiments or treatments (Scopes, 2013). Additionally, they may be made without affinity tags, maintaining the natural structure and functions of the protein (Waugh, 2005). Additionally, there is little chance of poisoning by infectious agents because they are produced in controlled laboratory settings, therefore disease-state testing is not necessary (Walsh, 2014). Together, these benefits demonstrate the effectiveness, security, and versatility of recombinant proteins in contemporary biotechnology.

**8. CONCLUSION**

In addition to meeting demand, using contemporary biotechnological methods to increase fish production has the potential to improve both the number and quality of fish raised in aquaculture systems. In aquaculture, biotechnology may be used to regulate fish sex and the breeding cycle, as well as to create stocks that are more nutrient-dense, disease-resistant, develop more quickly, and are more tolerant of abiotic changes. In addition to improving the fish's health, this offers customers further nutritional advantages. With the creation of innovative vaccines and probiotics that lessen dependency on antibiotics and lessen environmental effects, the integration of biotechnological advancements also extends to illness treatment. Additionally, biotechnological technologies make it easier to monitor and enhance the quality of the water, which guarantees better raising circumstances and lowers mortality rates. In general, the use of aquaculture biotechnology plays a key role in tackling the issues of nutrition and food security, promoting sustainable aquaculture methods that benefit the environment and the world's population.

**9. DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

Author(s) hereby declares that no generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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