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ROLE OF RECOMBINANT DNA TECHNOLOGY IN FISHERIES AND AQUACULTURE DEVELOPMENT

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Abstract:-*Recombinant DNA (rDNA) consists of DNA molecules which are formed by laboratory methods of genetic recombination to bring together genetic material from multiple sources. Recombinant technology is an important biotechnological tool which brings in the desirable genes together in the perspective of enhancing aquaculture production. The technology is effectively utilized in the production transgenic fish species for better growth and survival. Another milestone achievement in terms of fish disease management is the development of DNA vaccines against various diseases affecting fish species. Further, genetically modified microbes were developed by incorporating the potential genes capable of degrading toxicants for environmental bioremediation. Recently evolved gene editing tools using CRISPR-CAS technology further revolutionized the utility of recombinant DNA technology in fisheries and aquaculture. The applications of rDNA technology in various spheres of fisheries and aquaculture are discussed in detail.*

Key words: rDNA technology, DNA vaccines, Transgenic fishes, gene editing, Bioremediation.

1. INTRODUCTION:-

Recombinant DNA technology is defined as "the joining together of DNA molecules from different organisms and inserting it into a host organism to produce new genetic combinations". DNA fragments are usually derived from different biological sources. Recombinant DNA technology is a progressing part of biotechnology which yield production of recombinant DNA molecules by isolating DNA from different species, cut and spliced together. Recombinant technology is effectively used in various areas of fisheries and aquaculture. For eg. fish vaccines against various pathogenic microbes; Recombinant therapeutic proteins and antigens that are not normally produced in live organism; production of genetically modified fish, which has more growth rate than normal ones. The technology is effectively contributing the development of fisheries field for blue revolution.

2. RECOMBINANT DNA TECHNOLOGY:-

Recombinant DNA technology is one of the essential component of biotechnology. It was developed by Boyer and Cohen in 1973. The technology constitutes five major steps [01].

1. Cutting the desired DNA using restriction enzymes.
2. Amplifying the gene of interest by PCR.
3. Inserting the genes into the suitable vectors.
4. Transferring the vectors into the host organism.
5. Obtaining the products of recombinant genes.

3.TOOLS IN RECOMBINANT DNA TECHNOLOGY:-

Restriction enzymes commonly called as ‘‘molecular scissor’’ play an important role in recombinant DNA technology. They recognize specific sequences and cut in the DNA molecule. eg. EcoR1 cuts at GAATTC and BamH1 cuts at GGATCC. DNA Ligase which is known as “molecular glue” is another enzyme used to attach two pieces of cut DNA together. Many other enzymes like Methylase, Topoisomerase, Alkaline Phosphatase, Kinase, Taq polymerase are used in recombinant DNA technology. Vector, which is a DNA molecule used to artificially carry foreign genetic material into another cell is as a vehicle of carrying the gene of interest. Host organism such as *E.coli* is used to make the copy or for the expression of the rDNA. [02].

4. GENETICALLY MODIFIED FISHES:-

A genetically modified organism (GMO) is any organism whose genetic material has been altered using genetic engineering techniques. The aim is to introduce a new trait which does not occur naturally in the species. Genetically modified fish are used for scientific research activities as model organism and widely used as pets, food and as bio sensors. Many fish species such as Atlantic salmon, Coho salmon, Common carp, Zebra fish, Rainbow trout, Loach, Gold fish etc., are manipulated with genetic engineering techniques and made as genetically modified fish with higher efficiency.

4.1. AQUADVANTAGE SALMON:-

The first patented genetically modified fish (GMO) is aquAdvantage salmon. Aqua bounty, a biotechnology company in USA got approval of FDA (Food and Drug Administration) to sell in the market [03]. It was developed by the addition of a single copy of the OPAFP-GHC2 construct which consists of a promotor sequence from ocean pout directing production of a growth hormone protein using the coding sequence from Chinook salmon [04]. The continuous expression of this transgene makes the fish grow round the year evenly instead of only during spring and summer seasons. The genetically improved fish possess three sets of chromosomes (triploid) [05]. This genetically modified aqua Advantage salmon attained its marketable size of 4-6 kg in 18 months wherein the normal salmon fish attains in 24-38 months. Environment Canada approved the production of the genetically modified variety for commercial purposes in Canada [06] and subsequently the Canadian food inspection

agency approved the sale of the fish in Canadian markets [07].

4.2. GLO FISH:-

Genetically modified GloFish made through rDNA technology are used as popular ornamental fish. GloFish is patented brand of genetically engineered florescent ornamental fish. The fish was developed as pollution detector by glowing in the presence of environmental toxin. It receives the glow from a gene which encodes for the GFP (Green Fluorescence Protein) which is extracted from jelly fish which naturally produce this bright green fluorescence and are available in five colors that are bright red, green, orange-yellow, blue and purple.[08]. The alterations took place when zebra fish embryos are injected with GFP and then as the modified fish breed, the gene is passed on to further generations [09]. But, the survival capacity of these constantly fluorescent fish is lesser than that of wild ones which makes the risk of sustained ecological impact is considered to be marginal [10].

4.3. ETHICAL ISSUES:-

The use of Genetically Modified (GM) fishes and the technology is surrounded by ethical concerns and situational judgment. The consumers are mainly concerned about the long term human health effects of the GM fishes. The notable GM fish risks to humans are the potential development of allergens and toxicity of GM fishes. Issues of concern include the capability of the GMO to escape and potentially introduce the engineered genes into wild populations altering the natural genetic diversity. Fish with high growth rate may develop a morphological abnormality similar to acromegaly in humans. [11]. In GM coho salmon (*oncorhynchus kisutch*), there are morphological changes and changed allometry that lead to reduced swimming abilities [12]. Genetically modified triploid fish are more susceptible to temperature stress tends to possess higher incidence of deformities (eg., abnormalities in the eye and lower jaw) as reported [13].

4.4 BENEFITS OF GENETICALLY MODIFIED FISHES:-

1. Increasing growth rates.
2. Improving feed utilization.
3. Increasing environmental tolerance.
4. Disease resistance.
5. Reproduction control.
6. Improved food quality characteristics.
7. Serves as pollution monitor.
8. Improving host resistance to a variety of pathogens, such as IHNV (Infectious Hematopoietic Necrosis Virus), BKD (Bacterial kidney disease) and Furunculosis. [14].

4.5. FUTURE PERSPECTIVE:-

1. Raising marine fish in fresh water ecosystem.

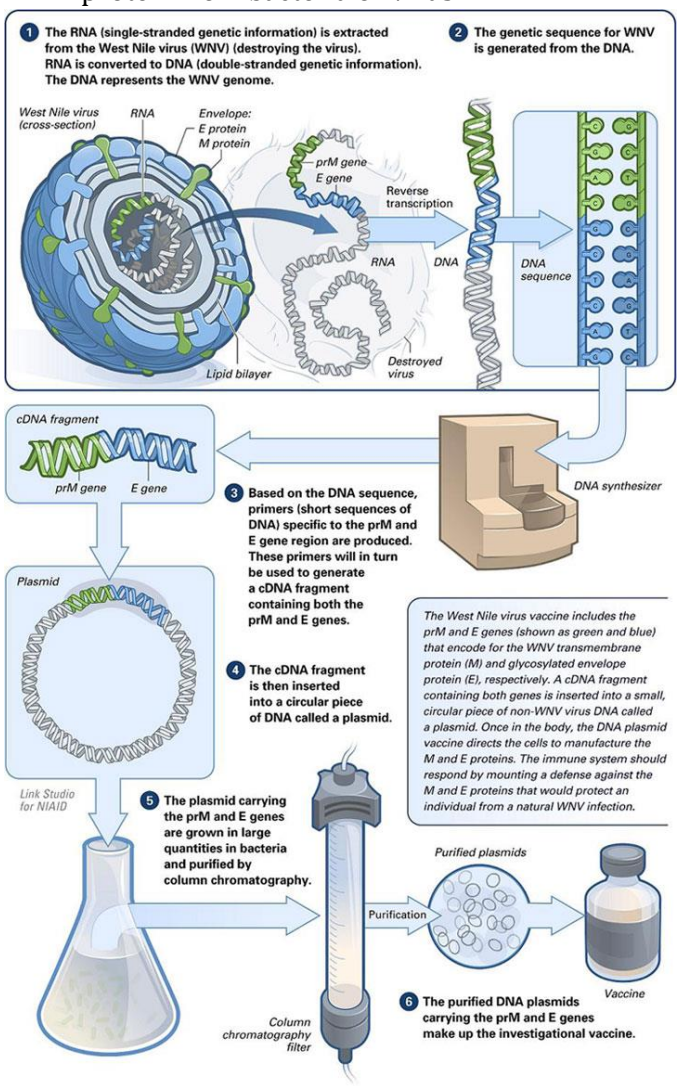
2. Manipulating the length of reproductive cycles for continuous breeders.
3. Increasing the tolerance of aquaculture species to wider ranges of environmental conditions.
4. Enhancing nutritional qualities and taste.
5. Controlling sexual maturation to prevent carcass deterioration as fish age. [14].

5. VACCINES:-

The vaccines which are produced by recombinant DNA technology which provide immense support in aquaculture development. The vaccine is the agent which stimulates the fish immune system to recognize the disease causing agent as foreign body and destroy it. Further, it recognizes and destroys associated microorganisms which may encounter in the future. The developed vaccines must be effective in preventing death and provide long term immunity. Vaccines produced by recombinant DNA technology are called as recombinant vaccines.

5.1. PRODUCTION:-

1. Identify and isolate specific gene from bacteria or virus.
2. Gene is inserted into plasmid DNA and ligated.
3. This engineered plasmid transformed into another bacterium.
4. Allow the bacterial culture to grow and produce the antigenic protein.
5. The vaccine is purified and recovery of protein from bacteria or virus.



5.2. DNA VACCINES:-

DNA vaccines contain DNA that codes for specific proteins or antigens from a pathogen.

Fig.1: Production of DNA vaccine (Source: Wikipedia, the free encyclopedia).

When they are injected in to the cells they tend to synthesize the antigenic proteins. Because these proteins are recognized as foreign material and are processed by the host cells and displayed on their surface, the immune system is alerted then triggers immune responses [15]. DNA vaccines consist of an expression plasmid that carries a specific gene that codes for a selected antigenic protein which when expressed in the host is expected to elicit a strong immune response. [16]. DNA vaccines are often more effective in protecting against viral infections and have especially efficient against fish [17]. DNA vaccines have been efficacious in the prevention of fish exposure to intracellular bacteria like *mycobacterium marinum*. [18]. The first DNA vaccine was against IHNV and tested in rainbow trout *(Oncorhynchus mykiss)*. DNA vaccine against IHNV which was licensed and commercialized in Canada. Salmonid alpha virus subtype 3 DNA vaccine is against pancreas disease virus in the European Union and is marketed as Clynav. This vaccine contains plasmid deoxyribonucleic acid as its active substance, which stimulates active immunity. DNA vaccines are considered safer than attenuated live vaccines as they are without of infection. They only express the antigenic protein segments and not the entire organism. [19].

5.3. ADVANTAGES OF DNA VACCINES:-

1. The main advantage of DNA vaccine is their ability to stimulate both the humoral and adaptive immune system.
2. DNA vaccines may also be used to treat malignancies and auto immune or genetic disorders.
3. When used for cancer therapy, plasmid DNA encoding a Tumor- associated antigen (TAA) can be designed to induce CTL (Cytotoxic T Lymphocyte) responses against cancerous cells expressing the antigen.[20].
4. In autoimmune disorders, DNA plasmids may encode immune modulatory proteins that could tailor the immune response.
5. DNA vaccines do not use microorganisms and therefore avoid the risk of reversion.

5.4. DISADVANTAGES OF DNA VACCINE:-

1. As with “killed” vaccines, and subunit vaccines, there is no transient infection.
2. DNA vaccination is likely to produce a mild immune response and require subsequent boosting.
3. DNA degradation may takes place by nuclease warrants well established supply chain system.
4. There is a possibility of inducing antibody production against DNA.
5. Affecting genes may control cell growth. [21].

Table 1. The DNA vaccines in fish species

Sl. no	Fish species	Pathogenic organisms	Genes used
1.	Rainbow trout	IHNV	IHNV-G, IHNV-G2 SVCV-G
2	Atlantic salmon	ISAV, IPNV, IHNV	Hemagglutinin-esterase (ISAV) IHNV-G
3.	Asian sea bass	*Vibrio Anguillarum*	OMP 38 gene
4.	Hybrid striped bass	*Mycobacterium marinum*	Ag58A
5.	Spotted sand bass	*Aeromonas veronii*	Omp 38, Omp 48
6.	Common carp	SVCV	SVCV-G
7.	Turbot	AHNV	VHSV-G, Capsid proteins (AHNV).

6. BIOREMEDIATION:-

Bioremediation is the process of cleaning of contaminated soil, water with the use of microorganisms. These tailored microbes act in environment by growth of microorganisms and degrade the target pollutants. Recombinant DNA technology used to produce alternative and high efficiency microorganism. *Pseudomonas putida* and *Nitrosomonas europaea* are the microbes which are commonly used in bioremediation. Isolation of the genes from these bacteria that promote bioremediation and then modifying and incorporating them into a suitable host such as *E. coli* is done for using as a bioremediation agent [23]. The super bug invented by Chakrabarty which could able to degrade organic solvents like toluene [24] which was the first patented genetically modified microbe. It can convert styrene oil into the biodegradable plastic Poly Hydroxyl Alkanoates [25]. This may be used in effective recycling of polystyrene. It also involves the bio control activities such as damping off diseases, pythium [26] and fusarium [27]. Sea pollution by oil spills by ships could also be prevented by these bacteria.

7. GENE EDITING (CRISPR-CAS):-

CRISPR gene editing is a genetic engineering technique which genomes of living organisms may be modified. It is simplified version of the bacterial CRISPR-Cas9 antiviral defense system. CRISPR stands for clustered regularly interspaced short palindromic repeats. They are direct repeats which found in DNA of bacteria and archaea [28]. Cas9 is a nuclease complexes with synthetic gRNA (guide RNA) which cuts desired cell genome. It is referred as enzymes from bacteria that control microbial immunity [29]. These sequences are derived from DNA

fragments of bacteriophages which had infected earlier. They are used to detect and destroy DNA from similar bacteriophages during infections. This technology can provide more benefits to aquaculture and fisheries. There occur high growth rate, fecundity and disease resistance of many species by gene editing using CRISPR-Cas9. It offers new opportunities to create de novo (a new) alleles [30]. It could also be used in aquaculture development by producing favorable changes in genome and curing genetic diseases. It has been successfully applied in several major aquaculture species of salmonidae (Atlantic salmon, rainbow trout) Cyprinidae (Rohu, grass, and common carp), Siluridae (channel and southern cat fish), pacific oyster (crassostrea gigas), Nile tilapia (*Oreochromis niloticus*), and gilthead sea bream (*sparus aurata*). There are many successful applications of this technology like growth, pigmentation development, disease resistance, immunity, muscle development, germ cell development, sterility, reproduction, sterile fish production, omega-3 metabolism etc.,[31]. This technology is promising new tool which will aid the growth and development of aquaculture sector in immense way.

8. CONCLUSION:-

The recombinant DNA technology provides successful applications in fisheries sector. The production of genetically modified fish which is enhanced by the use of genetic engineering techniques yielded desirable results in the field of aquaculture. There will be production of therapeutic proteins for control of fish diseases. It can develop fish with new traits by gene editing technology. DNA vaccines are of great help in controlling disease occurrence in aquaculture. With the advent of new tools such as CRISPR cas and improved sequencing methods, recombinant DNA technology is a tool for way forward in the development of fisheries sector in the country.

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