Chronicle of Aquatic Science
Open Access Peer Reviewed Journal
Vol. 1, Issue 5
https://chronicleofaquaticscience.in/
Popular Article
Mahapatra et al. (2023)
Manuscript Id: CoAS_V1IS5_07

OPEN ACCESS

Revolutionizing Aquaculture by developing Genetically Modified Aquatic Organisms

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How to cite this article:

Mahapatra, S., Piyushbhai, M. K. and Binesh, A. 2023. Climate Change and Their Impact on Food Security. *Chronicle of Aquatic Science* 1(5): 74-82.

ABSTRACT

Genetically modified organisms introduced with foreign gene of interest to the host genome to improve various traits such as muscle growth, resistance to various condition like disease, thermal diffraction tolerances factor, salinity tolerance and enhance feed conversion ratio. In the present article we described the steps for developing the genetically modified aquatic organism, their applications, risk associated with their potential release in nature environment and regulatory organization associated with it.

KEYWORDS

Genetically modified aquatic organisms, Aquaculture, risk assessment, regulatory bodies for GMO

Introduction

GMOs are termed genetically modified organisms with the introduction of any foreign gene of interest to the host genome. GMO has been successfully produced in aquaculture sector for the better production of fish muscle and nutritionally improved species. The Aqua-advantage salmon is a great example of GMO produced in aquaculture sector as it has been accepted as fish food worldwide (clifford,2014). Many other fish species have also been produced in the aquaculture sector from warm water as well as cold water categories. Tilapia, channel catfish, medaka, carp and zebra fishes are the fish species which have been used by several researchers to produce genetically modified fish species with better disease resistance capacity, weight gain factor, thermal diffraction tolerances factor (in case of cold-water fish), better food conversion ratio etc (Karthik et al, 2006; Rasal et al,2016).

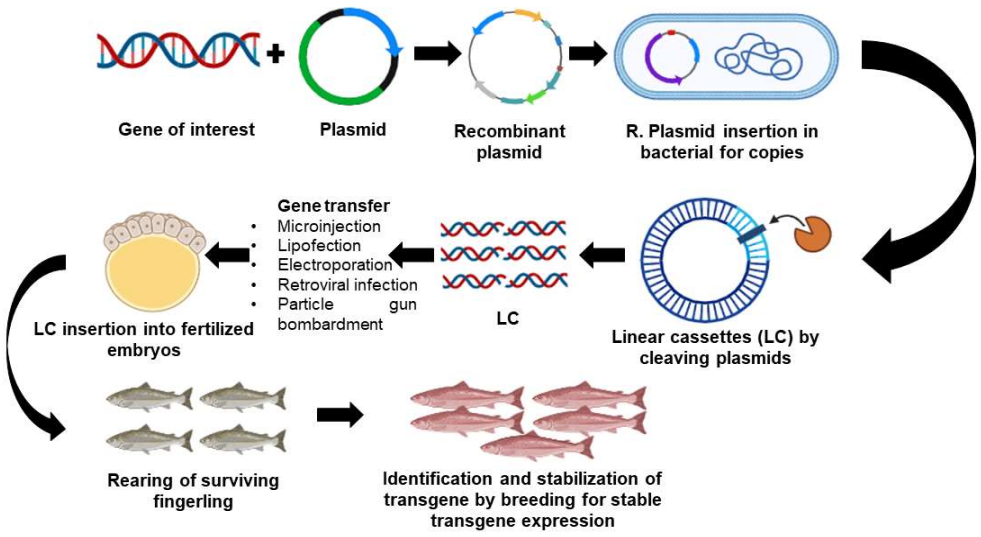

Figure 1. Steps for Genetically Modified Fish production.

Steps used to produce GMO

1. The starting step to produce transgenic or genetically modified fish species is the selection of a correct host species and the gene of interest which will be improved with the execution of transgenesis process. As the example of selection of fish species any fish species can be selected using which the gene of interest could be expressed potentially. For example, salmon, carp, medaka, channel catfish, trouts, IMCs, tilapia, Chinese carps etc. as the example of

gene of interest any type of single or multiple genes of interest can be selected (Saxena and Jha, 2013). For example, growth hormone gene, anti-freeze protein gene, disease resistance gene etc.

2. The second and most important step is the designing of genes for insertion to the selected host species. As fishes are eukaryotes, for the expression of the inserted gene it needs a promoter sequence. There are several types of promoters that have been used in the case of fishes but the ability of expression among the host species differs. Basically, two types of promoters have been used. Such as – constitutive promoters and inducible promoters. Constitutive promoters are the categories which doesn't require any external stimuli for expressing gene of interest whereas inducible promoters require external stimuli such as – light, chemical reagents, temperature and any type of mechanical injuries. Constitutive promoters are unregulated while inducible promoters are regulated by application of external stimuli. Some examples of constitutive promoters include cytomegalovirus (CMV), Rous sarcoma virus long terminal repeats (RSV-LTR), active and chicken δ-crystalline. Some examples of inducible promoters include mouse metallothionein and rainbow trout metallothionein. Some other types of promoters which have been used from piscine origin are flounder anti-freeze promoter, carp β-actin promoter, H3 promoter and salmon MT-B promoter. Using both the gene protein and promoter sequence, the gene construct will be produced, which will be further used to produce recombinant plasmid, which will be used for the insertion into the host.
3. The production of many clones of these designed genes is a necessary part as with this step the gene numbers will be multiplied to billions of copies. For this the designed genes were inserted to the bacterial strains. In a preferable environment these genes can multiply to several copies.
4. After the multiplication of these genes, it's a mandatory step to validate whether all the genes multiplied are of transgene category or not. Validation of this confirmation of transgene can be done in two ways. Likely with the help of selectable marker gene or with the help of reporter gene. Selectable marker genes are the segments in the designed gene which represent the suitable traits, such as – antibiotic resistance gene, which when inserted with the designed gene will express a protein with that antibiotic resistance character. Reporter

genes are the segments which can easily report the required trait in the designed gene, such as – GFP (Green Fluorescent Protein) which can be easily recognized by their fluorescence activity. In bacteria the commonly used reporter gene is *E. coli lac Z*, which indicates the required trait in the designed gene by changing the color to blue when grown in X-gal medium.

5. After the validation of the transgenes these should be isolated from the bacterial cells for further use. The circular shaped plasmids are then generally converted to linear forms with the help of ligases for further insertion into the selected host organism.
6. The linear transgenes inserted to the target organism prior to the first cleavage completion during fertilization.
7. Then the hosts usually incubated in the preferable conditions and the fry survival used to notice. The fittest fries let to increase up to a standard size during which the fishes can show their characteristics.
8. After the growth of these fish fries the transgenic individuals can be screened through many types of methods such as – real-time PCR analysis, southern blotting, northern blotting, western blotting, in-situ hybridization, slot/dot blot and PCR (most preferable method).
9. Transgenic organisms after screening can be maintained with a successful breeding strategy. Transgenic individuals can selectively bred with other transgenic individuals for the successful transmission of transgenes from one generation to the other generations gradually (Wang et al, 2021).

Applications of GMO in Aquaculture

Transgenesis or GMO production has been developed in aquaculture industry for the modernization in the industry to overcome the disease problems, lesser growth rate, pollution problems, and selective traits in the further generations. Transfer of cloned genes have been developed in several fish species which includes zebrafish, loach, rainbow trout, medaka, carp, catfish. But globally two transgenic fishes have been adopted by several countries. Aqua-advantage salmon and Glo fish are the two GMO fish species which have been accepted by several countries. Aqua-advantage salmon had first been developed by AquaBounty technologies, USA. Aqua-advantage salmon is basically Atlantic salmon with chinook salmon growth hormone and the promoter sequence of ocean pout (Jena et al,2017). pUC18 plasmid was used for the cloning and expression of this genome. This modification in the gene construct resulted

in healthy and good fish growth without affecting its quality. Generally, these fishes take three years to grow upto the marketable size but with this transgene it took only 16 to 18 years to attain marketable size which make this transgene a very widely acceptable technology globally. To maintain the stability of this transgene aqua-advantage salmon again back crossed with wild type and concluded with the presence of transgene in its second and fourth generations.

Glo fish are basically zebrafish with fluorescent activity. It was first developed at National university of Singapore by Dr. Zhiyuan Gong in 1999. They inserted the GFP (Green Fluorescent Protein gene) from jelly fish to the zebrafish embryo and let them integrate to the fish genome (Gong et al,1995). These transgene fish usually flourish when environmental toxins are present. It has the advantage of detecting pollution. As these are patented species, which are globally available, they have different trademarks, namely "Electric Green", "Starfire Red" and "Sunburst Orange" (Rasal et al,2016). Many other fish species have also been developed through Transgenesis but because of some ethical issues it could not be able to globalize.

Risk Assessment of GMO in Aquaculture

GMO globalization mainly depends on risk factors and ethical issues. The risk assessment of GM fish can be done in two ways – health risk assessment and environmental risk assessment. Health risk assessments mainly depend on the contents what are present in any GM food (Hallerman and Kapuscincki,1995). It may contain some foods which could be allergen to some humans. So, the labelling of GM food should be proper so that any person can go through these labels before using these GM foods. Reportedly some GM foods contain allergens which could create serious health hazards with renal, pancreatic, hepatic and reproductive issues (Poulsen,2004). Environmental risk assessment mainly includes the escape of GM fishes to the natural environment which can create scarcity and competition over food with other wild type species. The escape of GM fish can cause disturbance in natural biodiversity (Aikio et al,2008). Any undesirable gene could also be spread through the gene pool of natural environment. Antibiotic resistance, cross-contamination, pesticide resistance insect and crossbreeding are some other environmental risks (Mahgoub,2019). To overcome these risks raised by transgenesis there are biological and physical containment factors. Biological containment factor mainly focuses on the production of sterile triploids or all-female

population of GM fish. By modifying hosts and vectors also these risks can be minimized. Physical containment factors include controlling the host species in their physical environment itself. GM fish should be cultured in a closed environment with biologically inactive fishes (Wong and Eenennaam,2008). The net size and filter size should be appropriate and checked regularly to avoid the escape of fish species. Culture of GM fish in an open environment should be avoided. The experimental tanks or aquariums should be covered for the avoidance of fishes jumping out from the experimental environment to outer space. With all the containment factors there are many organizations working together to check these risks. Internationally as well as nationally many organizations are in force.

Regulations of GMO

GMO regulation is an inherent step before releasing these species to the natural environment. The Cartagena protocol on biosafety is in force under the convention on biological diversity (CBD) to check the movement of living modified organisms between countries internationally. This protocol works based on advanced information agreement (AIA) and biosafety clearing house (BCH). The exporter countries should mention all the information regarding the living modified organisms prior to the selection of that species to import. India has been a member of this protocol since 2003 among all the 171 countries (Bartley and Hallerman,1995; Aerni,2004 and Dadgarnejad et al,2017).

Nationally the regulation of GMO has been conducted by the Rules, 1989 (Rules for the manufacture, use, import, export and storage of hazardous micro-organisms, genetically engineered organisms or cells, 1989) under environment protection act, 1986 (Monien and Cai,2018). Two departments are in the force for the execution of these above rules, namely "The ministry of environment, forest and climate change" and "Department of Biotechnology". The other departments which are also take role are "Ministry of Science and Technology", Government of India and State Governments (Raju,2007). There are six competent authorities which take part in these roles with advisory, approval and monitoring capacity. They are The RDAC (The Recombinant DNA Advisory committee), IBSC (Institutional Biosafety Committee), RCGM (Review Committee on Genetic Manipulation), GEAC (Genetic Engineering Approval Committee), SBCC (State Biotechnology Co-ordination Committee), DLC (District Level Committee) (Jha and Shanka,2017). Before releasing any GMO or transgenic organism

to the natural environment review, approval and monitoring is necessary. These rules and regulations are of very much needed role to overcome the risks that could be raised because of transgenesis technology.

Conclusion

Transgenesis is an advanced technology to meet the demands of growing population worldwide. All the sectors like medical, pharmaceuticals, agricultural, veterinary and aquaculture sectors are now accepting this technology for the improvement in their production. Among all the sectors the aquaculture sector can benefit by this technology as fishes produce more numbers of eggs as compared to the other animals. With acceptance of these types of technology it's a mandatory act to accept all the rules and regulations for the avoidance of risk factors associated with this technology.

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