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Application of Gene Transfer in Freshwater Fish: A Review

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

In the field of aquaculture, genetic engineering technology has been widely used in fish for production with the aims of increasing the growth rate of fish, regulating gonadal maturity, sex differentiation and sterility. One of the modern techniques that is expected to be a useful tool in the development of aquaculture is gene transfer technology. Transgenic fish have been established, especially for the purpose of enhancing the growth rate in aquaculture. Aquaculture production is expected to be increased several times to meet future food needs in the form of fish due to the increase in human population. This technique has been applied to species that have economic value. This review describes the variation of gene transfer methods, persistence and expression of the transferred genes, their applications and future prospects of gene transfer research in aquaculture.

Keywords: Aquaculture; Genetic Engineering; freshwater fish; gene transfer; GMO.

1. INTRODUCTION

Biotechnology has advanced at a fast pace in the previous decade, affecting practically every aspect of life, including aquaculture [1]. The generation of fish with rapid growth in a short period of time is the standard for the success of fish farming. The production target might be in the form of weight produced in raising activities, or it can be in the form of number of fish produced to determine the survival rate, especially for hatchery operations [2]. To

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accomplish this, efforts must be made to develop superior fish seeds, as indicated above.

To satisfy society's needs, we need a technology which is both effective and efficient including the use of biotechnology. One way that can be done is the use of transgenic technologies in fish [3]. Transgenic or recombinant DNA technology rDNA is genetic engineering that allows the recombination or recombination of genes from different sources. The goal of these GMOs is to get the required features while also increasing productivity. Until now, carp, tilapia, catfish, catfish, and carp have been used in gene transfer activities [4].

In the field of aquaculture, genetic engineering technology has been widely used for consumption fish. There are several advantages of genetic engineering, namely increasing the growth rate of fish, regulating gonadal maturity, sex differentiation and sterility; increase resistance to pathogens, adapt fish to new environments, change the biochemical characteristics of fish meat so as to create the desired meat taste, change metabolic pathways so that feed efficiency occurs [3]. Transgenic fish have also been used in ecotoxicology, with the potential to develop more advanced and integrated systems for monitoring chemical health effects [5]. Therefore, a review of this journal was carried out with the aim of a study to determine gene transfer technology in freshwater fish to obtain high production.

2. GENE TRANSFER FACTORS

The factors that influence the technique of producing transgenic fish are based on several stages based on Kusrini [3], namely: 1. Determination of fish species. 2. Selection of a specific gene with a specific product of the desired gene. 3. Isolation of DNA containing the target gene or gene of interest (GOI). 4. Isolation of bacterial DNA plasmid to be used as vector. 5. Manipulation of DNA sequences by inserting DNA into vectors. (a) DNA cutting using restriction endonuclease enzymes. (b) Splicing to vectors using DNA ligase. 6. Transformation into host microorganism cells 7. Cloning of foreign cells and genes. 8. Identify the host cell containing the desired recombinant DNA. 9. Storage of cloned genes in DNA libraries. 10. Addition and multiplication of the purified recombinant gene into each egg or sperm of fish selected as transgenic fish. 11. Artificial fertilization by combining the eggs and sperm in certain containers in water media.

The success of gene transfer in fish eggs and sperm can be identified by fluorescent microscopy. Things that are needed at this stage are:

- Identification of individuals who are suspected of being successful as transgenic fish.
- Identification of genetic traits in the F-1 offspring.
- Identify the phenotypic condition of the offspring.

3. GENE TRANSFER TECHNIQUE

I. Sperm Electroforation

The principle of this method is to make reparable holes in the cell membrane with the help of a vibrating electric current (electric pulse). The cell is suspended in a DNA solution, and this solution can enter the cell through the holes that have been formed. The electrophoresis technique has been used in several economically important species, for example in a study of catfish which transferred growth hormone genes to fish sperm by administering an electric shock (125 V/cm and 50 V/cm with pulse counts of 3 and 5) in fish sperm [6]. Mutiara catfish provides a great opportunity for African catfish GH genes to enter and join the DNA of the host fish genome during the electroporation process.

II. Microinjection Technique

Microinjection is the most widely used method because it has high success. It has also been carried out on fish by several researchers including As in the study on medaka fish, a solution of DNA fragments containing gene fragments was injected into the nucleus of an immature egg, while the chorion was still soft because the medaka egg would become very hard after being fertilized. The eggs are incubated in vitro [7].

Another study found that the microinjection method was successful in the introduction of antisense GnRH in rainbow trout to increase reproduction. Another research found that addition of D6 desaturase and phytase can increase fat metabolism and phosphorus metabolism [1,8].

Transgenic are divided into two types, namely auto transgenic (introduced foreign genes are from the same species) and allotransgenic

(introduced foreign genes are from different species) [2]. The application of transgenic technology to catfish in Indonesia has not been carried out.

III. Sperm as gene carrier

Spermatozoa is a specific method designed to transfer foreign DNA into oocytes and sperm are di-rectly involved in the fertilization process. DNA is bound to the postacrosomal region by specific protein components and will join the parental genome after fertilization. Gene binding by sperm can be carried out in a state of motile sperm and a high enough DNA concentration [9].

4. GENE TRANSFER APPLICATIONS IN FRESHWATER FISH AQUACULTURE

In aquaculture, genetic characteristics such as increased growth rate, resistance to cold temperatures and disease, and resistance to low dissolved oxygen levels can be introduced to fish of important economic value. The genes that have been successfully transferred to fish are presented in Table 1. Genetic characters that can increase growth rate, resistance to cold temperatures and disease, resistance to low dissolved oxygen gas, and feed conversion efficiency have been achieved in several fish species and introduced to fish of important economic value for profitability.

5. BARRIERS TO GENE TRANSFER IN FISH

Fish whose genetic material has been artificially engineered in the laboratory are called genetically modified organisms / GMOs [15]. Controversy also occurs among several scientists who conduct research on genetic engineering [16-20]. The controversy is related to the many risks that arise in various aspects of people's lives. However, in its development to date, GMOs still cause rejection from various parties [21].

Although the use of transgenic technology is recognized as having the ability to express foreign genes and opening up options for producing a large number of industrial products such as the commercial pharmaceutical industry, there are still concerns [22]. Products of genetically modified organisms (GMOs) or (genetically modified / GE) have been developed and traded in many developed countries such as the United States and the United Kingdom. One of the most serious threats is that the species itself could become extinct due to unstable patterns of population development (called the "Trojan horse gene effect") [8].

In PP No. 21/2005 [23] also states that GMOs, both domestic and foreign, which will be tested before being distributed in Indonesia, must be accompanied by basic information as an indication that the product meets the requirements of environmental safety, food safety and feed safety. This information explains the description and purpose of use, genetic and phenotypic changes that are expected to be detected, clear taxonomic identities, which are detrimental and endanger human health as a result of the production, preparation, storage, distribution, and use of GMO food processes; while the safety of GMO feed is the condition and effort needed to prevent the possibility of adverse impacts and endanger the health of animals and fish as a result of the process of production, preparation, storage, circulation, and utilization of GMO feed.

Table 1. Gene Construction in Several Fish Species

Species	Gene construction	Results	Reference
Mutiara catfish	pTarget-CMV-CgGH	Growth boost	[6]
Catfish (*Clarias* sp.)	mBP-tiGH	Growth boost	[10]
Mutiara catfish	PhGH (*Pangasius hypophthalmus* Growth Hormone)	Resistant to disease	[11]
Tilapia	1α (EF1α) promoter	Increase fish color brightness by green fluorescent protein (GFP) expression	[12]
Tilapia	Growth hormone	Growth boost along with lower feeding rate (FR) and lipid and energy content than control fish.	[13]
Siamese catfish	Lysozyme gene	Resistant to disease	[14]
Rainbow trout	Glucose transporter and heksokinase	Carbohydrate metabolism	[8]

6. CONCLUSION

The conclusions of this review are: (1) gene transfer in freshwater fish has enormous potential, especially in overcoming food shortages for the world's population and helping to preserve the environment despite the lack of species development in the general population; (2) Gene transfer techniques include electrophoresis, microinjection technique (3) gene transfer applications in freshwater fish aquaculture include increasing growth, increasing resistance to cold temperatures, increasing fish resistance to pathogens.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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