Title: Bioengineered Animals and Microorganisms

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ABSTRACT

Genetically engineered animals are revolutionizing public health through biomedical, environmental, and food applications. They are crucial for developing new diagnostic techniques and drugs for human diseases, offering clinical and economic benefits. They improve human health and food security by enabling healthier meat and milk. Livestock are more efficient at converting feed to animal protein, reducing waste production. Genetic engineering enhances animal welfare by providing disease resistance and overall health. Microorganisms play a crucial role in food product improvement, eliminating carcinogenic compounds, inhibiting pathogenic bacteria, producing healthier natural sweeteners, and synthesizing beneficial compounds like carotinoids.

KEYWORDS: Bioengineered animals; Bioengineered fish; Bioengineered microorganisms.

I. BIOENGINEERED ANIMALS

Transgenic technology has made it feasible to enhance commercially important livestock species by transplanting genes from similar or unrelated species. Using biotechnology, genetic improvement may be accomplished in a single generation, as opposed to the numerous generations required by traditional animal breeding procedures. Although numerous methods for transferring genes have been developed, the bulk of transgenic animals are presently generated utilizing four methods: nuclear transfer, microinjection, viral vector infection, and embryonic stem cell transfer. The nuclear transfer procedure involves inserting the whole genetic material from a donor cell's nucleus into a mature, unfertilized egg that has had its nucleus removed. The embryo is then implanted in the womb of a foster mother, where it will develop into a mammal with the same genetic make-up as the donor cell [1]. A little bit of foreign DNA carrying one or more genes is introduced into the male pronucleus when an egg is fertilized. The egg must be at the single-cell stage to ensure that the transgene is present in every somatic cell of the animal. The embryo is then implanted in the womb of a surrogate mother [2]. In the process of retroviral infection, a viral vector is employed to convey a gene. Retroviruses are commonly used in the process of DNA transfer because of their inherent ability to infect cells [3]. Embryonic stem cells are extracted from blastocysts and cultivated in the stem cell transfer method. A chimaera animal is formed by inserting cultured cells into the inner cell mass of a blastocysts stage embryo, which is then implanted in the foster mother [4]. It's critical to remember that these procedures just give tools for the creation of new animal strains with fresh genetic material, not new species. Transgenic pigs that produce meat with decreased fat content and transgenic cows that produce milk with improved composition are two examples of genetically engineered animals. The primary goals of livestock genetic engineering initiatives are improved production efficiency and better animal feeding products.

I.A. MODIFIED MILK IN TRANSGENIC DAIRY CATTLE

Bovine milk has been compared to an almost perfect diet due to its abundance in vitamins, calcium, and vital amino acids [5]. Vitamins A, B, C, and D are a few of the vitamins present in milk. Milk contains the highest amount of calcium of any dietary source, and two servings of milk or other dairy products daily will satisfy an adult's calcium needs [6, 7]. About 80% of the protein in milk is made up of caseins, which are also very nutritious and useful [8]. Calcium, magnesium, iron, and zinc are just a few of the cations that the caseins have a great affinity for. Milk contains four different kinds of naturally occurring caseins: S1, S2, S3, and S4

[8]. They form massive micelles, which are what give milk its physicochemical characteristics. The micelles' structure can vary slightly depending on the casein ratio, and this can alter the milk's functional characteristics. For the purpose of making cheese, the casein concentration of the milk is crucial since it increases cheese yield and nutritional quality [9]. According to estimates, increasing milk's casein concentration by 20% would enhance cheese output and bring in an extra $190 million per year for the dairy industry [2]. A single copy of each of the genes encoding the s1/s2, and -casein proteins is present in dairy cattle, and are the two most significant caseins [10]. Improved heat stability is the result of smaller micelles, which are caused by increased milk -casein concentration. Due to the increased phosphorylation of -caseins and their ability to bind calcium phosphate, milk calcium levels are affected [11, 12]. Transgenic mice have been the primary focus of research into changing the composition of milk to improve nutritional or functional properties. Despite not always mimicking ruminant levels of protein expression, mice are effective models for studying protein expression in mammary glands [13]. Brophy et al., 2003 [14] employed the nuclear transfer approach to develop transgenic cows with extra copies of the genes CSN2 and CSN3, which produce bovine - and -caseins, respectively. CSN2 and CSN3 genomic clones were discovered in a bovine genomic library. According to previous mouse studies [15], CSN3 had relatively low expression levels. The CSN3 gene was coupled to the CSN2 promoter in a CSN2/3-fusion construct created by the researchers to boost CSN3 expression. The CSN2 genomic clone and the CSN2/3-fusion construct were co-transfected into bovine fetal fibroblast (BFF) cells, and the two genes demonstrated coordinated expression. Nuclear transfer was used to generate nine entirely healthy and functioning cows, with transgenic cells acting as donor cells. According to Brophy et al., 2003 [14], over expression of CSN2 and CSN2/3 in transgenic cows boosted -casein levels by 8-20% and 100%, respectively).

I.B. TRANSGENIC POULTRY: EGG AS BIOREACTOR

A great deal of study has gone into utilizing mammals and birds as bioreactors. Mammals may now be used as bioreactors thanks to the creation of transgenic mice and the identification of tissue-specific promoters [16, 17]. Clark et al. proposed using transgenic swine mammary glands to create therapeutic proteins in milk in 1987 [18]. Despite the high expression of foreign protein in milk and significant milk output, the use of mammary glands as bioreactors has certain limitations, including the lengthy process of producing a stable line of transgenic founder animals and the high expense of milk purification of foreign protein [19].Furthermore, researchers have long studied utilizing chicken eggs for the production of foreign proteins. The fact that the bulk of the proteins in egg white are regulated by a single gene called ovalbumin is one of the numerous advantages of employing eggs as bioreactors. Furthermore, egg white has a high protein content, is naturally sterile, and has a long shelf life [20, 21].

The infrastructure for growing, collecting, and processing chicken eggs is currently in place [19]. A bacterial gene was recently successfully inserted, produced, and released in the egg white of transgenic chicken by a team of researchers from the biotech business AviGenics, Athens, Georgia [21]. The *E. coli* -lactamase (EC 3.5.2.6) reporter gene was used as the transgenic because eukaryotic cells can easily secrete and measure it. The transgene was expressed using the replication-deficient retroviral vector NLB from the avian leucosis virus (ALV). The cytomegalovirus (CMV) promoter was used to control the insertion of the -lactamase coding gene into the viral vector pNLB-CMV-BL. The biological activity of the protein -lactamase was confirmed, which was shown to be secreted in the blood and egg white and whose expression levels were consistent over four generations of transgenic chickens. These findings suggest that the chicken egg is a promising bioreactor candidate because foreign proteins may be expressed and secreted there. The major purpose of the chicken model is to identify, isolate, and define gene enhancers and promoters that are active and drive tissue-specific protein expression in adult oviducts, as well as to develop more efficient non-viral-based ways for producing transgenic chicken.

II. BIOENGINEERED FISH

Fish are thought to be the safest for human eating of all transgenic, domesticated animals that have been created so far, and they are anticipated to be the first transgenic animal to be approved as a food item [22]. For the commercialization of Atlantic salmon with a growth hormone (GH) gene from Chinook salmon, the business Aqua Bounty has submitted an application to the FDA, which is currently being reviewed [23]. The primary challenge to achieving this objective is a better understanding of the potential dangers associated with the release of transgenic fish into the wild, and as of yet, little study has been done to address these issues [24]. Sterilizing all transgenic fish is one way to prevent their spread in the wild, but there is currently no effective

way to sterilize them completely [25]. The following is a description of some of the transgenic techniques being investigated to increase antifreeze property and improve growth rate.

II A. INCREASING ANTIFREEZE PROPERTY IN FISH

Fish species like ocean pout and winter flounder in freezing water secrete antifreeze proteins (AFPs) and antifreeze glycoproteins (AFGs) to protect their bodies from freezing. These proteins lower the freezing point of the fish's serum, attaching them to the ice surface and blocking ice crystal formation. Four types of AFPs and at least one AFG have been identified.

Most aquaculture-important fish, such as Atlantic salmon and tilapia, do not produce antifreeze proteins naturally, so they cannot survive and be raised in areas of the world where water temperatures drop below freezing, posing a major problem for sea cage farming along the northern Atlantic coast [26].The creation of commercially important transgenic fish, particularly frost-resistant salmon, would significantly increase the area accessible for fish farms, increase production, and cut consumer prices. The creation of commercially important transgenic fish, particularly frost-resistant salmon, would significantly increase the area accessible for fish farms, increase production, and cut consumer costs. Flounder AFPs are tiny polypeptides of the Type-I AFP family with two isoforms: skin-type and liver-type. Hew et al. (1999) [26] created a transgenic stable line of Atlantic salmon with cold tolerance capability using the winter flounder liver-type AFP gene. The transgenic pioneer fish showed consistent AFP expression and amounts of physiologically active protein, resulting in three generations of transgenic salmon. The expression of AFP was liver specific and showed seasonal variation similar to that of winter flounder, but the levels of AFP in the blood of these fish were low (250 g/ml) compared to natural AFP concentrations in winter flounder (10-20 mg/ml), making the salmon freeze resistant [26]. The current research aims to develop gene constructs that enhance the transgene's copy number and hence raise AFP expression levels in critical organs, resulting in improved antifreeze properties in farm fish.

II B. IMPROVING FISH GROWTH RATE

Many salmon species, including many others, have had their genes for fish growth hormone cloned and characterized [27, 28] Transgenic tilapia fish (*Oreochromis niloticus*) were created by scientists from the University of Southampton in the United Kingdom. These fish were modified with growth hormone genes from several salmonids. In their experiment, Rahman *et al*. 1998 [29] employed a variety of construct types, but the one that produced the greatest outcomes was the one that contained a Chinook salmon GH gene under the control of the ocean pout antifreeze promoter, fish egg microinjections using fertilized eggs. The construct was successfully integrated into the founder (G0) tilapia's genome, and the transgene was then passed on to the G1 and G2 generations, according to the research. A three-fold increase in growth rate and a 33% increase in food conversion ratio were seen in transgenic tilapia that expressed the transgene, which would lower the cost of production for farmers. A desirable characteristic for commercial transgenic fish is infertility at maturity, which this transgenic tilapia also demonstrates.

III. BIOENGINEERED MICROORGANISMS

For more than 5000 years, both consciously and unconsciously, humans have used spontaneous fermentation to preserve a range of foods, including bread, alcoholic beverages, dairy products, vegetables, and meats. However, scientists only recently—within the past century—realized that the process of fermentation was carried out by the action of microbes and that each bacterium in charge of a particular food processing could be separated and identified. Today, with the help of cutting-edge bioengineering techniques, it is possible to characterize significant food strains with great precision, to isolate and enhance genes involved in fermentation, and to transfer advantageous features between strains or even between other species.

III A. ELIMINATION OF CARCINOGENIC COMPOUNDS

In the food business, brewer's yeast (*Saccharomyces cerevisiae*) is one of the most significant and often utilized microorganisms. This microbe is cultured for its cells and cell components in addition to the final products it produces during fermentation [30]. Currently, the fermentation of bread and alcoholic beverages uses yeast most frequently. Recombinant DNA technology has made it possible to modify yeast to have new traits

while also getting rid of unwanted byproducts. Ethyl carbamate, often known as urethane, is one of the unfavorable by-products created during the yeast fermentation of food and drink [31]. Due to this, the alcoholic beverage industry has committed a significant portion of its budget to research aimed at lowering the amount of ethyl carbamate in its goods [32]. The production of urea, which results from the breakdown of the abundantly present amino acid arginine in grapes, is what causes the spontaneous reaction between ethanol and urea that produces ethyl carbamate. Arginase, an enzyme that catalyses the breakdown of arginine found in yeasts used in wine production. If this enzyme can be inhibited, arginine won't be converted to urea, and urea won't be able to combine with ethanol to generate ethyl carbamate. The enzyme Arginase (EC 3.5.3.1) is encoded by the CAR1 gene in industrial yeast [32]. A transgenic yeast strain with the CAR1 gene inactivated was created by Kitamoto et al., 1991 [33] to lessen the generation of urea in sake. By inserting an inefficient CAR1 gene and DNA homologous recombination, preventing it from performing its intended purpose, sequences that are similar to sections of the Arginase gene, the researchers were able to create the mutant yeast strain. As a result, urea was removed and the fermentation of sake no longer produced ethyl carbamate. The removal of ethyl carbamate from wine and other alcoholic beverages can be accomplished using the same method [33].

III B. INHIBITION OF PATHOGENIC BACTERIA

To increase safety, cleanliness, and efficiency in the production of fermented foods, the use of starter and protection bacterial cultures is a common practise in the food industry today [34]. A starter culture is a liquid made composed of a specific combination of microorganisms that is used to kickstart industrial fermentation. Starter cultures provide a desirable aroma or texture to food, whereas protective cultures do not change the qualities of food but rather prevent the growth of unwanted harmful germs [35].To make food processing more viable, the same microbe should be used for starter and protection cultures, but unfortunately, this is not always possible. The strains of microorganisms that are currently used in starter and protective cultures can be improved using genetic engineering techniques, allowing for the addition of novel traits and the removal of undesired ones [36].

The three main objectives of genetic engineering research on starting cultures are to improve process stability, efficiency, and product safety [35]. The pH level in the culture increases during the fermentation process of various foods, such as mould-ripened cheese, as a result of lactic acid being broken down by fungus. *Listeria monocytogenes* and other food-borne pathogenic bacteria can flourish in this alkaline medium [37]. The adoption of starter cultures, which can also act as protection cultures and stop the proliferation of such hazardous microbes, might significantly increase the safety of food items. The enzyme lysozyme (EC 3.2.1.17) can be an effective agent for the inhibition of Listeria in food. Van de Guchte *et* al., 1992 [38] integrated the gene responsible for lysozyme formation in a strain of the bacterium *Lactococcus lactis*. After genetic transformation, this bacterial strain was able to express and secret lysozyme at high levels. The researchers cloned lysozyme-encoding genes from *E. coli* bacteriophages T4 and lambda in wide-host-range vectors and expressed in *L. lactis.* Biologically active lysozyme were produced and secreted by the transgenic L. lactis strains, suggesting that these bacteria can be used both as a starter and protective culture [38]. The transgenic *L. lactis* strains generated and released biologically active lysozyme, indicating that these bacteria can be employed as a starter and protective culture [38].

III C. NATURAL SWEETENER PRODUCED BY MICROORGANISMS

While methods to improve food flavor have been around for a while, it has only recently been realized that microbes can also be employed to produce and improve flavor. Today, synthetic chemicals are used in large number of flavoring methods for foods and beverages [39]. Bioflavors, or flavors created through biological processes, are growing in popularity among consumers as public awareness of the risks associated with synthetic chemical use rises [40, 41]. Although thousands of natural volatile and synthetic scents are known, only a small number are frequently utilized and produced on an industrial scale. There are numerous ways to make bioflavors, including (1) product extraction from plant materials and (2) the employment of particular microbes that have been bioengineered for their biosynthesis. Microorganisms can produce bioflavors on a wide scale at a low cost, without the need for plant material, and with the protection of natural resources in mind [42].

ERYTHRITOL

Industrial erythritol production has grown due to the development of electrochemical processes, which produce erythrose and erythritol through the decarboxylation of arabinoic or ribonic acid. A more natural method involves biotechnological fermentation, resulting in higher yields. Erythritol is derived from fermentation processes by fungi or lactic acid bacteria, with common pathways including *Trigonopsis, Candida, Pichia, Moniliella, Yarrowia, Pseudozyma, Trichosporonoides, Aureobasidium,* and *Trichoderma*. Separation and purification steps are crucial for erythritol use as a food additive. A patent outlines separation from fermenting microorganisms, ion exchange chromatography, crystallization, and activated-carbon treatment for erythritol fraction recovery [43].

TAGATOSE

The manufacture of biotechnological tagatose by enzymatic isomerisation is favoured over chemical techniques. Although L-arabinose isomerase (l-AI) is a biocatalyst source for biological D-tagatose production, its bioconversion efficiency is restricted due to metal ion requirements and low thermostability. By modifying the functional characteristics of l-AI, protein engineering and genomic techniques can improve bioconversion efficiency. Individual protein variations can be evaluated using high-throughput screening or selection techniques, allowing for particular mutants with higher catalytic activity. To circumvent safety concerns, the L-arabinose isomerase gene can be transferred to GRAS hosts such as C. Glutamicum, Corynebacterium ammonagenes, and Bacillus megaterium. More study is required to investigate novel biocatalyst sources derived from GRAS microorganisms [44].

CONCLUSION

Genetic engineering enables scientists to study gene function by altering biological systems, leading to the creation of human disease models like Alzheimer's, ALS, Parkinson's, and cancer. These models provide valuable insights into disease development and potential treatments. However, genetic engineering raises ethical issues beyond health and welfare affecting animal integrity and dignity. Some types may be restricted from commercial use, requiring stakeholder involvement. Genetically engineered microorganisms (GEMs) offer cost and resource benefits in food production, but consumers' integration of sustainability may lead to inconsistencies between GMO labeling and their intent for sustainable food choices. Safety must be ensured, and regulatory agencies worldwide must align safety evaluation and categorization approaches to avoid unnecessary trade barriers caused by inconsistencies in global regulations.

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