

# BIOENGINEERED ANIMALS AND MICROORGANISMS

## Abstract

Genetically engineered animals are revolutionizing in various public sectors including disease diagnosis, gene therapy in various diseases, environmental sector, and animal based food products. Genetic engineering are crucial for developing new techniques for disease diagnosis and cure for various human diseases, and drug production, offering clinical and health benefits. They improve human health through genetic modifications and drug development, they also provide food security by production of healthier meat, milk and animal products. 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.

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## I. BIOENGINEERED ANIMALS

Transgenic technology has made it feasible to enhance various livestock species by transplanting genes from similar or unrelated species making it commercially important. Using biotechnology, genetic improvement may be accomplished in a single generation, as opposed to the numerous generations by traditional animal breeding procedures and advanced *in vitro* fertilization technique. Although numerous methods for transfer of genes have been developed, the bulk of transgenic animals are presently generated utilizing four methods: direct nuclear transfer method, use of various viral vectors for gene transfer, micro-injection, and embryonic stem cell gene transfer method. In direct nuclear transfer procedure nucleus is directly transferred to unfertilized egg having its nucleus removed. The fertilized egg is then developed into embryo and embryo is implanted into the a foster mother to develop into a individual with the same genetic make-up as the donor cell from which nucleus has taken [1]. A little bit of foreign DNA carrying one or more genes may introduced into the male pronucleus when an egg is fertilized. The gene transfer should ensure that fertilized egg must be single-cell so that the transgene will appear in every somatic cell of the animal. After that embryo is implanted in to the womb of surrogate mother for the proper development of embryo in to young one [2]. In the process of retroviral gene transfer, a viral vector is employed to convey a gene. Retroviruses are used DNA transfer because of their inherent ability to infect cells [3]. Embryonic stem cells are extracted from blastocysts and cultivated for modification. 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 improved composed milk are two examples of genetically engineered animals. The primary goals of livestock genetic engineering initiatives are improved production efficiency and better animal feeding products.

**1. 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 copies of the CSN2 and CSN3 genes, which produce bovine protein - and -caseins protein 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 and the CSN2/3-fusion construct were co-transfected into fibroblast cells of fetal bovine (BFF), 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 transgenicallt produced cows boosted -casein levels by 8-20% and 100%, respectively.

- 2. 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 mice with transgene 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 transgenic protein in milk and significant milk output, the use of mammary glands as bioreactors has certain limitations, including the lengthy process of producing a stability of transgenic animals and the expense of milk purification of foreign protein is high [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 white part of chicken egg for transgenic chicken by research team from biotech company 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 RNA viral vector NLB from the avian leucosis virus (ALV). The cytomegalovirus (CMV) promoter part was used to control the integration of the -lactamase coding gene into the viral vector pNLB-CMV-BL. The activity of the protein -lactamase was confirmed, which was shown to be released in the blood and white portion of egg. The 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 isolate, identify and define the gene for promoters and enhancers that are active and drive tissue-specific protein expression, as well as to form efficient transgenic chicken using non-viral-based ways.

## II. BIOENGINEERED FISH

Among all transgenic, domesticated animals created so far fish are safest food for human being consumption, and they are anticipated for the first transgenic animal food to be approved [22]. For the Atlantic salmon commercialization with a growth hormone (GH) gene from Chinook salmon, the business Aqua Bounty [23]. The primary challenge to achieving this objective is understanding of the potential dangers associated with the release of transgenic fish into in to the wild food chain, 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.

**1. Increasing Antifreeze Property in Fish:** Fishes like ocean pout and winter flounder in freezing water secrete proteins with antifreeze property , this proteins are called antifreeze proteins (AFPs) and antifreeze glycoproteins (AFG) which protect their bodies from freezing. Antifreeze protein and glycoprotein low down the freezing point in serum of fish by attaching to the surface of ice and hence block the formation of ice crystal. Four types of AFPs and at least one AFG have been identified.

Some important fishes like salmon fish of atlantic and tilapia fish , had no genes for antifreeze proteins and glycoproteins naturally, so this fishes cannot be aquacultured in freezing temperature as they cannot be survived and raised in areas with temperature dropdown below freezing point, posing a major problem for farming of above fishes along the northern coast of atlantic [26].The creation of commercially important particularly frost-resistant salmon transgenic fish, would significantly increase the area accessible for fish farms, increase production, and cut consumer prices. The creation of commercially important transgenic fish, 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 gene expression of AFP was specific in liver and also showed seasonal variation in their expression same as winter flounder, but the levels of AFP in the serum of the transgenic fish were low which is around 250 microg/ml when compared to natural AFP concentrations in winter flounder which is between 10-20 mg/ml, hence making the transgenic salmon fish 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.

**2. 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

Humans are using micro-organism form thousands of years back for, both consciously and unconsciously, spontaneous fermentation to preserve a wide range of foods, like bread, dairy products, alcoholic drinks, dairy products like curd, vegetables, and meats. However, scientists only recently—within the past century—identified that the process of fermentation in foods was due to the action of micro-organisms. and that each bacterium involved in a particular food processing could be separated and identified. Today, with the help of cutting-edge bioengineering techniques, characterization of significant strains in food with great precision is possible, also to isolate the genes required for fermentation, and transfer their advantageous features between strains or even between other species is possible and current perspective for research.

**1. Elimination of Carcinogenic Compounds:** In the food business, *Saccharomyces cerevisiae* one of the brewer's yeast is the most significant and often utilized microbial species. This microbe is cultured for its cells and cell components in addition to the final products it produces during fermentation [30]. Currently, this species is used frequently for the fermentation of all types of breads and alcoholic drinks. Now with rDNA (Recombinant DNA) technology it possible to modify this yeast species to have new beneficial traits and also getting rid of unwanted byproducts. Ethyl carbamate, often known as urethane, is one of the unfavorable by-products created during the fermentation of food and drink by yeast [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. Inhibition of this enzyme causes no expression of arginine thus, arginine won't be converted to urea, and urea won't be able to combine with ethanol to generate ethyl carbamate. The Arginine enzyme was encoded by the gene *CAR1* in industrially used yeast species [32]. A 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].

- 2. Pathogenic Bacteria Inhibition:** For safety increase, cleanliness, and efficient production in fermented products, protectional bacterial cultures and starter cultures are in common in practice in food industries [34]. A starter culture involves liquid which is composed of a specific combination of microbes 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 culture 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 lysozyme involves in inhibition of *Listeria* contaminations in food. Van de Guchte *et al.*, 1992 [38] created bioengineered bacteria *Lactococcus lactis* containing gene for lysozyme production. After genetic transformation, this bacterial strain was able to express and secret lysozyme at high levels. The transgenic *L. lactis* strains generated and released biologically active lysozyme, indicating that these bacteria can be used in both protective culture and starter culture [38].

- 3. 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 and synthetic scents are known, but at industrial level only a small number are frequently utilized and produced. There are numerous ways to produce bioflavors, firstly extraction from plant materials and secondly 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].

#### IV. ERYTHRITOL

Erythritol production at industrial level has grown due to the development of electrochemical processes, which produce erythrose and erythritol through the decarboxylation reaction of arabinoic acid. The natural method involves fermentation, resulting in higher yields. Erythritol is obtained from fermentation by fungi or lactic acid bacteria, with common pathways including other fungi like *candida* species. *Pseudozyma* etc. A patent outlines separation of erythritol product from fermenting microorganisms *via*. ion

exchange chromatography, crystallization, and activated-carbon treatment for erythritol fraction recovery [43].

## V. 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 ammonigenes*, and *Bacillus megaterium*. More study is required to investigate novel biocatalyst sources derived from GRAS microorganisms [44].

## VI. 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.

## REFERENCES

- [1] Alsaggar, M., & Liu, D. (2015). Physical methods for gene transfer. *Advances in genetics*, 89, 1-24.
- [2] Wheeler, M. B. (2003). Production of transgenic livestock: promise fulfilled. *Journal of animal science*, 81(15\_suppl\_3), 32-37.
- [3] Whyte, J. J., & Prather, R. S. (2011). Genetic modifications of pigs for medicine and agriculture. *Molecular reproduction and development*, 78(10-11), 879-891.
- [4] Hochedlinger, K., & Jaenisch, R. (2003). Nuclear transplantation, embryonic stem cells, and the potential for cell therapy. *New England Journal of Medicine*, 349(3), 275-286.
- [5] Karatzas, C. N., & Turner, J. D. (1997). Toward altering milk composition by genetic manipulation: current status and challenges. *Journal of Dairy Science*, 80(9), 2225-2232.
- [6] Rinzler, C. A. (2009). *The new complete book of food: a nutritional, medical, and culinary guide*. Infobase Publishing.
- [7] Kandra, P. (2022). The Current and Future Prospects of Animal Biotechnology Applications in Food. In *Recent Advances in Food Biotechnology* (pp. 43-58). Singapore: Springer Nature Singapore.
- [8] Brophy, B., Smolenski, G., Wheeler, T., Wells, D., L'Huillier, P., & Laible, G. (2003). Cloned transgenic cattle produce milk with higher levels of  $\beta$ -casein and  $\kappa$ -casein. *Nature biotechnology*, 21(2), 157-162.
- [9] McMahon, D. J., & Brown, R. J. (1984). Composition, structure, and integrity of casein micelles: a review. *Journal of Dairy Science*, 67(3), 499-512.
- [10] Bawden, W. S., Passey, R. J., & Mackinlay, A. G. (1994). The genes encoding the major milk-specific proteins and their use in transgenic studies and protein engineering. *Biotechnology and Genetic Engineering Reviews*, 12(1), 89-138.

- [11] Dalgleish, D. G., & Law, A. J. (1989). pH-induced dissociation of bovine casein micelles. II. Mineral solubilization and its relation to casein release. *Journal of Dairy Research*, 56(5), 727-735.
- [12] Jimenez-Flores, R., & Richardson, T. (1988). Genetic engineering of the caseins to modify the behavior of milk during processing: a review. *Journal of dairy science*, 71(10), 2640-2654.
- [13] Colman, A. (1996). Production of proteins in the milk of transgenic livestock: problems, solutions, and successes. *The American journal of clinical nutrition*, 63(4), S639-S645.
- [14] Brophy, B., Smolenski, G., Wheeler, T., Wells, D., L'Huillier, P., & Laible, G. (2003). Cloned transgenic cattle produce milk with higher levels of  $\beta$ -casein and  $\kappa$ -casein. *Nature biotechnology*, 21(2), 157-162.
- [15] Persuy, M. A., Legrain, S., Printz, C., Stinnakre, M. G., Lepourry, L., Brignon, G., & Mercier, J. C. (1995). High-level, stage-and mammary-tissue-specific expression of a caprine  $\kappa$ -casein-encoding minigene driven by a  $\beta$ -casein promoter in transgenic mice. *Gene*, 165(2), 291-296.
- [16] Gordon, J. W. (1989). Transgenic animals. *International review of cytology*, 115, 171-229.
- [17] Swift, G. H., Hammer, R. E., MacDonald, R. J., & Brinster, R. L. (1984). Tissue-specific expression of the rat pancreatic elastase I gene in transgenic mice. *cell*, 38(3), 639-646.
- [18] Clark, A. J., Simons, P., Wilmot, I., & Lathe, R. (1987). Pharmaceuticals from transgenic livestock. *Trends in Biotechnology*, 5(1), 20-24.
- [19] Ivarie, R. (2003). Avian transgenesis: progress towards the promise. *TRENDS in Biotechnology*, 21(1), 14-19.
- [20] Tranter, H. S., & Board, R. G. (1982). The inhibition of vegetative cell outgrowth and division from spores of *Bacillus cereus* T by hen egg albumen. *Journal of Applied Microbiology*, 52(1), 67-73.
- [21] Harvey, A. J., Speksnijder, G., Baugh, L. R., Morris, J. A., & Ivarie, R. (2002). Expression of exogenous protein in the egg white of transgenic chickens. *Nature biotechnology*, 20(4), 396-399.
- [22] Niiler, E. (2000). FDA, researchers consider first transgenic fish. *Nature Biotechnology*, 18(2), 143-143.
- [23] Zbikowska, H. M. (2003). Fish can be first—advances in fish transgenesis for commercial applications. *Transgenic Research*, 12, 379-389.
- [24] Muir, W. M., & Howard, R. D. (1999). Possible ecological risks of transgenic organism release when transgenes affect mating success: sexual selection and the Trojan gene hypothesis. *Proceedings of the National Academy of Sciences*, 96(24), 13853-13856.
- [25] Razak, S. A., Hwang, G. L., Rahman, M. A., & Maclean, N. (1999). Growth performance and gonadal development of growth enhanced transgenic tilapia *Oreochromis niloticus* (L.) following heat-shock-induced triploidy. *Marine Biotechnology*, 1, 533-544.
- [26] Hew, C. L., Fletcher, G. L., & Davies, P. L. (1995). Transgenic salmon: tailoring the genome for food production. *Journal of Fish Biology*, 47, 1-19.
- [27] Du, S. J., Gong, Z., Fletcher, G. L., Shears, M. A., King, M. J., Idler, D. R., & Hew, C. L. (1992). Growth enhancement in transgenic Atlantic salmon by the use of an “all fish” chimeric growth hormone gene construct. *Bio/technology*, 10(2), 176-181.
- [28] Devlin, R. H., Johnsson, J. I., Smailus, D. E., Biagi, C. A., Jönsson, E., & Björnsson, B. T. (1999). Increased ability to compete for food by growth hormone-transgenic coho salmon *Oncorhynchus kisutch* (Walbaum). *Aquaculture Research*, 30(7), 479-482.  
Rahman, M. A., Mak, R., Ayad, H., Smith, A., & Maclean, N. (1998). Expression of a novel piscine growth hormone gene results in growth enhancement in transgenic tilapia (*Oreochromis niloticus*). *Transgenic research*, 7, 357-370.
- [29] Gibson, B., Geertman, J. M., Hittinger, C. T., Krogerus, K., Libkind, D., Louis, E. J., ... & Sampaio, J. P. (2017). New yeasts—new brews: modern approaches to brewing yeast design and development. *FEMS Yeast Research*, 17(4), fox038.
- [30] Ough, C. S. (1976). Ethyl carbamate in fermented beverages and foods. I. Naturally occurring ethylcarbamate. *Journal of agricultural and food chemistry*, 24(2), 323-328.
- [31] Dequin, S. (2001). The potential of genetic engineering for improving brewing, wine-making and baking yeasts. *Applied microbiology and biotechnology*, 56, 577-588.
- [32] Kitamoto, K. A. T. S. U. H. I. K. O., Oda, K., Gomi, K., & Takahashi, K. O. J. I. R. O. (1991). Genetic engineering of a sake yeast producing no urea by successive disruption of arginase gene. *Applied and environmental microbiology*, 57(1), 301-306.
- [33] Gardner, N. J., Savard, T., Obermeier, P., Caldwell, G., & Champagne, C. P. (2001). Selection and characterization of mixed starter cultures for lactic acid fermentation of carrot, cabbage, beet and onion vegetable mixtures. *International journal of food microbiology*, 64(3), 261-275.
- [34] Geisen, R., & Holzapfel, W. H. (1996). Genetically modified starter and protective cultures. *International Journal of Food Microbiology*, 30(3), 315-324.



- [35] [36] Hansen, E. B. (2002). Commercial bacterial starter cultures for fermented foods of the future. *International journal of food microbiology*, 78(1-2), 119-131.
- [36] Gahan, C. G., O'Driscoll, B., & Hill, C. (1996). Acid adaptation of *Listeria monocytogenes* can enhance survival in acidic foods and during milk fermentation. *Applied and environmental microbiology*, 62(9), 3128-3132.
- [37] van de Guchte, M., Kok, J., & Venema, G. (1992). Gene expression in *Lactococcus lactis*. *FEMS microbiology reviews*, 8(2), 73-92.
- [38] Vanderhaegen, B., Neven, H., Coghe, S., Verstrepen, K. J., Derdelinckx, G., & Verachtert, H. (2003). Bioflavoring and beer refermentation. *Applied Microbiology and Biotechnology*, 62, 140-150.
- [39] Armstrong, D. W., & Yamazaki, H. (1986). Natural flavours production: a biotechnological approach. *Trends in Biotechnology*, 4(10), 264-268.
- [40] Cheetham, P. S. J., & Wootton, A. N. (1993). Bioconversion of D-galactose into D-tagatose. *Enzyme and Microbial Technology*, 15(2), 105-108.
- [41] Krings, U., & Berger, R. G. (1998). Biotechnological production of flavours and fragrances. *Applied microbiology and biotechnology*, 49, 1-8.
- [42] Moon, H. J., Jeya, M., Kim, I. W., & Lee, J. K. (2010). Biotechnological production of erythritol and its applications. *Applied microbiology and biotechnology*, 86, 1017-1025.
- [43] Roy, S., Chikkerur, J., Roy, S. C., Dhali, A., Kolte, A. P., Sridhar, M., & Samanta, A. K. (2018). Tagatose as a potential nutraceutical: Production, properties, biological roles, and applications. *Journal of Food Science*, 83(11), 2699-2709.