

12. Genetic modification and biotechnology

Today's topic will focus on genetic modification and biotechnology, the lecture is part of module number 1 - animal genetics, which is part of the ISAGREED project. This presentation was supported by an Erasmus plus KA2 Collaborative Partnership Grant entitled: Innovating the structure and content of the curricula in the field of animal genetic and food resources management using digitisation.

Genetic modifications can be defined as targeted changes in the genetic information of an organism. Using these procedures, genetically modified organisms, also known as GMOs, are created. The most modern approach to genome modification is gene editing. Gene editing is a process that allows highly targeted genetic modification of different parts of the target genome. An example of gene editing is CRISPR/Cas9 which finds applications in gene therapy as well as in food production using precision fermentation.

The presentation will cover the history and origins of these technologies, understanding how their processes work and how they can be used in food production.

Gene editing procedures were first described in 1979 when a stretch of DNA was replaced by a synthetic stretch of DNA. DNA editing methods have evolved from editing by Zinc-finger proteins binding to DNA through HDR and NHEJ technology targeting both homologous and non-homologous recombination. In 2012, separate tracks of gene editing research using effectors similar to TALE transcriptional activators merged with research on the bacterial immune system to implement the CRISPR-Cas9 RNA-guided DNA endonuclease technology that is currently revolutionizing gene editing.

The CRISPR repeat spacer array is part of the CRISPR-Cas system, which serves as a defense against viruses and other foreign DNA. The CRISPR-Cas system consists of two main parts: the CRISPR array and the Cas proteins. The CRISPR array contains spacers (short stretches of DNA) that are separated by repeats. These spacers are stored in the cell's memory space and serve as a record of previous infections by viruses or foreign DNA. Cas proteins can recognize the spacers and subsequently destroy the virus or foreign DNA.

TracrRNA is a small RNA molecule that binds to CRISPR RNA (crRNA) and Cas proteins. TracrRNA, together with RNase III, cleaves crRNA into small fragments to form single-guide RNA (sgRNA), which binds to Cas proteins and allows them to recognize the target DNA sequence.

For the formation of a functional editing unit, the Cas9 molecule is required to associate with the gRNA guidance system. The complex targets the recognition of a PAM sequence adjacent to the target sequence triggering the recognition process of the target DNA sequence by the gRNA guide molecule.

If the Cas9 and gRNA complex recognises the complementary sequence following the PAM stretch, the DNA strand is unwound and the nucleases are activated. If there is a mismatch between the DNA strand and the gRNA guide sequence, the process does not proceed.

In the case of nuclease activation, the HNH and RuvC domains of the Cas9 system are activated to form a break on the DNA double strand, at a specific site guided by the gRNA sequence. The resulting situation can be exploited in several ways.

The CRISPR/Cas9 system itself causes DNA strands to break at the site where the change is to be made. Once the DNA strands are broken, a donor fragment with the desired sequence is delivered into the cell. This donor fragment is then used to repair the broken DNA strands. With the HDR system, homologous recombination is used to repair the broken DNA strands. Using the NHEJ system, the broken DNA strands are joined together using protein factors.

These proteins bind to the broken DNA strands and join them together. NHEJ protein factors are able to repair broken DNA strands without using homologous recombination.

The use of the CRISPR-Cas9 system is not only in creating changes on the DNA strand but also in changing the expression of a specific gene, for example.

The SAM (Synergistic Activation Mediator) system is a protein complex that is used to increase transcriptional activity. The SAM system consists of a nuclease-dead SpCas9 (dCas9) and an RNA guide as a carrier to transport SAM effector domains to target gene promoters. This system enables site-specific transcriptional activation of the gene.

The future use of gene editing is in many directions. In addition to the development of new species of plants and animals and radical ecological interventions such as the sterilization of mosquitoes already implemented, we can identify the following research directions.

CRISPR-Cas9 can be used to identify new target proteins for drugs. This technology allows researchers to precisely alter or delete genes in cells and monitor how these changes manifest themselves at the cellular level. In this way, researchers can identify new target proteins for drugs and develop new drugs to treat different diseases.

Use of CRISPR-Cas9 for gene therapy applications, where the system can be deployed directly "IN VIVO" and can alter the causal mutant allele in target genes in real time to restore the production of functional gene products in a variety of genetically determined diseases.

In the field of synthetic biology, CRISPR-Cas9 technology provides the ability to modify metabolic pathways to bring cells to a specific state or to promote their growth beyond in vitro conditions.

Cell based food is a type of food that is made from cells isolated from animals and grown in a laboratory. The aim is to create a product that is similar to traditional meat, poultry, fish, milk or eggs. This technology is seen as a possible alternative to intensive animal farming, which may pose an ethical or environmental problem in the future.

The cell selection procedure for cell-based food depends on the type of product to be created. Typically, animal cells that have the ability to proliferate and differentiate into the desired tissues, such as muscle or fat cells, are selected. Cells can be obtained from a variety of sources such as biopsies, embryonic cells, stem cells or engineered cells.

The next step is cell production. The cell proliferation process in cell-based meat production involves placing selected animal cells in bioreactors, where they are supplied with nutrients and growth factors to promote cell division and growth. Cell proliferation can take several days or weeks, depending on the cell type and growing conditions. Once the desired number of cells

has been reached, the cells can differentiate into muscle or adipose tissues, which form the basis of cell-based meat. Cell proliferation is one of the most costly and complex phases of cell-based meat production, and methods are being developed to optimise and streamline it.

Harvesting in the cell-based food process is the stage where cells or tissues are removed from the bioreactors and processed into the final product. Harvesting can vary depending on the type of cell-based food being produced. For example, in cell-based meat, cells or tissues may be harvested manually or mechanically, while strict hygiene and safety standards must be observed. Harvesting may also involve additional steps such as cleaning, sterilisation, freezing, packaging or combining with other ingredients. Harvesting is subject to regulation by the competent authorities that control the quality and safety of cell-based food.

The last step of production is the formulation of the product. This is the stage where the cells or tissues are processed into a final product that has the desired organoleptic and nutritional properties. This process may involve different steps such as freezing, drying, fermentation, extrusion, 3D printing or the addition of other ingredients such as vegetable proteins, fibre, colourings or flavourings. For example, in cell-based meat, 3D printing can be used to create a muscle-like structure and texture. The formulation of the product affects the quality, shelf life, safety and acceptability of the product.

The process of preparing cell based meat can also be graphically illustrated using the following diagram.

The first step is to biopsy the muscle from live animals or via biopsy. The cells are sorted into muscle, connective tissue and adipose cells as appropriate. A separate step is the immortalization process.

The immortalization process in cell-based food production is a process that allows cells to grow and divide indefinitely under laboratory conditions. This process is necessary to obtain enough cells for food production. There are several ways to achieve cell immortalisation, for example by using telomerase, oncogenes or viruses. Each method has its advantages and disadvantages in terms of efficacy, safety and ethics.

Placing the cells in a bioreactor ensures oscillating movement of the medium in the chamber to ensure an even distribution of nutrients and oxygen among the cells. The bioreactor also mimics the physiological conditions of tissues, such as pressure, tension and strain, which stimulate cell growth and differentiation.

The selection of the scaffold in the cell-based meat production process is an important step that affects the morphology, function, and quality of the meat produced.

A scaffold is a three-dimensional structure that provides support and stimulation to cells for growth and differentiation in a directed tissue arrangement that can, in some cases, take the shape of the final product.

Scaffold must meet several criteria such as biocompatibility, edibility, nutritional value, texture, porosity, mechanical properties, degradability and low cost. There are many types of scaffolds, which vary according to material, shape and method of preparation. Some of them are created by 3D printing technology, as shown in the figure, using different materials and technologies.

Textured Soy Protein (TSP) is an edible protein material derived from soybeans that has a porous texture and a fleshy texture. TSP can promote the growth and differentiation of muscle cells and form 3D tissue without the need for additional growth factors.

Cell sheet technology is a technique that allows the creation of scaffold-free cell-based meat using temperature-sensitive culture dishes. Cells are attached to the dish at 37°C and released at 32°C as a thin layer. Multiple layers of cells are then stacked on top of each other to form a 3D tissue with its own extracellular matrix.

Microcarriers are small particles of different materials (e.g. collagen, alginate, gelatin) that serve as a substrate for the cells. Microcarriers can be used to increase cell density in bioreactors or to form 3D tissues through aggregation or weaving.

The texture of the final cell-based meat products depends on the cell type, growing method and processing. Some products are made from a single layer of cells that have a thin and soft consistency suitable for meat slices, nuggets or sausages. Other products are made from multiple layers of cells that form a three-dimensional tissue with a texture similar to muscle fibres. These products have a tougher and more fibrous texture, suitable for steak.

The final cell-based meat products can also be modified by adding vegetable proteins, fats, colours or flavourings to achieve the desired taste, flavour and appearance. Some products can be cooked, fried or grilled in the same way as traditional meat.

The final cell-based meat products are still in the research and development phase and are not available to the general public. Currently, there are only a few approved products that have received regulatory approval for sale to consumers. In Singapore, these are chicken pieces from Eat Just, which are composed of 70% cultured chicken cells and 30% plant-based proteins.