THE POTENTIAL FOR USING MICROORGANISMS IN FOOD BIOTECHNOLOGY

BONCIU ELENA[1*], ROSCULETE ELENA[1], OLARU AUREL LIVIU[1], ROSCULETE CATALIN AURELIAN[1]

[1]University of Craiova, Faculty of Agronomy, 19 Libertatii Street, Craiova, Romania

*Corresponding author email: elena.agro@gmail.com

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ABSTRACT

Microorganisms are complex systems, mono or multicellular, with prokaryotic or eukaryotic cells, endowed with their own metabolism and genetic continuity, very diverse in shape, size and metabolic activity. The microorganisms used in biotechnologies are known as useful microorganisms, used in the form of starter cultures for fermentation processes which are the basis for the development of the food industry: fermentative yeasts used in the beer production, wine, ethyl alcohol, bakery; lactic and propionic bacteria used in the dairy industry and in food preservation, etc.

The agri-food field is one of the great beneficiaries of the development of biotechnology, being able to provide in the near future most of the raw materials needed for human food. Technological processes for obtaining food, with some exceptions (sugar industry, oil plants industry, milling industry), are biotechnologies that are based on the use of microorganisms or their metabolites.

INTRODUCTION

Biotechnology includes a wide range of diverse technologies and they may be applied in each of the different food and agriculture sectors. It includes technologies such as gene modification (manipulation) and transfer; the use of molecular markers; in-vitro vegetative propagation of plants; embryo transfer and other reproductive technologies in animals or triploidisation in fish. It also includes a range of technologies used to process the raw food materials.

Microorganisms are an integral part of the processing system during the production of fermented foods. Microbial cultures can be genetically improved using both traditional and molecular approaches, and improvement of bacteria, yeasts and moulds is the subject of much academic and industrial research. Traits which have been considered for commercial food applications in both developed and developing countries include sensorial quality (flavour, aroma, visual appearance, texture and consistency), virus (bacteriophage) resistance in the case of dairy fermentations, and the ability to produce antimicrobial compounds (e.g. bacteriocins, hydrogen peroxide) for the inhibition of undesirable microorganisms. In many developing countries, the focus is on the degradation or inactivation of natural toxins (e.g. cyanogenic glucosides in cassava), mycotoxins (in cereal fermentations) and anti-nutritional factors (e.g. phytates). In agriculture but also food industry, it is a particular interest for those microorganisms with potentially serious implications for food security (Cotuna et al., 2015; Paraschivu et al., 2013; Paraschivu et al., 2015; Paraschivu et al., 2017).

Biotechnologies can be divided into traditional biotechnologies and modern biotechnologies (Bonciu, 2012; Briggle, 2012).

Traditional biotechnologies have a long history (starting with 6000 BC; they are based on traditional techniques of cultivation of microorganisms and do not exclude the use of modern techniques (DNA cloning, cell cultures, etc.). On the other hand, modern biotechnologies have a shorter history (about 50-100 years) and use modern methods (recombinant DNA technique, cell cultures, etc.).

Modern biotechnologies have a vast field of exploitation in modern industry (Olaru et al., 2020; Rosculete et al., 2019; Salceanu and Olaru, 2016, 2017). The world's production of exogenous enzymes, used in almost all industries, the synthesis of essential amino acids by involving bacteria, the production of alcohol used as a substitute for oil or the production of biomass by capitalizing on the fermentation of fast-growing plants.

Private and public investments in biotechnology are typically aimed at five broad goals, namely improvements in (1) food production (agricultural and food biotechnology), (2) environmental health (environmental biotechnology), (3) materials production (industrial biotechnology), (4) human health (biomedical technology), and (5) security and national defense (Briggle, 2012).

MATERIAL AND METHOD

This is a review about microorganisms used in food biotechnologies. Microorganisms are generic terms for the group of living organisms which are microscopic in size, and include bacteria, yeasts and moulds.

Once a microorganism has been selected, either by classical methods or by the use of molecular modification and screening techniques is necessary its cultivation under conditions which ensure the expression of specific characteristics, practically useful. In this way, the use of a microorganism in modern biotechnology is based on the classical principles of cultures microbial, known and elaborated for a long time.

RESULTS AND DISCUSSIONS

Knowing the particularities of microorganisms allows the development of applications in various fields of agriculture, food industry, environmental protection, etc. Biotechnology has long been identified with industrial microbiology, including aspects of culturing microorganisms only, preservation, improvement of the conventional method (mutation and selection of mutants of interest) and their practical use for the production of specific products (Bonciu 2012; Bonciu et al., 2020). Subsequently, the field of biotechnology has expanded, including plants, animals, viruses and cell cultures as well as recombinant DNA technology applied for industrial purposes. Of the more than 100,000 species of prokaryotic and eukaryotic microorganisms currently known, only a few hundred species are used to produce useful substances.

The implementation of advanced biotechnologies in the food industry aims to achieve the following objectives:

- obtaining safe and quality foods, probiotic and nutraceutical foods;
- making food bio products with a therapeutic role for different target segments of consumers (children, the elderly, hypertensive people, diabetics) but also some foods with a role in preventing diseases or usable as adjuvants in the treatment of diseases;
- obtaining food ingredients and additives through biotechnological processes;
- creating less energy-consuming biotechnologies than classical technologies;
- greening of the technological processes of the food industry by using advanced biotechnologies.

Biotechnology in the food processing sector targets the selection and improvement of microorganisms with the objectives of improving process control, yields and efficiency as well as the quality, safety and consistency of bioprocessed products (Bonciu, 2017; Musa Maryam et al., 2017).

Numerous biotechnologies based on the activity of microorganisms have been known since antiquity and used in the manufacture of bread, beer, wine, vinegar, cheese, etc.

Currently, with the help of microorganisms are obtained over 200 products on an industrial scale, only by microbial way:

- alcohols: ethyl, methyl, butyl, isopropyl;
- acids: citric, lactic, gluconic, acetic;
- proteins, amino acids;
- enzymes, vitamins;
- antibiotics, biological pesticides;
- hormones, interferon, insulin, etc.

The use of microorganisms in biotechnologies has many advantages: they grow quickly and can be easily grown in large quantities on media containing the appropriate organic substances; have the ability to constantly maintain their physiological properties, under certain culture conditions, producing easily and in large quantities, the enzymes necessary to transform the substrate in the desired direction; makes these changes, with the formation of useful products, in relatively simple and inexpensive conditions.

Fermentation is the process of bioconversion of organic substances by microorganisms and/or enzymes (complex proteins) of microbial, plant or animal origin. It is one of the oldest forms of food preservation which is applied globally. Indigenous fermented foods such as bread, cheese and wine, have been prepared and consumed for thousands of years and are strongly linked to culture and tradition, especially in rural households and village communities. It is estimated that fermented foods contribute to about one-third of the diet worldwide (http://www.fao.org/biotech/c11doc.htm).

The industrial production of enzymes from microorganisms involves culturing the microorganisms in huge tanks where enzymes are secreted into the fermentation medium as metabolites of microbial activity. Enzymes thus produced are extracted, purified and used as processing aids in the food industry and for other applications. Purified enzymes are cell free entities and do not contain any other macromolecules such as DNA.

Genetic technologies have not only improved the efficiency with which enzymes can be produced, but they have increased their availability, reduced their cost and improved their quality. This has had the beneficial impact of increasing efficiency and streamlining processes which employ the use of enzymes as processing aids in the food industry (http://www.fao.org/biotech/c11doc.htm).

Biotechnology in the production of enzymes bring desirable changes in food and a range of other high value-added products: genetically modified starter cultures, genetically modified foods. (Bessin, 2000). The rapidly increasing availability of genomic sequence information for many organisms, including food-related bacteria, yeasts, and molds, allowed for the introduction of large-scale proteomic technologies to identify the majority of proteins that a microbial cell synthesizes. In food biotechnology, proteomics is used for bioprocess improvement, validation, and quality control.

Fermentation is one of the oldest methods of food preservation, bringing together the art of traditional practices with modern scientific understanding and development. They contribute great flavor, nutritional value, and variety to diets. Fermented foods are foods that have been subjected to the action of microorganisms or enzymes. They constitute about one-third of our diet and originated several 1000 years ago, when microorganisms were introduced incidentally into local foods (Campbell-Platt, 2014).

Yeasts are single-celled organisms of the eukaryotic type that multiply by mitosis (budding) or meiosis (sporulation) and have as their main characteristic the quality of producing the fermentation of simple sugars with anaerobic formation of

ethyl alcohol and carbon dioxide. Other advantages induced by the use of yeasts in food biotechnologies:

- have a very important role and are used in the food industry in the manufacture of wine, beer, fermentation spirit, bread and derived products, compressed yeast;
- have a valuable chemical composition and are used in industrial microbiology to obtain proteins that can be used in human nutrition (type SCP - Single Cell Protein), or to obtain feed yeasts for animal feed, yeasts containing 5.5% protein, B vitamins, amino acids;
- may lead to lysed, plasmolysed extracts, used as food additives or which enrich the culture media intended for the cultivation of selected microorganisms.

During fermentation processes, microbial growth and metabolism (the biochemical processes whereby complex substances and food are broken down into simple substances) result in the production of a diversity of metabolites (products of the metabolism of these complex substances). These metabolites include enzymes which are capable of breaking down carbohydrates, proteins and lipids present within the substrate and/or fermentation medium; vitamins; antimicrobial compounds, organic acids (e.g. citric acid, lactic acid) and flavour compounds (http://www.fao.org/biotech).

Some enzymes such as such as superoxide dismutase, ascorbate peroxidase, etc., are useful for protection of the cell systems of plants from the cytotoxic effects of the active radicals. (Babeanu et al., 2008; Marinescu et al., 1999; Soare et al., 2017).

The field in which microbiology as a science most influences social development is the food industry (Baltz et al., 2010). Microorganisms play a very important role at this level, being used as food agents for the production of wine, cheese, bread, beer, etc. (Figure 1 and Table 1).

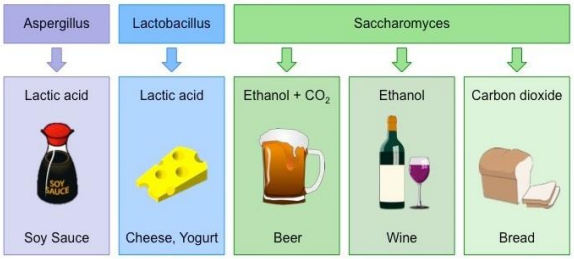

Figure 1. Some products of fermentation (http://www.old-ib.bioninja.com.au/)

Thus, the primary function of *Saccharomyces* in bread-making is the production of carbon dioxide to make the dough rise.

Table 1

The potential of different groups of microorganisms to obtain useful substances

Group	Species	Products obtained (e.g)
Bacteria	*Methylophilus methylotrophus* *Acetobacter sp.* *Lactobacillus bulgaricus* *Propionibacterium sp.* *Streptococcus sp.* and *Lactobacillus sp.* *Pseudomonas denitrificans*	Monocellular proteins Vinegar Yogurt Pressed cheese Dairy products Vitamin B12
Yeast	*Saccharomyces cerevisiae* *Sacch. carlsbergensis* *Eremothecium ashbyii*	Bread, wine, Light beer Vitamin B2
Molds	*Aspergillus sp.* *Trichoderma reesii* *Aspergillus niger* *Penicillum roquefortii* *Penicillum camambertii*	Pectinases, Proteases Enzymes Organic acids Roquefort type cheese Camembert cheese
Algae	Spirulina sp. Chlorella sp. Scendesmus sp.	Single cell proteins, Carotene, Pigments

Yeast acts on sugars in the dough, breaking them down by fermentation to make carbon dioxide and ethanol. When the bread has risen to a desired height, the bread is baked in an oven to kill the yeast and evaporate the ethanol.

In wine production, yeast is added to crushed grapes and put into a tank, when the oxygen is consumed aerobically, fermentation occurs. Carbon dioxide escapes from the tank while the ethanol stays behind. The yeast cells are not killed by heat as this would affect the taste (may be killed later by the concentration of ethanol). Different strains of yeast produce different flavours and the strains used are capable of withstanding higher concentrations of alcohol (>10 – 15%).

In beer production, the barley grains are wetted in order to cause germination, which triggers the breakdown of starch to maltose – making a liquid called malt. More water is added to make a sweeter-tasting liquid called wort, and then hops are added to give the liquid a bitter taste. The mixture is boiled and cooled before yeast is added, which breaks down the maltose into glucose. Fermentation by yeast produces ethanol and carbon dioxide, and the beer is finally pasteurised (heated) to kill any remaining yeast cells.

Taxonomists have classified algae according to their colour - green, red, blue, brown - and most include pigments such as chlorophyll a, b and c, β-carotene, phycocyanin, xanthophyll and phycoerythrin. All these pigments have a great potential for use in the food industry, but also in pharmaceuticals or cosmetics (Dufossé et al., 2005).

By combining the genes of microorganisms with different properties, the researchers obtained new types of carotenoid pigments, which are not found in nature, at least until now. These pigments are the multi-hydroxylated ones and are considered to be very effective as antioxidants, in terms of human consumption.

The pink-fluorescent food dye is obtained from the red microalgae *Porphyridium phycobiliproteins*. Extracts from this organism are of great therapeutic importance, containing polysaccharides with anti-inflammatory and antiviral properties, long-chain unsaturated fatty acids, carotenoids and fluorescent phycobiliproteins. The color navy blue can be obtained as a food dye from the red microalgae *Porphyridiump hycocyanin*, which also contains sulfanated polysaccharides, carotenoid pigments and lipids.

On the other hand, the food industry is not based solely on appearance, so some researchers have turned their attention to studying how microorganisms are involved in determining the taste of food. The most important food - bread - is based on a microbial ingredient - yeast. The fermentation process of the dough includes different species of microorganisms, both yeast and lactic acid bacteria, which are responsible for the taste of the final product. The way these microorganisms metabolize carbohydrates is the most important factor that determines the taste of bread.

In principle, there are two types of components that are responsible for the taste of bakery products: the first category includes non-volatile components (organic acids produced by heterofermentative bacteria), which have the role of acidifying the environment, lowering the pH and thus conferring a specific taste; the second category is volatile components and includes alcohols, aldehydes, ketones, esters and sulfur-based chemical species. All these products are obtained during fermentation and help define the taste of the dough (Rehman-ur-Salim, 2006). It should be noted, however, that the type of flour used to obtain the dough has a significant role in creating taste, through the way microorganisms interact with its components.

Currently, the use of microbial organisms in the food industry and beyond has led to the development of technologies to discover and enhance their potential. The purpose of food biotechnology is to discover new sources of nutrients, but especially methods by which these sources can become available to the energy circuit. In this sense, microorganisms have a very

important role, most having the ability to reintegrate into the circuit of an ecosystem the substances that result in the energy needed to maintain it. Food biotechnologies can intervene in these types of processes to boost the metabolisation of certain compounds with the formation of others.

The importance of microorganisms in the original microbiota as well as in the contamination is decisive both for ensuring the quality of food and for their safety, respectively for the consumer (Belyaev et al., 2004). Therefore, for controlling their use in the interest of technological management it is imperative to acquire all the information on the necessary conditions and essential influencing factors in their biology (Avram, 2008).

Through bioengineering and biotechnologies, with the help of yeasts valuable substances can be obtained such as: interferon, with cytostatic and antiviral effect; B vitamins, with complex therapeutic effect. Animal husbandry also benefits from the modern biotechnologies (Cola M. and Cola F., 2018; Cola M. and Cola F., 2019). Improving some production characteristics or some quality determinations in the meat or milk industry uses many biotechnological processes (Cola F. and Cola M., 2018; Cola M. and Cola F., 2019).

CONCLUSIONS

Traditional biotechnologies are used successfully in the food industry. At their base are the fermentation processes, the decomposition of organic substances (carbohydrates, organic acids, alcohols, amino acids, nitrogenous bases) under aerobic or anaerobic conditions and the formation of intermediate products (lactic acid, acetic acid, butyric acid, formic acid, ethanol, butanol, propanol, acetone).

Different types of microorganisms: yeasts (alcoholic fermentation), lactic acid bacteria (lactic fermentation), butyric bacteria (butyric fermentation), propionic bacteria (propionic fermentation) are involved in the fermentation process and they have a great potential for use in food biotechnology. Thus, microorganisms are used in dairy processing and fermentation cheese, wine and beer production, bakery, vinegar and juice production, etc.

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