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IMPROVING TRAINING IN FOOD MICROBIOLOGY

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Abstract

Microbiology is a fundamental discipline in university programs aimed at training specialists - microbiologists of a wide profile. The main goal of any of these programs is to find an appropriate balance between the basic concepts of microbiology and the practical skills necessary for the work of specialists, including professionals in the food industry.

Training also includes requirements for the organization of a laboratory for microbiological work, quality control of food and raw materials in accordance with regulatory standards, including sampling, the use of traditional and new rapid detection methods, identification and quantification of dangerous microbes in samples. Particular attention is paid to control methods the presence of antibiotics in foods that cause multiple drug resistance of pathogens, ways to reduce microbiological hazards in food products. On the other hand, with increasing knowledge of probiotics, reliable results of the content of useful microbiota in products are needed. Students, masters, as well as professionals to improve their skills through the International Center for Biotechnology at Moscow State University take part in the training. Educational and methodical manuals on methods of microbiological work, detection of dangerous infections, colonizing products, permissible levels of their presence in the product, state quality standards have been published.

Over the past decade, most microbiology courses in the field of food have developed, quality control methods have been improved, in addition to basic knowledge of the microbiology of food, critical thinking skills have been acquired in solving safety problems, as well as in the creation of biologics and food additives that enhance biological value for the benefit of human health.

***Key words**: Methods of control, Food safety, Training of food microbiologists.*

1. Introduction

Food safety is a task that humanity is trying to solve throughout the historical period. At all stages of his development, a person was closely connected with the surrounding world. In the 4th century BC, Hippocrates in the in the "Diet" wrote: "Our food should be a medicine, and the medicine should be food". However, cooking, preserving food with the help of microorganisms and damaging it are two sides of one process. In 1857, Pasteur opened a new era in food microbiology, scientifically proving that it is microorganisms that cause food spoilage and indirect infectious diseases of humans and animals. But since the emergence of a highly industrialized society, the dangerous interference of man in nature has sharply increased, it has become more diverse and now threatens to become a global danger for all mankind. According to the concept created by the founders of microbiology Louis Pasteur and Robert Koch, infection underlies some diseases, and therefore microbes are something to be combated. However, in addition to really dangerous microorganisms, we are constantly accompanied by those who help us live, be strong and healthy (the teaching of the Nobel Prize winner Élie I. Metchnikoff). As early as 1903, Metchnikoff proposed to use microbial antagonist cultures to combat pathogens [6].

Nutrition is one of the most important interfaces between a person and the external environment. The use of food should not lead to food poisoning, and the products themselves should not contain hazardous ingredients. Ensuring the safety of food raw materials and food products is one of the main trends determining the health of the population and the preservation of its gene pool. In this regard, the introduction of a system for ensuring food safety problem became a challenging task in recent years. In 2005, the International Standard Organization (ISO) established the standard ISO, 2200 "Food Safety Management Systems. Requirements" and in 2013 was established "Certification

scheme for food safety systems" in compliance with ISO 2000:2005 and technical specifications for sector PRPs. The Russian version of this standard is GOST R ISO 2000-2007 [4]. It ensured the unification of requirements for HACCP systems and their convergence with the requirements of other standards for management systems.

Recent years have seen a revolution in views regarding the role of microbes, which has remained unnoticed to general public. In the coming third millennium, the constant impact on the human body of environmentally unfavorable environmental factors, radiation effects, industrial poisons, psycho-emotional overloads lead to a worsening of the epidemiological situation, an increase in the number of infectious, cardiovascular and nervous diseases [2, 3, and 15]. The excessive and sometimes uncontrolled use of chemotherapeutic drugs, including antibiotics, is accompanied by a decrease in immunity, leading to a prolonged nature of recovery. The solution to this problem is to create probiotics - drugs based on living microorganisms that suppress the growth and development of pathogenic microbiota [16].

The gastrointestinal tract (GIT) of a person acts as a link between nutrition and all other physiological functions of the body. When the GIT is enriched with "useful" microbes, healing begins from within. Microbiocenosis of the intestine is the center of the microecological system of humans. In the human body, about 100 trillion microorganisms live, the number of which is 13 times higher than the number of cells in the human body [1, 14], and their mass is about 2 - 3% of body weight. A healthy human microbiota is 85 - 98% consists of lactic acid bacteria [15]. Intestine is an additional endocrine organ. Probiotics are taken as a healthy population as prevention, and for the treatment of various diseases of the gastrointestinal tract (GIT), such as diarrhea, including antibiotic-associated diarrhea, obesity. The changes in the microbial community of the intestine are most closely associated with mood disorders: depression and anxiety disorder. Probiotics that can alleviate the condition of patients suffering from mental illness, such as: anxiety, depression, chronic fatigue, autistic spectrum disorders, and eating disorders, are called psychobiotic [3, 18]. Food products are common sources of probiotics (Figure 1). Functional food products (FFPs) with probiotic microorganisms, in particular lactic acid bacteria are defined by the Russian standard as containing not less than 5×10^7 colony forming units of a probiotic in a daily dose [8].

According to Russian data, probiotic milk products account for about 10% of the total volume of production of fermented milk products [10].

Lactic acid bacteria have a long history of use in the preservation of food and feed. We have all probably

Figure 1. Food products as sources of probiotics: 1 - Lebanese Leben and Laban; 2 - Fermented carrots; 3 - Kurunga (Ulan-Ude, Buryatia) and Acidophilus; 4 - Kefir, Sour cream, Curdled milk, Cottage cheese (Russia); 5 - Mature cheeses; 6 - Sauerkraut

eaten them several times, since they are present in cheese, yoghurt, sour milk and on fruits. The microorganisms are harmless to humans and animals and have generally recognized as safe (GRAS) status. Species of *Lactococcus* are closely associated with food centuries as starter cultures in the food industry and some strains consist a human symbiotic microbiota. LAB are cultures of strategic importance to the nation's health.

In the modern society, food security is a growing problem for many consumers. The concept of rational and balanced nutrition, which contributes to increasing cultural consumption and balancing reasonable human needs with environmental needs, requires an increase in the range of food products in order to enhance their safety and biological value and to improve quality control methods. The main tasks of the state policy in the field of healthy nutrition are:

- Development and introduction in the food industry of innovative.
- Technologies, including bio- and nanotechnologies.
- Improvement of quality control mechanisms of food products and food raw materials produced on the territory of the Russian Federation and imported from abroad.
- Development of educational programs for various groups of the population on healthy nutrition.
- Training of specialists for work on quality control of products.

The President Decree of May 7, 2012 "Strategy of improving the quality of food products in the Russian Federation until 2030" was issued on priority: creating conditions for the production of new products with given quality characteristics, promoting healthy nutrition principles. The introduction to this paper highlights the urgency of the topic and shows the need for a structural modernization of teaching for the formation of a systematic knowledge of food microbiology for training experts in this important field.

2. Improving training in food microbiology

2.1 History of the establishment of the Department of Microbiology at the Lomonosov Moscow State University

The Department of Microbiology was established in 1924 on the basis of the Department of Plant Physiology at the Biological Department at the Physics and Mathematics Faculty of the Moscow State University. Its founder is Professor Eugene E. Uspensky (1889 - 1941), a major specialist in the field of physiology of microorganisms, algology, soil, and water microbiology. Professor Uspensky developed the structure of the educational process at the department, organized practical classes in microbiology, and industrial practice for students.

During the period of 1938 - 1967 the Chair was headed by Professor, Member of the USSR Academy of Sciences Vladimir N. Shaposhnikov, and the founder of technical microbiology in the USSR, which includes all aspects of the industrial use of microbes. He was the first to formulate the theory of biphasic development of fermenting bacterial cultures. Knowledge of the laws of development and growth of cultures of microorganisms made it possible to control the course of many microbiological processes, including those used in biotechnology for the creation of food products. Professor Shaposhnikov was the organizer of a number of industrial microbiological productions: lactic, acetic and butyric acids, acetone and butanol, etc. Names of Eugene Uspensky and Vladimir Shaposhnikov are associated with the formation and development of a system of teaching microbiology at Moscow State University, and the establishment of microbiologist schools that largely determined the development of microbiology in our country. Since 1947, the department started to work on the producers of certain antibiotics physiology, which production was just beginning in the country. The scientific research in this field was headed by Professor Nikolai S. Egorov, Honored Scientist of the USSR. Significant changes have occurred in the educational process.

2.2 Program for teaching students in microbiology

Microbiology is assigned a special role in the system of natural sciences and in general in the life of society in connection with the problems of food and energy resources, obtaining drugs for: medicine, food industry, agriculture, mining and environmental protection. The extensive development of the education sector includes both traditional and a modern approaches. Department trains students specializing in the subjects' microbiology, and microbiology and biotechnology of microorganisms. Teaching program includes lectures on: general and industrial microbiology, cytology, physiology, biochemistry, genetics and ecology of microorganisms, molecular biology, electron microscopy, and taxonomy.

The curriculum also includes practical exercises and works in the microbiology workshop. Development of new research methods made it possible to significantly expand the range of tasks of the microbiology workshop, including continuous cultivation, directed synthesis of antibiotics, the formation of proteolytic and amylolytic enzymes by microorganisms, etc. Also works are carried out related to the protection of the environment from industrial waste pollution, food safety, the original methods of obtaining a number of enzymes, amino acids, biopreservatives and therapeutic and prophylactic preparations protected by copyright certificates and patents.

Teachers of the Department of Microbiology, are also the employees of the Russian Academy of Sciences Institute of Microbiology and leading Russian institutes of Biology Science. For a number of years, the students of the Department have also been acquainted with microbiology practice in leading scientific institutes in Germany, France and the Netherlands. Work on the title of Bachelor and Master on a variety of topics are performed on the basis of the Department, as well as various research institutes and laboratories of the world.

The teachers prepared 4 separate courses of lectures on general microbiology and conducted a comprehensive laboratory work for students of all specialties of the biological profile of Moscow State University within the framework of a general microbiological program (for more than 300 students per year), cooperating with the International Biotechnology Center for the improvement of professional skills of microbiologists for the food industry with the issuance of a certificate.

Department of Microbiology conducts a variety of educational work with the Department of Advanced Education skills at Moscow State University, with the classes with students of other institutions of Moscow, and schoolchildren. Employees popularize achievements in the field of microbiology at seminars, on radio and television. During its existence, the department has trained more than 1,500 qualified experts who work in Russia and around the world (European, African and Latin American countries, Bangladesh, India, Iran, and China). Former graduate students, graduates, masters became major scientists, organizers of science, and experts in food production too.

2.3 Specialization in food microbiology

The training program includes basic knowledge in the field of microbiology, national food safety standards for the control of the food and raw materials quality, food microbial contamination, presence of microor-

Table 1. Microbiology training program for bachelors

Name of lecture modules	Main tasks	Hours
Industrial microbiology	Physiology of individual groups of microorganisms. Regulation of the metabolism of microorganisms. Scheme of typical microbiological production.	550
Microbiology with the basics of biotechnology	Relationship of biotechnology with other biological disciplines. Methods of cultivation (processes and apparatus) in biotechnology. Cellular and molecular levels of biotechnology. Methods of Genetics and Molecular Biology in Biotechnology.	60
Fundamentals of microbiological production	Raw materials, modern technological equipment and stages of the technological process. Preparation of culture, cultivation and allocation of the target product. Obtaining microbial biomass. Extracellular and intracellular purified metabolites.	48
Food safety	Students are trained in the analysis of the food products and food raw materials, study separate groups of bacteria that colonize food products and contribute to problems associated with the presence of opportunistic pathogens, master the methods of their cultivation on special diagnostic environments.	60
Special course	Selected sections of biochemistry. Laboratory management and safety engineering of microbiological research Cytology and functional morphology of microorganisms. Ecology of microorganisms Molecular microbiology. Actual problems of microbiology	24 12 24 36 24 12
Practical work in the field of food microbiology	The goal of the task is to master the methods of working with microorganisms. their identification and regulation of the synthesis of metabolites	60
Performing an independent qualification work for obtaining a bachelor's degree on selected topics	Obtaining uncultivated forms of bacteria. Quality control of products and raw materials. Bacteriocins as biopreservatives Probiotics Bacterial toxins.	60

ganisms that are harmful to health and the availability of beneficial microorganisms that increase the biological value of products. Following new special courses on food microbiology were included in the training program for students: "Physiology of individual groups of microorganisms", "Regulation of the metabolism of microorganisms", "Biotechnology and microbiology industry", "Ecology of microorganisms", "Food safety", "Bacteriocins as biopreservatives", "Probiotics", and "Bacterial toxins".

Duration of the course of lectures on Industrial Microbiology for students of the 4th year (bachelors) is 550 hours, and lectures on special courses - 60 hours. Actual problems of modern microbiology are given for masters and graduate students as an option Lectures are supplemented with visual illustrations that help students to better learn the material, and with an intention to cause interest in learning. Theoretical courses are supplemented by laboratory practice.

Students are also trained in the sanitary-bacteriological analysis of the environment, food products and food raw materials. In classes, students mainly study individual groups of bacteria that colonize foods and contribute to problems associated with the presence of opportunistic pathogens in the bacterial population, and master the methods of their cultivation on special diagnostic media.

2.3.1 General training in bachelor's degree

Preparation of microbiologists begins with the course "Microbiology with the basics of biotechnology" includes 60 lecture hours and 60 hours of practical training (Table 1).

The purpose of this course is to introduce students to the unique world of prokaryotic microorganisms (bacteria and archaea). The tasks of the discipline are to form the students' understanding of the:

- Cytological and morphological features of prokaryotes.
- Diversity of prokaryotic metabolism.
- Distribution of prokaryotes in nature and the role of these organisms in global physico-chemical, climatic and ecological processes.
- Microorganisms in human practical activities: biotechnology, the impact on human health of its internal microbiota and microbiota of the human environment.

Thus, during the general course, the bachelors get acquainted with the problems and directions of development of biotechnology, in particular, food microbiological technologies. The graduate and post-graduate studies training in the field of food microbiology is carried out during lecture special courses and practical classes.

The main course in this direction is the lecture special course "Biotechnology and Industrial Microbiology" - 48 hours a week. The aim of the course is to develop deep knowledge in the field of microorganism biotechnology, or more precisely to:

- Increase the ability to reasonably apply knowledge in the field of molecular microbiology in order to obtain new food products; Master the analysis skills in order to solve the original problems of microorganism biotechnology;
- Improve the ability to critically evaluate and interpret the achievements of theory and practice in the field of microbial biotechnology;
- Promote innovations in the specialized field of activity.
- Perform quality control of products and raw materials including: bacterial contamination (permissible levels), presence of opportunistic pathogens, content of residual quantities of antibiotics, and number of beneficial bacteria - probiotics in accordance with the requirements of regulatory documents on methods of detection and control by sanitary-epidemiological rules and regulations [2, 8, 12]. Obviously, the main feature of microbiological standards is the range of tolerances, according to which, taking into account the recommendations ISO, it is customary to determine the quality of products. The reports of WHO research groups indicate that microbiological standards aimed at protecting the health of consumers, must be as much as possible consistent throughout the world. However, full unification is excluded due to the need to take into account local conditions. The undifferentiated application of "soft criteria" in situations dangerous from the epidemic point of view, causes the risk of food poisoning. And vice versa: excessive "tightening" of microbiological criteria unnecessarily restrains production. This proves the need to classify products according to the degree of epidemic danger (risk level).
- Perform sampling, apply methods of detection and quality control in accordance with sanitary and epidemiological rules, and in accordance with regulatory documents approved by the state departments of Rospotrebnadzor. The usual methods of determining the total number of mesophilic bacteria colonizing food products (the Koch method) are long, require a large number of sterile Petri dishes, and often are not reliable.

For this purpose Department of Microbiology in recent years use the method of bacterial contamination (CFU) using the Buck Truck (Bacteriological analyzer, SY-LAB, Gerate GmbH, Austria) device has quickly won the market because of its versatility and reliability. The Buck Truck 4300 device registers two parameters: the M parameter (medium impedance) and the E parameter (electrode impedance), which are taken into account separately or in combination (Figure 2). Using this method for recording the growth of microorganisms ensures the universal use of the device. The measuring system of the device is highly sensitive to microbial metabolites and allows measurements even in selective nutrient media.

Figure 2. Bacteriological analyzer BuckTrak 4300 (SY-LAB Gerate GmbH,Austria)

It is known that viable but non-culturable cells (VBNC) are formed under stressful conditions in natural or artificial environments (fresh, marine, tap water, soil, fermented meat and dairy products) [9]. The presence of VBNC makes it difficult to assess microbial contamination, and their presence in food products can be dangerous for human and animal health. The ratio of the number of live to dead cells was determined after staining the samples with a set of fluorescent dyes Live/Dead® (Baclight™ Life Technologies, Carlsbad) on an Opton (Zeiss, Germany) luminescent microscope with 320 magnification. The results of the Pakhomov *at al.*, study [11], of the samples showed that 33.6%

of *Klebsiella pneumoniae*, and 33.4% Alcaligenes *faecalis* were detected as died. This method allows the estimation of the true dissemination of experimental samples, the presence of undesirable bacteria in the products and the index of probiotic cultures (Figure 3).

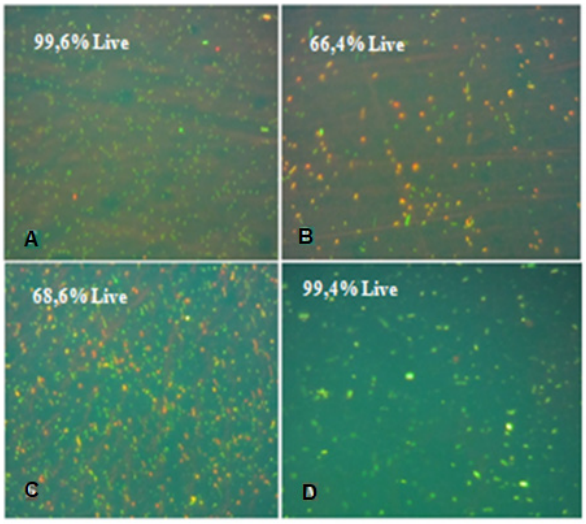

Figure 3. Living (green) and dead (red) cells of the investigated microorganisms after 1 year of incubation, painted with a set of Live / Dead, an increase of 320 times:
A - *Enterobacter aerogenes* GISK 418; B - *Klebsiella pneumoniae* 1954; C - *Alcaligenes faecalis* 415; D - *Proteus vulgaris* HX 19222 [11]

The method of determining the residual quantities of chloramphenicol (chloromycetin) and the tetracycline group of antibiotics in the animal origin products were improved, except for the microbiological method, by the highly effective method of liquid chromatography and immunoenzymatic methods [7, 8].

The cultural-morphological and physiological-biochemical properties of the cultures are studied by classical microbiological methods and Bergey's Manual of Systematic Bacteriology [19]. Also, for identification of cultures are used rapid molecular genetic methods on an automatic sequencer (Genetic Analyzer 3500XL, Applied Biosystems, USA). The screening of nucleotide variations in genomic sequences of the 16S pRNA gene of isolated bacterial strains was performed using the GenBank NCBI database with the BLAST program.

However, the stability of the nucleotide sequence of the 16S rRNA gene does not allow unambiguous identification of closely related species. For example, among lactobacilli there are many species and subspecies of phylogenetically close groups of *Lactobacillus casei, L. plantarum, L. buchneri,* and *L. acidophilus*, which are difficult to differentiate accurately, which leads to the search for new genetic markers. The topology of the phylogenetic trees of the *groEL, rpoB,* and *rplB* genes of the considered strains of lactobacilli is identical to the traditional phylogeny of *16S rRNA*, with the exception of the dendrogram based on the analysis of the rplB gene which indicates the possibility of using the analysis of the nucleotide sequence of the rplB gene as a genetic marker for the identification of lactobacilli [17]. The importance of lactobacilli in the food and medical industry as starter cultures and probiotic strains requires special attention to their correct taxonomic identification, since this is the main proof of the safety of products containing live microorganisms or their metabolic products [10, 16].

For example, professional food microbiologists should be able to respond quickly to crises in the field of food safety and use the most advanced and rapidly developing technologies. As diagnostic tests using ciliary *Tetrahymena pyriformis* or laboratory animals [5]. Mice are convenient to use for laboratory experiments, since they have small dimensions, high fecundity, and a short period of life. The biological characteristics of these rodents, including the characteristics of nutrition and the activity of the digestive system, are similar to those of humans. Toxicological studies are conducted at the Interfaculty Laboratory of Moscow State University.

Studies of the microbiome-intestine-brain axis are generally carried out using rats, which are popular for scientific research on psycho-biotics. Comparing the intestinal microbiota of rat patients with microbiota of healthy rats, it is possible to evaluate its interrelation with a certain disease by neurochemical characteristics [13], 15]).

Training materials are useful tools in both curriculum development and open access for both small and large groups, but different target groups require their own approach. Textbooks and teaching aids have been published (see Figure 4).

Figure 4. Training materials for Microbiology:
1 - Archean [20], 2 - Ecology of microorganisms [21], 3 - Molecular microbiology [22], 4 - Protoplast fusion of lactic acid bacteria *Lactococcus lactis* [23], 5 - Bacteriocins synthesized by lactic acid bacteria [24], 6 - Lactic acid bacteria and their differentiation [25]

Conclusions

- In summary, a training program on specific aspects of food safety management was developed, targeting both students (bachelor, master), also postgraduate and professionals, who are interested in improvement of knowledge; for example, sampling and monitoring, hygienic design or conservation. Currently, one of important tasks in education is the ability of a teacher to interest students, to stimulate them, to create the motivation needed for qualitative mastering of the material. A profound form of mastering knowledge is possible only when the teacher creates the necessary conditions for an informed and interested student to learn.

- Training courses in microbiology in the field of food safety should provide the graduate with a set of competencies necessary for professional work in modern conditions. Active development of the education sector includes traditional skills and a modern approach.

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