Theory and practice of legal regulation in the creation and use of genetically modified microorganisms

Voeikova Tatyana Alexandrovna

Candidate of Biological Sciences, Lead Research Officer

National Research Center "Kurchatov Institute", Moscow;

State Research Institute of Genetics and Selection of Industrial Microorganisms of the National Research Center "Kurchatov Institute", Moscow;

Skorokhodova Alexandra Yurievna

Candidate of Biological Sciences, Senior Research Officer

Federal Research Center "Fundamentals of Biotechnology" RAS ", Moscow.

Kuligin Vladislas Sergeevich

Engineer-Researcher, Postgraduate

National Research Center "Kurchatov Institute", Moscow;

State Research Institute of Genetics and Selection of Industrial Microorganisms of the National Research Center "Kurchatov Institute", Moscow

Abstract. The article discusses the issues of safety when using genetically modified microorganisms (GMM) on an industrial scale, producers of biologically active substances, the principles of legal regulation in the field of GMM circulation. The analysis of the practical use of the requirements of Russian and international legislation in the construction of a GMM producer of succinic acid based on the *Escherichia coli* K-12 strain is carried out.
Keywords: genetically modified microorganisms; biosafety; genetic engineering; *Escherichia coli* K-12.

The use of advanced technologies in the field of creation and use of genetically modified organisms (GMOs), including genetically modified microorganisms (GMM), poses complex and urgent issues of biosafety before modern society. On the one hand, the development of genetic engineering methods has ensured the successful solution of many problems in agriculture, industry and medicine, but at the same time, the possibility of editing genomes and changing DNA molecules raises serious concerns regarding the creation of hybrid molecules with new, previously non-existent combinations of genes. As a result, variants of more pathogenic bacteria and viruses may arise that are resistant to currently available antibiotics and other drugs. The introduction of GMM into the environment, in particular into the soil, is highly unpredictable. It

is believed that at present 5% of all microorganisms actually existing in nature are known - bacteria, actinomycetes, fungi, yeasts. Consequently, it is impossible to unequivocally determine how GMM will act on as yet unexplored species of microorganisms. When using GMM in animal husbandry, the life cycle of animals and humans must be considered as a whole.

In this regard, the problems of ensuring biosafety come to the fore and are a top priority for all mankind. Legislation in the field of GMM regulation, control over genomic research, risk assessment of the use of genetic editing technologies of organisms, all these issues are the subject of close attention of governments and international organizations that cooperate in the development of regulatory documents and general principles of biosafety. One of the important documents uniting the efforts of many countries in biosafety issues is the Cartagena Protocol, adopted in January 2000 in Montreal and entered into force in 2003. In accordance with the precautionary principle, the purpose of the Protocol is to promote the provision of an adequate level of protection in the field of safe the transfer, handling and use of living modified organisms resulting from the application of modern biotechnology and with the potential to adversely affect the conservation and sustainable use of biological diversity, also taking into account risks to human health and with particular attention to transboundary movements. In 2023, Russia plans to join the Cartagena Protocol on Biosafety, which regulates the interstate flows of GMOs on a global scale. Joining the Cartagena Protocol can give Russia an advantage in terms of increasing its export potential and ensuring the safety of imported agricultural products. However, in order to join an international document, Russia needs to harmonize legislation with international norms, experts say.

Obtaining biologically active substances necessary for medicine, agriculture, and industry is a priority task for the microbiological industry of the Russian Federation (RF), as indicated by the active legislative activity of the RF government in relation to the regulation of genetic engineering activities in recent years. In RF, the legal policy in the field of genetic engineering is based on Federal Law № 86-FZ, 1996 "On State Regulation in the Field of Genetic Engineering", which, with some changes, is still in effect [1]. Additions and clarifications were made in accordance with Federal Law № 358-FZ, 2016 "On Amendments to Certain Legislative Acts of the Russian Federation in terms of improving state regulation in the field of genetic engineering." The need to further update the provisions of Federal Law № 86-FZ and bring it in line with international law was announced in 2021 at a meeting of the section "Normative and legal regulation in the field of GMO circulation" of the expert council of the Federation Council Committee on agrarian and food policy and environmental management. It should be noted the important generalizing role of the RF Government decree of 22.04.2019 N 479 "On approval of the Federal Scientific and Technical Program for the Development of Genetic Technologies for

2019 - 2027" [2]. Of great importance in promoting GMM producers to the market of domestic biotechnological industries is the Order of the Ministry of Agriculture RF dated October 30, 2020 N 655 "On approval of the Methodology for the production of molecular genetic research of genetically modified agricultural microorganisms used for breeding and (or) growing on the territory of the Russian Federation"[3]. The order approves new research methods that are necessary for the registration of modified plants, animals and microorganisms. These methods were developed in connection with the Decree of the RF Government № 839 of 2013 "On state registration of genetically modified organisms intended for release into the environment, as well as products obtained using such organisms or containing such organisms, including the specified products. imported into the territory of the Russian Federation"[4]. An important document contributing to the registration of GMOs is the All-Russian Classifier of Transformational Events, 2015 (ARCTE) [5]. ARCTE is used to classify information on the characteristics of transformational events for registration of GMOs intended for release into the environment. Thus, the legislative framework of RF in relation to legal regulation when using GMM is being improved.

A comparative analysis of regulatory documents regarding the rules for regulating the use of GMM in the RF, the United States and the European Union (EU) showed similarities and differences in relation to a number of positions. In the United States, the legal framework is the most liberal and developed in detail; in the EU, increased attention is paid to safety issues, which leads to lengthy procedures for introducing strains into biotechnological production. RF legislation is currently less detailed and often restrictive despite good prospects for the development of the microbiological industry in RF.

The industrial use of microorganisms in the United States is regulated by the Environmental Protection Agency (EPA) and the Toxic Substance Control Act (TSCA) of 1976, which introduced new regulatory rules for microbial biotechnology products in 1997 [6]. This law states that the object of regulation is the organism itself, and not the method of obtaining it. In the EU, control is carried out by the European Food Safety Authority (EFSA), and the main document governing the use of GMM is Directive 2009/41/EC [7]. The regulation of the use of GMM in the EU and RF is carried out on a different principle, namely, the subject of regulation is the method of obtaining GMM - genetic engineering. In this, US legislation is fundamentally different from EU and RF legislation.

Consider the legal regulation regarding the use of recipient strains, on the basis of which GMMs are created, in the USA, EU and RF. In the US and the EU, there are lists of safe recipient microorganisms recommended for use and creation of GMM based on them. In the US, this is the GRAS (Generally Recognized as Safe) list, in the EU - QPS (Qualified Presumption of

Safety), i.e. list of microorganisms generally recognized as safe. Strains that have received the status of GRAS and QPS, as well as producers created on their basis, can be used in biotechnology without additional safety checks of recipients, which contributes to the rapid introduction of strains into industrial production. The principle of selection for inclusion in the lists of safe microorganisms is different. In the USA it is based on the characteristics of the strain, in the EU the selection is based on the species as a taxonomic unit. Therefore, *E. coli* K-12 strains, widely used in laboratories around the world, are non-pathogenic and have lost the ability to multiply in the intestines of warm-blooded animals, are approved in the United States for use in industrial biotechnology as safe recipient strains. In the EU, *E. coli* K-12 is not included in the QPS list because other strains of this species may show pathogenic properties. Under US law, not all genetically engineered strains are regulated by TSCA. If only the genes of the organism itself or closely related species, between which there is a natural exchange of genetic material in nature, were used in the construction of the strain, then such organisms are not considered GMM, since they do not carry foreign heterologous genes and are not transgenes. In the EU and RF, all genetically engineered strains are considered GMM. There is no list of recipient strains safe for use in the Russian regulatory framework. The creation and legislative approval in RF of a list of recipient strains and GMM safe for industrial biotechnology, created on the basis of these strains, is a necessary step for the rapid advancement of genetically engineered producers to the industry market.

Requirements for genetic material introduced into recipient strains are the same in WHO, US, EU and RF regulations. Thus, the introduced genetic material should be of minimum size; not contain genes encoding toxins or virulence; antibiotic resistance genes; sequences with unknown functions; genes affecting the immune system, determining the synthesis of allergens that negatively affect human health; do not increase the survival rate of the microorganism in the environment.

As an example of the practical use of the principles of creating a safe GMM that meets the requirements of Russian and international legislation, at the Research Center"Kurchatov Institute" - State Research Institute of Genetics on the basis of the *E. coli* K-12 strain, a GMM strain of *E. coli* K-12 SGM2.0Pyc-int, a producer of succinic acid (SA). The strain contained in the chromosome a heterologous pyruvate carboxylase gene - *pyc*A from the Bacillus *Subtilis strain*, i.e. was transgenic. To obtain a GMM producer, modern methodologies of precision chromosomal recombination engineering were used in combination with traditional methods of molecular genetics and microbiology.

The original *E. coli* K-12 strain synthesized SA from glucose under anaerobic conditions in an amount of ~ 5% of the total amount of mixed acid fermentation products. In *E. coli* cells,

the activity of pyruvate carboxylase required for efficient SA biosynthesis is absent. At the first stage of the genetically engineered design of the SA producer, a plasmid with a heterologous pyruvate carboxylase gene, *pyc*A, from the *Bacillus subtilis* 168 strain, which increased the synthesis of oxaloacetic acid, the SA precursor, was introduced into the original strain. The plasmid contained the gene for resistance to the antibiotic ampicillin, which was used as a selection marker for the selection of cells containing the *pyc*A gene. In addition, a number of genes encoding enzymes involved in other reactions of mixed acid fermentation were removed from the strain, which made it possible to direct the flow of necessary metabolites for the synthesis of SA. An intermediate strain was obtained, designated *E. coli* SGM2.0 [pPYC], with an increased efficiency of anaerobic synthesis of SA [8].

At the second stage of construction according to the norms concerning the characteristics of the genetic material introduced into the recipient strains, the plasmid with the gene for resistance to the antibiotic ampicillin was removed. The *pycA B. subtilis* subtilis gene required for SA biosynthesis was integrated into the chromosome of the strain under the control of the strong constitutive P_L promoter of the lambda phage, which ensured a high and constant level of pyruvate carboxylase synthesis. The resulting strain was designated *E. coli* K-12 SGM2.0Pyc-int [9]. To create this strain, more than 20 genomic editing events were performed. The correspondence between the expected and introduced changes in the genome GMM was established using high-throughput sequencing technology using read mapping, genomic assembly, whole-genome and local alignment of nucleotide sequences.

Evaluation of the biosynthetic characteristics of the strain showed that the strain is able to use various carbohydrates for the efficient production of SA, and the proportion of the target compound synthesized anaerobically by the strain reached 88% of all secreted metabolites. This level of synthesis is comparable to that of the best modern SA producers based on *E. coli.*

The next stage consisted in carrying out a comparative analysis of the characteristics of the initial *E. coli* strain K-12 and GMM, obtained under the influence of a number of environmental factors, such as UV irradiation, temperature regime, behavior in water, soil, and wastewater. These indicators are a necessary characteristic of the strain when introduced into industrial production and environmental monitoring. It was shown that both strains are sensitive to extreme environmental factors. Compared to the original strain, GMM is characterized by a reduced viability under UV irradiation and an increase in temperature. When cultivated in soil, the growth of both strains is significantly inhibited within a week. In the sewage from the Moscow collector, containing in addition to microorganisms and various chemical substances, the process of death of the GMM strain occurs within three days, while the original strain forms

separate colonies within a week. The results show that GMM does not compete with obligate microorganisms present in soil and wastewater.

Thus, the E. coli K-12 SGM2.0Pyc-int strain meets international and Russian industrial production requirements, does not contain mobile genetic elements in plasmids, antibiotic resistance genes, is characterized by a high level of biosynthesis of the target product, and its viability in natural conditions was not increased. In accordance with the regulations, the *E. coli* strain K-12 SGM2.0Pyc-int was deposited in the National Bioresource Center of the All-Russian collection of industrial microorganisms of the RC "Kurchatov Institute" - State Research Institute of Genetics.

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