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REVIEWS

Mycology at the Institute of Microbiology, Russian Academy of Sciences: History and Prospects for the Future

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Abstract—This review deals with the historical development of mycology at the Winogradsky Institute of Microbiology, Russian Academy of Sciences. Starting from the works of Academician G.A. Nadson, the review considers from the historical perspective the main achievements of the researchers of the institute in the field of mycology, including such important subfields as the cytology, genetics, physiology, and biochemistry of mycelial fungi and yeast. The review concerns itself with the major theoretical ideas generated by the team of the Laboratory of Experimental Mycology in the course of their studies of micro- and macromycetes. Special attention is also given to recent developments in biotechnology and medicine, including the development of new drug preparations from biologically active substances of fungi.

The science dealing with fungi is termed *mycology*, but no unambiguous and comprehensive definition of the distinctive features of the organisms referred to as fungi has yet been given. In terms of one of the recent definitions, fungi are eukaryotic heterotrophic organisms that absorb dissolved nutrients from the environment, i.e., behave as osmotrophs, in contrast to animals. Fungi are characterized by insignificantly differentiated tissues and cell walls. Most fungi form mycelia at the vegetative developmental stage. During their life cycle, fungi also give rise to dormant cells (spores) that form as a result of asexual or sexual reproduction. The cell walls of fungi contain the aminopolysaccharide chitin, and glycogen occurs in the mycelium cytosol [1]. Fungi are believed to be the most ancient organisms among extant eukaryotes [2].

Studies of fungi have been performed since time immemorial. The ancient Greek scholar Theophrastus (quoted in [3]) was the first to mention fungi in his works. For a long time, fungi were not regarded as a self-contained kingdom of life, and they were classified into the plant kingdom. This was due to the extreme heterogeneity of the physiological and biochemical properties of fungi and of the chemical composition of their cells. In addition, fungi, in various respects, are similar to plants, animals, and prokaryotes. These similarities presented serious difficulties for mycologists. The French botanist A. Veian (quoted in [3]) wrote in the 18th century that "fungi were invented by the devil to disturb otherwise harmonious Nature and to confuse and frustrate researchers." Fungi finally acquired the status of a special kingdom in the 19th century, when sufficient experimental evidence was provided [4]. A new "system of life" proposed in the late 20th century consists of six kingdoms (*Animalia, Protozoa, Bacteria, Fungi, Plantae*, and *Chromista*), and fungi established themselves as a self-contained kingdom in this system [5].

Much attention was given to *Fungi* at the end of the 20th century and the beginning of the 21st century. The data accumulated by this period suggested that fungi, due to the considerable heterogeneity of their physiological and biochemical properties [4], might be used as the main producers in biotechnological systems, instead of plants, animals, and bacteria. Of evident biotechnological importance were also the high growth rates of fungi, their high biomass-accumulating potential, and the applicability of fungi to multipurpose environmentally friendly production processes that could be carried out without any constraints. Fungi acquired additional weight as a result of numerous studies that revealed them to be indispensable sources of drugs with wound-healing, anti-AIDS, immunomodulating, and especially anticancer activities. Based on these developments, a new subfield of medicine, termed pharmaceutical mycology [6], took shape by the early 1990s.

A significant contribution to these developments was made by the Institute of Microbiology, Russian Academy of Sciences. Studies of fungi were initiated when the institute was founded, under the guidance of G.A. Nadson, its first director and a full member of the Academy of Sciences of the USSR. Research on mycelial fungi and yeast was carried out in the following directions: (i) studies on the morphology and physiological and biochemical properties of fungi and (ii) the development of new biotechnologies aimed at produc-

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ing biologically active substances. In the 1980s, a third direction was introduced that was concerned with the production of drug preparations from fungal biomass.

In 1934, the studies conducted at the Institute of Microbiology (then under the Academy of Sciences of the USSR) concentrated on radiation microbiology, and the main research was done with yeasts, which belong to higher fungi (*Ascomycetes*). This research was guided by Nadson. As early as in 1920, he published his classic work "On the Effect of Radium on Yeast Fungi in Connection with the General Issue of the Effect of Radium on Living Matter" [7]. The central subject of subsequent research was the experimental modification of hereditary properties of microorganisms and the formation of new races, which was exemplified by the effects of radium and X rays on the yeast *Saccharomyces cerevisiae* (race XII). This research raised a major issue to be tackled by biologists of that epoch—the question of whether new forms of life could be created by man [8].

Of paramount importance for the further development of radiobiology in Russia were Nadson's works on the mode of action of radium on fungal cells. Based on his observations on irradiated yeast cells, Nadson described the morphological changes caused by irradiation. He demonstrated for the first time the existence of the death zone, the inhibition zone, and the stimulation zone, which form during the development of yeast on solid medium under irradiation. These studies also elucidated the dependence of the biological effects on the radiation dose [9]. These studies were continued by M.N. Meisel' and his associates, who used modern cytological approaches [10]. The results of the theoretical research done by Nadson in the field of radiobiology were so important that Meisel' rightfully pointed out that Nadson "made an invaluable contribution to microbiology and radiobiology" [10].

Nadson's research in the field of radiobiology was also of practical importance. For instance, he bred a "radiorace" of baker's yeast with an enhanced sporulation capacity, whereas the conventional race of *S. cerevisiae* used in industry completely lost this capacity, which is necessary under industrial conditions [11].

The numerous disciples of Nadson conducted further studies in the same field. They investigated the mutagenic effects of physical and chemical factors. V.I. Kudryavtsev established for the first time that exposure to low temperatures (freezing) results in morphological changes in yeast, which form amoeboid cells. This peculiarity is transmitted from generation to generation as a stable inheritable trait. Meisel', another associate of Nadson, showed that inheritable changes also occur in fungi in the presence of chloroform and potassium cyanide and under the influence of a combination of coal tar (a chemical factor) and UV radiation (a physical factor). Of considerable interest were the works by Academician A.A. Imshenetskii that saw daylight in the late 1930s. He revealed for the first time that the products of metabolism that accumulate in the medium during the growth of a yeast culture can activate the mutation process. These studies provided an explanation for the earlier data that industrial fungal cultures obtained from postidiophase cells vary in their physiological properties. N.A. Krasil'nikov, who also was Nadson's disciple, obtained new *Sporobolomyces* and *S. cerevisiae* strains at supraoptimal cultivation temperatures.

Special attention should be given to Nadson's cytological studies. For the first time, he investigated radiation-induced morphological changes in mitochondria and the capacity of these cell organelles to perform reparation processes. This research was further done by M.N. Meisel', Corresponding Member of the USSR Academy of Sciences, who headed the Department of Comparative Cytology of Microorganisms at the Institute of Microbiology. The department gave much attention to yeast. Prominent cytologists including V.I. Biryuzova, G.A. Medvedeva, M.N. Poglazova, etc., worked at this laboratory. They conducted their studies using phase-contrast and anoptral devices, the equipment required for fluorescent and UV microscopy, and electron microscopes. The main research direction of the department was the investigation of structure–function relationships in microbial cells under the influence of various physiological factors, including extreme conditions. It was yeast, primarily *S. cerevisiae*, that was investigated in greatest detail.

The research done at laboratory headed by Meisel' revealed that the radiological effect observed after irradiation depends not only on the extent of the primary damage inflicted on the cell and subcellular structures, e.g., mitochondria (as shown by Nadson earlier), nuclei, chromosomes, and the cytoplasm. For the first time, attention was given to secondary biochemical processes that occur in the cell after irradiation. The role of reactivation processes that necessarily follow irradiation was stressed. Prerequisite for a yeast cell's viability is the integrity of its membranes, whose destruction inevitably occurs under illumination. This results in the extrusion of a number of metabolites from the cell, which promotes the synthesis of other metabolites [12]. Yeast cells at the predivision or the initial growth stage are particularly sensitive. Mitochondria, microsomes, membranes, and cell walls become more fragile and lose their content upon illumination. The enzyme activities of mitochondria are disrupted. For instance, the oxidative phosphorylation rate is decreased [13]. Major changes (e.g., sterol accumulation) occur in the membrane composition [14]. The fact that the sterol content is enhanced under illumination was used by Meisel' and associates for practical purposes. They established that the sterol yield can increase to 200% of the control value in the presence of pantothenic acid. Thus, a new biotechnological process aimed at synthesizing ergosterol (provitamin D_1), which is essential for the human organism, was developed during the period under consideration. In addi-

tion, a method of increasing the inositol yield in yeast using X-ray irradiation was suggested [15].

By the early 1980s, the laboratory headed by Meisel' had initiated new research directions that dealt with the effect of IR irradiation on saccharomycetes and relevant airborne bioactivators. The latter direction was of practical importance and received special attention at the laboratory. Numerous studies confirmed the idea that plants can release a number of vitamins into the air. Yeast test cultures were suggested for detecting such "airborne" vitamins [16, 17]. For example, *Endomyces magnesii* could be used to quantitatively determine the pyridoxine content. Vitamins migrate through the air during such industrial processes as bread baking, beer brewing, and yeast dehydration. They exert a considerable influence on these processes, which can be detected using the yeast test cultures suggested by Meisel' and coworkers.

Apart from the major theoretical contribution made by Meisel' (summed up in a number of seminal works [13, 18]), we should also emphasize the practical applications of his research. It was on his initiative (and with his active personal involvement) that the production of fluorescent dyes and indicators was launched in the USSR. Subsequently, these substances were widely used in medicine, sanitation, and biotechnology, particularly for the purpose of developing express tests. Based on the data on the functional and cytological changes in yeast upon transition from aerobic to anaerobic conditions, the research team of the comparative cytology laboratory developed tests to be used in industrial fermentation processes and in bread baking industry. Of practical importance were also their studies on the cytology and metabolism of yeast upon its transition to the anabiotic state. These studies were further conducted by M.E. Beker, Academician of the Academy of Sciences of the Latvian Soviet Socialist Republic, and his disciples [19].

A contribution to mycological research at the Institute of Microbiology of the Russian Academy of Sciences was also made by V.I. Kudryavtsev, the head of the laboratory that concentrated on the systematics of yeast. As early as in 1934 he launched an extensive research program concerning the yeast flora of the Far East. Subsequently, field studies on the same subject were also conducted in the cities of Yerevan and Megri (on the Araks River), on the Kola Peninsula, and in Michurinsk Region. Yeast collections were also put together during private visits to the Crimea and Siberia. A wide variety of natural and artificial substrates were tested, including the mucilaginous exudate of trees, soil, berries, juices used in wine production, fermented milk products, liquors, honey, jams, preserves, etc.

The research was done by Kudryavtsev and a large number of his followers over the course of many years, resulting in a new yeast classification system and the monograph *Yeast Systematics* [20], one of the first manuals on this subject published in Russian. The value of this book was additionally enhanced by the amazingly precise images of yeast drawn by the prominent painter N.K. Sonina and by easy yeast identification techniques described in it. Therefore, the manual was widely used by specialists in this field.

After Kudryavtsev's death, his laboratory was headed by O.L. Rudakov. The laboratory initiated a new research direction that was concerned with the biology of mycophilic fungi and their practical potential. The yeast museum inherited from Kudryavtsev was supplemented with mycophilic species of fungi that were collected by Rudakov during his numerous field studies in various regions of the USSR; he also exchanged specimens with foreign collections of fungi.

Although the number of mycophilic species of fungi occurring in nature does not exceed 2000, these organisms are of particular importance because they are natural antagonists of phytopathogenic fungi. Using chemicals to protect plants from pathogenic fungi may not be the optimum strategy because they can disrupt the natural network of interorganismic relationships. Rudakov and his research team made considerable progress in the 1980s in their research on the goal-directed employment of efficient fungicide doses to control mycophilic fungi. They also obtained strains of mycophilic fungi that actively destroyed plant pathogens.

A major part of the research activities of this laboratory focused on another technological development using mycophilic fungi. These organisms serve as sources of antifungal antibiotics, yeast-decomposing enzymes, and acylases [21]. In addition, mycophilic fungi modify the biochemical properties of their hosts, including, in the first place, plant pathogens. This enables us to obtain producers of new biologically active compounds. These studies were considered "a promising, potentially fruitful research direction" [21] whose practical employment was initiated on a large scale in agricultural mycology and biotechnology in the 20th century.

Nadson's ideas on mutagenesis in fungi in conjunction with the considerable practical potential of mutants in technological microbiology were brought into focus again by Academician Imshenetskii. In 1941, the department formerly headed by Academician V.N. Shaposhnikov was taken over by Imshenetskii. Its new name was the Department for Experimental Studies on the Variability of Microorganisms; subsequently, it was renamed the Department for the Physiology of Mutants. The department focused on experimental research on the variability of microorganisms, with special attention to mycelial fungi and yeast. These studies were a major contribution to the research on inheritable changes in fungi and they promoted the work aimed at selecting suitable cultures for industrial uses [22].

In terms of this research direction, studies were also conducted for the purpose of obtaining enzymes from mycelial fungi. A proteolytic enzyme with fibrinolytic activity was obtained from an *Aspergillus terricola* cul-

ture. The preparation termed terrilytin was produced on the basis of this enzyme. This preparation successfully passed large-scale clinical tests concerning its effects on burns and wounds. It was recommended for treating patients and produced under industrial conditions. It was suggested that the same preparation should be used to eliminate blood clots, but prerequisite for this role of the preparation was a decrease in its toxicity. Toxicity tests were carried out on fibroblast cultures (using a culturable strain of the L clone), which, in this epoch, was at the forefront of experimental research [23].

I.D. Kasatkina, L.I. Solntseva, S.Z. Brotskaya, and their coworkers established the important fact that significant physiological changes in mutant fungi are typically accompanied by changes in colony morphology. For instance, it was shown that *Penicillium chrysogenum* strains forming folded colonies produce more gluconic acid than the strains characterized by the original smooth colony shape. The representatives of the laboratory Brotskaya, L.G. Loginova, L.A. Kuzyurina, Solntseva, N.F. Kuranova, Kh.Z. Stan'kov, Imshenetskii, and K.Z. Perova actively conducted experiments to obtain strains of *A. nidulans*, *A. niger*, and *A. oryzae* that form active amylases, proteinases, and pectinases. UV irradiation of industrial strains of *Aspergillus niger* made it possible to obtain the T-1 and T-2 mutants, which yield more citric acid than the original strain [24]. This research was of paramount practical importance because it confirmed the idea that the useful activities of strains used as superproducers in industry can be further enhanced by mutations.

Another research direction of A.A. Imshenetskii's laboratory concerned itself with the production of polyploid fungal forms using acenaphthene, colchicine, and camphor. As a result, polyploid yeast cultures (*Candida scottii*, *C. guilliermondii*, *C. utilis*, and *Pullularia pollulans*) were obtained. They were characterized by various polyploidy degrees (ranging from diploidy to tetraploidy). These strains were of practical interest. For example, *C. guilliermondii* had enlarged cells, which facilitated their separation. Such yeast could be stored for a longer time (and were more stable in lyophilized form) than the haploid strain. In addition, the mutant forms accumulated more biomass, riboflavin, and pollulan. Polyploid cultures were more resistant to deleterious factors than haploid cultures. For instance, the polysaccharide-synthesizing activity of polyploid *P. pollulans* strains was to a lesser extent dependent on the cultivation conditions (temperature, pH, aeration, and carbon sources). Hence, the resistance of fungal cultures to deleterious factors was conditional on their polyploidy. Obviously, this also applied to industrial strains, whose stability was regarded as the main prerequisite for using them as producers [25, 26]. Presently, the results of the theoretical research of Imshenetskii's associates are used in a number of biotechnologies aimed, e.g., at producing pollulan and several other yeast polysaccharides for medical purposes.

Mycelial fungi were the subject of the research done at the Department for the Physiology and Biochemistry of Thermophilic Microorganisms, founded (in 1960) and headed by Prof. L.G. Loginova. In order to obtain active thermostable substrate-degrading enzymes, the research team of this department investigated ascomycete fungi, including *Aspergillus fumigatus*, *A. terreus*, etc. Of special interest was the superthermophilic fungus *Myceliophthora thermophila*, isolated from its natural substrates in Central Asia. The fungus degraded cellulose and hemicellulose. From these thermophiles, researchers subsequently obtained the active enzyme preparation celloterrin and a protein–enzyme complex (PEC) that was capable of degrading cellulose to glucose. The PEC obtained from *M. thermophila* was developed as a livestock feed additive. The same thermophilic fungus became a useful experimental model that subsequently enabled researchers to develop a theory to explain the thermophily phenomenon in fungi [27].

From these facts it is evident that fungi were constantly in the focus of attention at the Institute of Microbiology ever since its foundation. Using these organisms, researchers revealed a number of important biological trends. Fungi acquired additional weight (as a subject of theoretical and applied research) more recently, which was due in part to the increasing impact of these lower eukaryotes on human health and biotechnology. It has been predicted that mycelial fungi will become the main biotechnological producers in the 21st century.

Active research on fungi at the Institute of Microbiology was initiated by the department that was founded in 1938 and originally called the Department for Fermentative Microorganisms. In 1943, this department (headed by Academician V.N. Shaposhnikov) was renamed the Department for Technical Microbiology. Presently, this department should be called the Biotechnology Department in accordance with its research goals.

Based on his seminal research on the physiology and biochemistry of heterotrophic microorganisms, Academician Shaposhnikov is to be considered one of the pioneers in the new field of science currently termed biotechnology. For more than 45 years, he was involved in setting up industrial facilities in the USSR that used processes carried out by microorganisms. These developments were based on the idea that developing a correct production design involving microbial metabolic activities "implies profound knowledge of the physiological basis of the metabolism of the organism involved" [28]. Shaposhnikov's idea that microbial metabolism is biphasic, suggested for the first time on the basis of his studies on acetone–butanol fermentation, should also be considered an important contribution made by him. The biphasicity principle was subsequently applied by Shaposhnikov and his followers to other kinds of fermentation. It was also used in production processes involving mycelial fungi as producers of

antibiotics, pigments, toxins, and other compounds. Shaposhnikov developed techniques that either prevent the transition of a microorganism to the second growth phase or, conversely, enable the microorganism to make the transition [29]. These works provided the foundations for Soviet biotechnology. In accordance with them, the secondary metabolism concept was subsequently developed. The first and second growth phases were denoted the trophophase and the idiophase (or the balanced growth phase and the secondary synthesis phase), respectively [30, 31]. Shaposhnikov also established a number of important facts that made it possible to change the patterns of biosynthetic processes in a goal-directed fashion and to enhance the product yield. This research enabled researchers to develop methods of continuous cultivation of fungi. Its results were widely used for the purpose of setting up various biotechnological processes in the USSR.

From 1963, this department was headed by Prof. M.N. Bekhtereva, and research on the physiology and biochemistry of fungi producing lipids and other biologically active substances was done under her guidance. The attention given to lipids was due to the following facts: (i) it was established that lipids always occur in living organisms; (ii) the successful development of lipidological methods made it possible to establish the chemical composition of a majority of known lipids; and (iii) data were accumulated concerning essential biological functions of lipids: they form barriers in biological systems and are involved in membrane-dependent transfer processes, excitation/irritation processes, thermosensory responses, fungal cell aggregation, transmission of intracellular signals, and membrane recycling [32, 33]. Of particular interest were the data that lipids are episemantic molecules [34] that provide information concerning the phylogeny and systematics of organisms, including fungi [4].

Apart from their general biological importance, fungi also have considerable practical potential. For example, they could be used as a replacement for plant oils. It was expedient to use biotechnological methods to obtain essential fatty acids (linoleic and linolenic acid) and eisosapolyenoic acids, taking into account the high demand of Russian medicine for preparations made from them.

Research on lipogenesis in mycelial fungi included studies on the lipogenic activity of mucorous and ascomycete fungi, their fatty acid composition, the selection of cultivation conditions for superproducers, and methods of increasing the desaturation degree of their lipids.

It was established that fungi produce as much oil as oil-bearing plants such as sunflower (57% of oil), soybean (25%), cotton (29%), and others. The greatest amounts of lipids were formed by the fungi of the genera *Cunninghamella*, *Mucor*, *Blakeslea*, and *Rhizopus* [35]. By varying the contents and chemical forms of sugar and nitrogen in the medium, the researchers succeeded in increasing the lipid yield in *C. japonica* to 60% of the biomass content [35]. However, although active oleogenic producers were obtained, it was mandatory to increase the desaturation degree (DD) of their lipids. Following are the factors tested for their effects on the DD of *C. japonica* lipids: cultivation temperature, medium aeration, carbon limitation, and a partial substitution of ethanol for glucose [35–39]. I.V. Konova *et al.* adjusted the temperature and aeration level to shift the ratio between tetraenoic and pentaenoic acid in the pseudofungi *Enthomophthora* and *Pythium*, favoring the synthesis of pentaenoic acid. Using *B. trispora*, it was shown that carbon limitation enhanced the contents of linoleic acid and desaturated C_{20}–C_{22} fatty acids. In *C. japonica* and *F. solani*, the lipid DD also increased in the presence of ethanol in the medium. Exogenous lipids contained in the medium also influenced the lipid DD and fatty acid composition. Another method developed to produce fungi with more desaturated lipids was based on obtaining mutants with a decreased number of conidia [37].

These data were used in biotechnological research and development activities of the laboratory. For instance, it was suggested that lipids with a high iodine index from the biomass of an antibiotic producer should be used as a replacement for plant (sunflower and soybean) oils for producing PFO60 alkyd resins [35]. During the same period, Bekhtereva's research team in collaboration with Academician Imshenetskii, Academician G.K. Skryabin, Prof. G.I. Samokhvalov, and Prof. M.G. Golysheva developed an industrial method for obtaining β-carotene (provitamin A), the neutral lipid of *B. trispora.* By optimizing the cultivation conditions of heterothallic strains of *B. trispora*, they obtained superproducers yielding 1.2 g/l of carotene [40]. Subsequently, the carotene yield was increased to 3–4 g/l by adding cotton oil and hydrocarbons, illuminating the mycelium with low-intensity daylight, increasing the glucose–nitrogen ratio, and supplementing the medium with the SH group activator L-cysteine [40–43]. Shortly before the collapse of the USSR, three plants (in Krasnodar, Verkhnedneprovsk, and Sverdlovsk) produced carotene.

The research team under the guidance of E.P. Feofilova investigated the mode of action of β-ionone in detail in the late 1980s. It was demonstrated for the first time that the addition of this carotenogenesis stimulator promotes, apart from carotene formation, the synthesis of carotenogenic proteins and RNA. Studies using transcription and translation inhibitors added to the medium against the background of β-ionone provided evidence that β-ionone stimulates de novo synthesis of carotenogenic enzymes in *B. trispora* at the level of RNA template translation by ribosomes [44]. The results of studies with trisporic acids (TA) also contributed to the intensification of carotene synthesis. TA are hormones regulating carotene synthesis in the fungi of the order *Mucorales*, and their formation is biosynthetically linked to β-carotene synthesis. The addition of β-ionone to the cultivation medium of *B. trispora*

increased the carotenoid yield almost threefold while drastically decreasing the TA amount synthesized. The addition of acetic acid and the optimization of the medium aeration level exert an analogous influence on the culture [45]. In collaboration with Samokhvalov, researchers synthesized chemical analogues of TA, detected the biological effects of specific chemical groups of TA on carotene biosynthesis, and determined the optimum TA concentration that provides for the maximum carotene yield in the culture of the (–) strain of the fungus [46].

Research on fungal lipogenesis also promoted other practical developments aimed at producing clinically useful lipids that can replace drugs produced abroad, such as Essentiale, Lipostabil, Vitamin F-799, etc. These preparations contain fatty acids as the active components. They are efficient medicines for atherosclerosis, hypertension, acute and chronic hepatitis, and alimentary canal diseases. The essential linoleic and linolenic acids are not synthesized by mammals and, therefore, are to be supplied with food. Instead of bovine tissues and marine animals, these fatty acids can be obtained from mycelial fungi (the kingdom *Fungi*) and pseudofungi (*Pythium*, *Phytophthora*), which have been recently classified into the kingdom *Chromista.* The preparations Gammalin K, Lipar, Pentarol, etc., were developed from the lipids of these fungi [47]. In addition, biotechnologies were developed for decontamination of the environment with the use of micromycetes that utilize lipid-containing waste accumulating at lipid-, milk-, and plant oil–processing facilities and on lipid-trapping filters of food production plants. The suggestion was made to use the group of mucorous fungi with high lipolytic activity to degrade the waste. Such biotechnologies are advantageous in economic terms because they produce a dual effect: they eliminate lipid-containing waste and yield fertilizers for agricultural purposes [47].

The 1990s were marked by a rapid development of biotechnology; fungi became the main biotechnological producers. The significant advances made by the Institute of Microbiology in the field of mycology in conjunction with the special attention given to fungi by the international scientific community promoted the foundation of a special laboratory (the Experimental Mycology Laboratory), set up in 1996 and headed by Feofilova, a disciple of Shaposhnikov. The laboratory dealt with a new research direction concentrating on the biochemical mechanisms that enable fungi to adapt to stress. These studies were related to the cutting-edge subfield of modern science that is referred to as biochemical adaptation of organisms to stress. The research in this subfield brought together specialists in various subfields of biology (including biochemistry and genetics), medicine, and chemistry.

Biomembrane lipids are known to be the main biomolecular targets of stress factors [48]. The Experimental Mycology Laboratory focused on the investigation of the cell lipid composition in various taxonomic groups of fungi under the influence of stress factors and stress protein–stabilizing compounds [49] termed chemical chaperons [50]. They include a large number of fungal cytosol carbohydrates belonging to noncyclic polyols and disaccharides (mannitol, erythritol, arabitol, glycerol, sorbitol, and trehalose) [50] that perform protective functions under stress and prevent membrane damage. Among these carbohydrates, trehalose [51] plays an essential role. Apart from the ordinary stress protector function (membrane stabilization), trehalose prevents protein denaturation [52]. The research on stress centered on the effect of typical natural factors, including light of different spectral composition, temperature oscillations, starvation, etc.

In terms of this research direction, it was necessary to unravel the mechanisms involved in lipid formation during cytodifferentiation. Special attention was paid to the complex character of this process, which is due to the existence of the sexual and the asexual cycle (involving up to five types of dormant cells).

Let us first consider the trends revealed in studies on the vegetative stage of development. For the sake of simplicity, we assume that it consists of two main phases: active growth and deceleration of growth and life-sustaining activities under the influence of a deleterious factor, e.g., starvation (nutrient depletion). Summing up the data obtained, we can draw the following conclusions:

(i) Synthesis of reserve lipids (triacylglycerols (TAG)) is an alternative to protein biosynthesis. In contrast, the formation of polar lipids proceeds in parallel with the synthesis of both alkali- and water-soluble proteins, and its rate reaches the maximum value during the intense growth of mycelium [53].

(ii) The lipids synthesized during the growth phase are desaturated to a large extent, readily oxidizable, and sensitive to reactive oxygen species (ROS). They include phosphatidylethanolamine (PEA), the predominant species; phosphatidylserine; cardiolipin; and phosphatidylcholine (PC), whose content is decreased. The deceleration of growth processes results in the predominance of PC and sphingomyelin, which are more saturated and resistant to oxidation. Hence, during the active growth phase, fungal membranes are more fluid and their lipids contain maximum amounts of diene and trienoic fatty acids. Upon the transition to the growth deceleration phase, the lipids accumulate more long-chain fatty acids [53].

(iii) The antioxidant activity (AOA) of fungal lipids reflects the state of the antioxidant pool in the membrane and its resistance to ROS [54] and represents a useful criterion of the status of membrane lipids in relation to the growth activity. The AOA value of membrane lipids reaches the maximum during the active growth phase. The growth-related AOA changes in membrane lipids differ from those in neutral lipids. The AOA of neutral lipids is very low during the tro-

phophase, suggesting a lack of antioxidants or their low content in the neutral lipid fraction [55].

These patterns are characteristic of the growth of fungi in submerged culture. Under natural conditions, fungi undergo a developmental cycle in which the active growth phase (bios) is followed by the formation of dormant cells denoted as spores. The transition to dormancy in response to stress results in drastic changes in the morphology and chemical composition of cells. These changes vary depending on the dormancy type. Fungi are characterized by two dormancy types: (i) the profound dormant state, which is predominantly characteristic of sexual spores (endogenous dormancy), and (ii) the superficial dormant state of vegetative spores (exogenous dormancy), which usually results from dehydration. Studies on the dormancy stage in the developmental cycle of *C. japonica* and *Blakeslea trispora* revealed significant differences in the lipid composition of the two kinds of dormant cells. Endogenous dormancy peculiar to zygospores is characterized by a high total lipid content (up to 40% of the biomass) and large amounts of PC (about 70% of the total phospholipid fraction) and TAG (over 90% of the total neutral lipid fraction). Sporangiospores lapse into the exogenous dormant state, and the total lipid and the TAG contents in this state are 7–8 and 2–3 times lower than in the endogenous dormant state. However, the PEA level is 4- to 5-fold higher in the exogenous dormant state. Moreover, sporangiospores are comparatively rich in lipids that regulate germination, including sterols (ST), sterol esters (STE), and free fatty acids (FFA). Research on ascomycete cells (conidia) in the exogenous dormant state (using *Aspergillus niger* conidia as an example) revealed that they contain considerably less lipids (7–9%) and their predominant components are TAG [57]. These data account for the fact that spores in the exogenous dormant state can be stored for a relatively short time and are characterized by quick germination, in contrast to, e.g., *Mucorales* zygotes, which assume the endogenous dormant state. Apart from energy-rich reserve substances (TAG), cells in the endogenous dormant state contain much PC, which prevents lipid oxidation, and the highly active antioxidant lycopene, which secures a prolonged survival of the cells [56].

These studies revealed differences between the processes of germination of cells in the exo- and endogenous dormant states. The differences are of considerable interest in terms of fungal cultivation. *Mucorales* zygospores and *Basidiomycetes* basidiospores exist in the endogenous dormant state. The germination of the zygospores of mucorous fungi is a very rare phenomenon. Less than 2–3% of them germinate. Even germination stimulators, such as temperature stress and toxicogenic compounds that damage membranes, do not facilitate the transition to the vegetative state. In contrast to conidia, fungal cells in the endogenous dormant state fail to germinate in water. In comparison to the zygotes of mucorous fungi, the basidiospores of basidiomycetes, e.g., *Agaricus bisporus*, germinate more successfully. For this purpose, basidiospores require exogenous carbon and nitrogen sources (asparagine and glucose), even though the lipid and soluble cytosol carbohydrate contents may amount to 19 and 12%, respectively, of the dry weight of the basidiospores involved. Temperature shock and incubation at low temperatures activate their germination [58]. However, even in this case the percentage of germinating basidiospores does not exceed 14%, while the percentage of germinating conidia (exogenous dormancy) is over 90%.

Research on dormant cells formed by mutants of *C. japonica* that differ in respect to their spore-forming capacity revealed a clear relationship between the lipid composition and sporogenesis intensity. Suppression of sporogenesis in mutants obtained by irradiating fungal cells with near-UV light results in the formation of spores with more saturated lipids. In contrast, the mycelium obtained from mutant spores contains more desaturated lipids rich in diene and trienoic acids [53].

This relationship was further investigated in a long series of studies on the effects of light with varied spectral composition on the ontogeny of fungi. It was assumed that daylight (especially its as yet almost uninvestigated green spectral region with a wavelength of 530 nm) is the most widespread natural stress factor. It was shown for the first time that continuous illumination of sporophores of fungi of the genus *Aspergillus* with green light increased the conidium number at the terminal developmental stage 2- to 3-fold. The spores forming in the light (L spores) differed from those in the control system in their lipid composition. As for the initial stage of spore formation, green light increased the lipid content of the conidia and influenced the phospholipid level. The phospholipids became more desaturated. Light with a wavelength of 650 nm (the red spectral range) produced the opposite effect. Of particular interest is the fact that L spores formed a mycelium that differed in the lipid composition from the control mycelium and possessed more active cellulolytic enzymes. Analogous data in support of the suggestion that L spores form mycelium with a changed lipid composition and modified metabolic activities were obtained with *Blakeslea trispora*. These studies made it possible to change the carotenoid yield [59]. Green light was shown to exert an influence on the capacity of reproductive cells to germinate. *A. japonicus* conidia forming under illumination exhibited a decreased gibberellin content, elevated TAG and sterol ester levels, and the capacity for rapid growth tube formation. However, the activation of growth processes during mycelium formation is accompanied by selective stimulation of gibberellin formation [60].

Apart from the theoretical novelty of the results obtained, they were of practical (biotechnological) importance because they enabled us to increase the spore yield and to obtain mycelium with elevated cellu-

lolytic activity. Illuminating spores with visible, especially green, light produced effects that also manifested themselves at the later ontogeny stage (at the mycelium stage, also termed the bios stage).

New facts were established with respect to the effects of temperature, a natural stressor. In the fungi of the subkingdoms *Eomycota* and *Neomycota*, changes were detected in the lipid acyl chains with regard to their saturation/desaturation level; the isomerization state, the chain length, and the phospholipid, ST, and STE contents. For example, hyperthermia resulted in an increase in the PC level and a decrease in the PEA content. The TAG level of the neutral lipids increased. An inverse trend of changes was observed under hypothermia. Patterns of temperature stress responses varied depending on the taxonomic position of the fungus involved. In *Mucorales*, temperature changes influenced the sphingomyelin level and promoted the formation of long-chain fatty acids not detected in these fungi earlier [61].

Apart from lipids, the team of the laboratory also investigated other biomolecules involved in the protection of fungal cells under stress. These are protector carbohydrates (PCa) located in the cytosol. It was established that the PCa composition varied depending both on the temperature and on the taxonomic position of the tested species of fungi. Adaptation to hypo- and hyperthermia in *Mucorales* (subkingdom *Eomycota*) is based on the regulation of trehalose synthesis, whereas this process also involves inositol in *B. trispora.* Mannitol and glycerol superproduction occurs in *Ascomycota* under hypothermia, while hyperthermia results in trehalose and inositol superproduction. *Basidiomycota* can use two systems of biochemical adaptation depending on the carbohydrate composition of the cytosol. In the absence of sucrose, glycerol and arabitol are involved in protecting cells under hypothermia and trehalose accumulates under hyperthermia (regulatory system 1). Regulatory system 2, which is peculiar to a number of basidiomycetes, including *Pleurotus ostreatus*, implies the involvement of sucrose, not glycerol or arabitol, in the response to temperature stress. In these studies, sucrose was detected for the first time in xylotrophic basidiomycetes. Formerly, sucrose was considered a plant-specific component (as far as eukaryotes were concerned).

Based on the data on the effects of high temperatures on the composition of lipids and protective carbohydrates, a hypothesis was suggested concerning the biochemical mechanisms that enable fungi to survive under hyperthermia [27]. The generally accepted hypothesis on micromycete thermophily proceeded from the data that micromycetes lack Δ15-desaturase, the main adaptive desaturase enzyme. It was assumed that, unlike mesophiles, thermophiles fail to synthesize linolenic acid and, therefore, cannot exist at low temperatures. However, our studies on the lipids of the superthermophile *M. thermophila* and the thermophiles *Curvularia lunata* and *Mucor pusillus* at low temperatures revealed that they contain insignificant amounts of linolenic acid (2%). In contrast to mesophiles, it occurred in neutral lipids, not phospholipids. In comparison to mesophiles, the thermophiles exhibited an extremely high sterol content, especially in microsomes and mitochondria, while the STE content was insignificant. The results obtained indicated that lipids of thermophiles are only involved in the adaptation of these fungi to high temperatures. Such lipids are also characteristic of thermophiles if they have to exist under hyperthermia. It follows that thermophiles lost, in the course of their evolution, two efficient mechanisms of biochemical adaptation, involving (i) Δ15-desaturase and (ii) regulation of lipid bilayer fluidity by adjustment of the sterol–sterol ester ratio. In addition, changes occurred in the composition of their protective carbohydrates. In contrast to mesophiles, thermophiles developed the capacity for trehalose synthesis during the active growth stage, which protects their membranes from high temperatures. It was established that trehalase, the trehalose-synthesizing enzyme of thermophiles, is thermostable. A new protein with a molecular weight of 45 kDa was detected in their mycelium. Presumably, this protein is a molecular chaperon, an analogue of heat shock proteins. In the light of these findings, fungal thermophily should not be considered a qualitatively new phenomenon. It seems more likely that thermophiles benefited by constantly using (at high temperatures) the adaptive mechanisms that transiently operate in mesophiles under stress [27].

The stress effect on the lipid composition of fungal membranes was also considered in terms of free radical oxidation. It was established that short-term interaction of *C. japonica* mycelium with the synthetic antioxidant (AO) 6-methyl-2-ethyl-3-hydroxypyridine alters the microviscosity, composition, and DD of the lipids. As for the phospholipid fraction, the PEA share increases and the quantity of choline-containing lipids decreases. AO increases the TAG and FFA content in the neutral lipid fraction. A prolonged action of AO results in an increase in the lipid content in fungal mycelium and in an enhanced level of desaturated C_{18} fatty acids. From the data obtained concerning the lipid composition, it was for the first time concluded that the addition of AO artificially "rejuvenates" fungal mycelium, arresting its development at the trophophase stage.

Based on the data on stress protection mechanisms in fungi, a number of biochemical criteria were suggested that are of much interest for fungal taxonomy. These data revealed that the representatives of the kingdom *Fungi* are extremely heterogeneous [4]. Of considerable interest in this context are the specific lipids of fungi (data obtained by I.V. Konova with coworkers), such as N-ethoxycarbonylphosphatidylethanolamine in the fungi of the genus *Absidia* and a previously unknown hydroxyacid-containing cerebroside detected in *Mortierella alpine*. As for *Linderina pennispora*, a representative of the family *Kiksellaceae*, its lipids pre-

dominantly contain *cis*-9-hexadecenoic acid, which supports the idea that fungi of this family differ from those of the order *Mucorales.* For the first time, data were obtained on the changes in the sterol compounds of fungal membranes that occur at some stages of morphogenesis in *Mucorales* and are directly related to the dimorphism phenomenon.

A large number of experiments dealt with the composition of the cell wall (CW) of fungi in relation to the responses of fungal cells to stress [62]. It was shown for the first time that fungal chitin differs from invertebrate chitin in elemental composition, crystallinity degree, and sorption capacity. Methods were developed to obtain chitin and chitosan from the CW of mucorous fungi and to prepare the chitin–glucan complex (CGC) from ascomycetes. A technique was proposed to vary the chitin–glucan ratio in the CGC by changing the cultivation conditions [63]. The maximum CGC amount was produced by ascomycete fungi on media with sucrose and ammonium nitrogen if the media are acidified to a pH value of 2 during the idiophase stage. Fungi synthesized more glucan than chitin on media with a high content of organic nitrogen. These data are potentially useful for developing methods of controlling the CW composition of fungi and the yield of the product of interest [62, 63].

Since the Experimental Mycology Laboratory was set up, its representatives have made a significant contribution to the development of modern biotechnology. Based on their theoretical conclusions concerning the influence of light on fungi, a new technique of obtaining spore inoculum was developed that is aimed at increasing the conidium yield and the activity of cellulolytic enzymes. The data obtained on the lipid and carbohydrate composition of conidia enabled researchers to formulate biochemical criteria that can be used under industrial conditions to determine the germination capacity of an inoculum. These criteria include the PC–PEA ratio and the glycerol, arabitol, and mannitol levels. The research team continued the studies (initiated by M.N. Bekhtereva) concerning the intensification of β-carotene synthesis at the Uralbiopharm Factory and showed the stimulatory effect of green light on the formation of this provitamin A. In collaboration with the Voykov Chemical Factory, we developed for the first time a profitable method of obtaining aminopolysaccharides from the CW of fungi under industrial conditions. A joint project involving the Uralbiopharm Factory resulted in developing and patenting a technique of producing lycopene from mucorous fungi. In collaboration with the Citrobel Company, a method for obtaining CGC from *Aspergillus niger* was developed and patented. Of considerable importance are the pioneering studies concerning the employment of ascomycete fungi to remove scale and other kinds of dirt from aircraft (a joint project with the Salut Company, a small-size enterprise).

Theoretical research on fungal biopolymers and the CW provided the foundations for pharmaceutical mycology, a new subfield of biotechnology. Developing and patenting two new drug preparations, mycoran and mycolipin, was an important achievement in this field. The preparation mycoran possesses antiburn and wound-healing activities. It was developed in collaboration with the Vishnevskii Surgery Institute and its employment for clinical purposes was permitted by an order of the Ministry of Health of the Russian Federation. It is currently used in Russian clinics [64, 65], including the burn therapy center of Hospital no. 36, which treated the victims of the terror attack in a Moscow subway line in 2004. Chitin and chitosan, the aminopolysaccharides of the CW of mucorous fungi, are the active components of mycoran. The other preparation, mycolipin, was developed together with the Oncology Center of the Russian Academy of Medical Sciences to treat prostate cancer [66]. Clinical tests carried out in 2002–2003 indicated that mycoran, apart from its antiburn activity, can be used for treating diabetics with purulent wounds that do not heal for a long time. The developers of these preparations were awarded gold and silver medals and certificates at Russian and international exhibitions.

Research on bioactive lipids, including pharmaceutically active fatty acids, is currently in progress. Of considerable interest in this context are the data obtained by I.V. Konova's team that *P. debarianum* exhibits high lipolytic activity. *P. debarianum* can be considered, therefore, a source of lipooxygenase in addition to polydesaturated fatty acids. It was demonstrated that fatty acids produce a stimulatory effect on the formation of γ-linolenic acid by mucorous fungi [67]. The laboratory team has initiated a new research direction aimed at developing biotechnological methods of cultivating *Basidiomycetes* and evaluating their nutritive value and clinical potential. These studies are conducted together with Russian professional cultivators of fungi. The development of a new type of cosmetics has also been initiated. Fungal biologically active substances are currently used for preparing ointments and creams such as a cream produced from the mycolycopene and β-carotene of mucorous fungi.

Further studies in the above areas of research are to be conducted by the laboratory team. However, the team plans to extend its research to new representatives of *Basidiomycetes.* Recently, these fungi have received special attention owing to the features they share with plants (the presence of betaine-containing lipids, sucrose, the cold shock proteins dehydrines, etc.), to their biologically active substances used in medicines and cosmetics, and to the high nutritive value of their fruiting bodies. In the future, studies will be conducted on the dimorphism phenomenon in *Mucorales.* In particular, the relationship between the composition of membrane lipids and sterols and the morphology of fungal cells (choosing between the yeastlike and the

mycelial habit) is to be elucidated. An interesting idea is to investigate the ergosterol endoperoxide of fungi (e.g., of *Absidia corimbifera*), whose presence can be considered a pathogenicity criterion. Another promising field of research that has not yet been sufficiently investigated deals with heterothallism, including the physiological and biochemical differences between the male and the female strains of zygomycetes. This research area, in combination with microbial endocrinology, one of the leading branches of biology, will form part of the new research directions to be developed at the Institute of Microbiology of the Russian Academy of Sciences.

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