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REVIEWS

# Trehalose: Chemical Structure, Biological Functions, and Practical Application

**E. P. Feofilova[a, 1], A. I. Usov[b], I. S. Mysyakina[a], and G. A. Kochkina[c]**

[a] *Winogradsky Institute of Microbiology, Russian Academy of Sciences, pr. 60-letiya Oktyabrya 7, k. 2, Moscow, 117312 Russia*

[b] *Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Leninskii pr., 47, Moscow, 119991 Russia*

[c] *Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, pr. Nauki 5, Pushchino, Moscow oblast, 142290 Russia*

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**Abstract**— Up-to-date information concerning the chemical structure and properties of trehalose, its natural occurrence and biological functions in plants, fungi, and prokaryotes, as well as its practical application, mainly in medicine and biotechnology, are reviewed. A special section deals with the role of trehalose and other protective polyols in stress processes in fungi.

*Keywords*: trehalose, protective carbohydrates, microorganisms, mycelial fungi, bacteria

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Trehalose was discovered in the mycelium of a fungal rye parasite *Claviceps* sp. in 1832 [1]. The French chemist Marcellin Berthelot was the first to isolate this disaccharide in considerable amounts as a sweet substance from the nests and cocoons of the insects *Larinus maculatus* and *L. nidificans*. This is where the name probably came from—cocoons of the beetle were called "Trehala manna" [2, 3], although there is another name for the disaccharide, mycose [4]. It turned out that trehalose is widely spread in nature and occurs in various species, from bacteria to invertebrates. The highest amount of trehalose (16 to 30%) is found in the spores of mycelial fungi and in yeasts [5, 6]. The presence of the sugar in mammals has long been doubted, although active trehalase, an enzyme cleaving trehalose in the intestine down to two glucose molecules [7] and acting as a marker of impaired kidney channels tubules [8], has been discovered in some humans back in 1965. A special gene involved in trehalose synthesis has also been discovered in many organisms with very low, hardly detectable trehalose levels. These data suggest that trehalose is a universal compound typical of all living beings.

In the middle of the past century, chemical synthesis of trehalose has been performed and vast knowledge on metabolism and natural sources of the disaccharide has been accumulated [9, 10]. For a long time trehalose had been viewed as an energy reserve, until evidence of its signal functions and ability to protect proteins and membranes under stress conditions, such as dehydration and heat shock, appeared. These data stimulated numerous works on specific physicochemical properties of trehalose and its role in living systems. The number of works focusing on trehalose is measured by thousands (for example, by the beginning of the year 2000, over 4600 studies mentioning the disaccharide have been reported [3]), and the most important results have been reviewed ([2, 11–13] and others). However, the main biological function of trehalose was comprehended but recently: the molecule of trehalose maintains and preserves life in a dormant state under unfavorable conditions (which probably applies to any living organism). The idea may probably be expressed more definitely: we owe trehalose the existence of nature on our planet despite all the strains our civilization crushes upon it. The main purpose of this review was to justify this statement and to demonstrate how the theoretical concepts on the protective role of trehalose promote the studies aimed at practical applications of trehalose, particularly, in the field of medicine. Data on trehalose interaction with other protective compounds (polyols) are analyzed for the first time with the *Mycota* kingdom as an example.

## CHEMICAL STRUCTURE AND PROPERTIES OF TREHALOSE AND ITS DERIVATIVES

The molecule of trehalose is built from two D-glucose molecules (Fig. 1). Similar to sucrose, an even more abundant disaccharide, trehalose is a nonreducing sugar since both glucose glycoside groups are blocked. Since monosaccharides may adopt an α or β-configuration and either a pyranose or a furanose

[1] Corresponding author; e-mail: feofilov@inmi.host.ru, biolog100@bk.ru

form, theoretically, 10 disaccharides comprising two D-glucose moieties linked via the 1 ↔ 1 bond are possible. The natural disaccharide is built from two identical α-D-glucopyranose moieties, its full name is α-D-glucopyranosyl-α-D-glucopyranoside, which is usually shortened to α,α-trehalose or simply trehalose. Isomeric α,β-trehalose and β,β-trehalose—different only by configurations of the glycoside centers—were synthesized; the corresponding disaccharides containing furanose forms of glucose are apparently unknown. A review by Birch [9] describes in detail how trehalose structure has been determined using the classical methods of carbohydrate chemistry.

Due to its high solubility in water–ethanol mixtures, trehalose may be easily isolated from biological materials by extraction with 70–80% ethanol upon heating. The disaccharide crystalizes as a dihydrate, mp 96–97°C, $[\alpha]_D$ +177–182, and may be identified in the form of trehalose octaacetate, mp 97–98°C, $[\alpha]_D$ +163. For quantitative determination of trehalose, either high-performance liquid chromatography with internal standards [14] or the effect of the highly specific enzyme trehalase in combination with D-glucose oxidase [15] are used. Trehalose has characteristic $^1H$ and $^{13}C$ NMR spectra, which may be efficiently used for trehalose detection and quantitative determination in biological samples [16, 17]. Other physicochemical characteristics of trehalose have been described in detail in a review by Otake and Wang [18]. Extraction from the biomass of baker's yeast has been proposed for preparative isolation of moderate amounts of trehalose [19].

Apart from free trehalose, several types of its derivatives occur in nature, including trehalose-6-phosphate, an intermediate product of its biosynthesis from uridine diphosphate glucose and glucose-6-phosphate [8, 10]. The products of glycosylation of trehalose molecules resembling similar derivatives of sucrose (raffinose, stachyose, etc.) are also known. For example, tetrasaccharides α-Glc-(1 → 4)-α-Glc-(1 ↔ 1)-α-Glc-(6 ← 1)-α-Gal, α-Gal-(1 → 6)-α-Gal-(1 → 6)-α-Glc-(1 ↔ 1)-α-Glc and β-Glc-(1 → 6)-β-Glc-(1 → 6)-α-Glc-(1 ↔ 1)-α-Glc and trisaccharides α-Glc-(1 → 4)-α-Glc-(1 ↔ 1)-α-Glc and β-Glc-(1 → 6)-α-Glc-(1 ↔ 1)-α-Glc were isolated from mycobacteria [20]. The presence of some of these oligosaccharides was noted in other bacteria, insects, and fungi.

Unusual derivatives of trehalose are glycolipids in which a trehalose residue constitutes the polar part of the molecule [21]. These glycolipids were initially detected in tuberculosis bacteria and were termed a cord factor, or a pathogenicity factor of the bacteria. In 1956 the structure of the cord factor was established to be trehalose 6,6'-dimycolate. The structure of mycolic acids was the subject of many subsequent studies. They were found to be branched $C_{60}$–$C_{90}$ acids with at least two chiral centers, in α- and β positions with respect

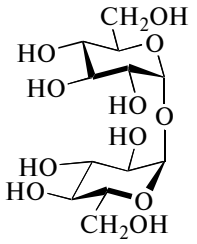


**Fig. 1.** Structure of the trehalose molecule.

to the carboxyl group, adopting R-configuration [22]. In the molecules of mycolic acids, α branches and meromycolate branches are distinguished (Fig. 2). In glycolipids isolated from various mycobacterial species or strains, α branches of mycolic acids differ only by length, while the meromycolate regions are more variable and may differ by their functional composition. Most often these regions contain *cis*-cyclopropane units, although oxygenated mycolates containing methoxy, keto, and epoxy groups occur as well [21] (Fig. 2).

From corynebacteria, 6,6'-diacyl trehalose derivatives similar to the cord factor were isolated f [23]. The constituent corynomycolic acids have a somewhat simpler structure than mycolic acids, although they also contain two chiral centers in R-configuration (Fig. 3) [22]. In these glycolipids, $C_{32}$ corynomycolic acid is found most often, although acids with shorter chains ($C_{24}$–$C_{30}$) and their dehydrated derivatives also occur. Still simpler acids were detected in maradolipids of the dormant larvae of the nematode *Caenorhabditis elegans*. They were the first representatives of 6,6'-diacylated trehalose derivatives isolated form an animal species [24].

Virulent mycobacteria also contain sulfoglycolipids based on the trehalose 2-sulfate. Other hydroxyls of the trehalose residue may be acylated by palmitic, stearic, or more complex acids, such as phtioceranic or hydroxyphtioceranic acids [21].

## TREHALOSE BIOSYNTHESIS

Trehalose biosynthesis occurs via several pathways [25]. In the cells of yeasts, basidiomycetes, insects, and a number of other organisms, the disaccharide is formed either from α-D-glucosyl phosphate and D-glucose under the effect of trehalose phosphorylase [26], or from UDP-glucose and glucose-6-phosphate with subsequent dephosphorylation [27]. In some bacteria, an enzyme catalyzing an intramolecular rearrangement of maltose to trehalose was found [28]. Other bacteria are capable of utilization of starch or maltodextrins as a starting material for trehalose synthesis. In this case, first maltooligosyl trehalose synthase (MTSase) catalyzes formation of a trehalose residue at the reducing end of maltooligosaccharide; then

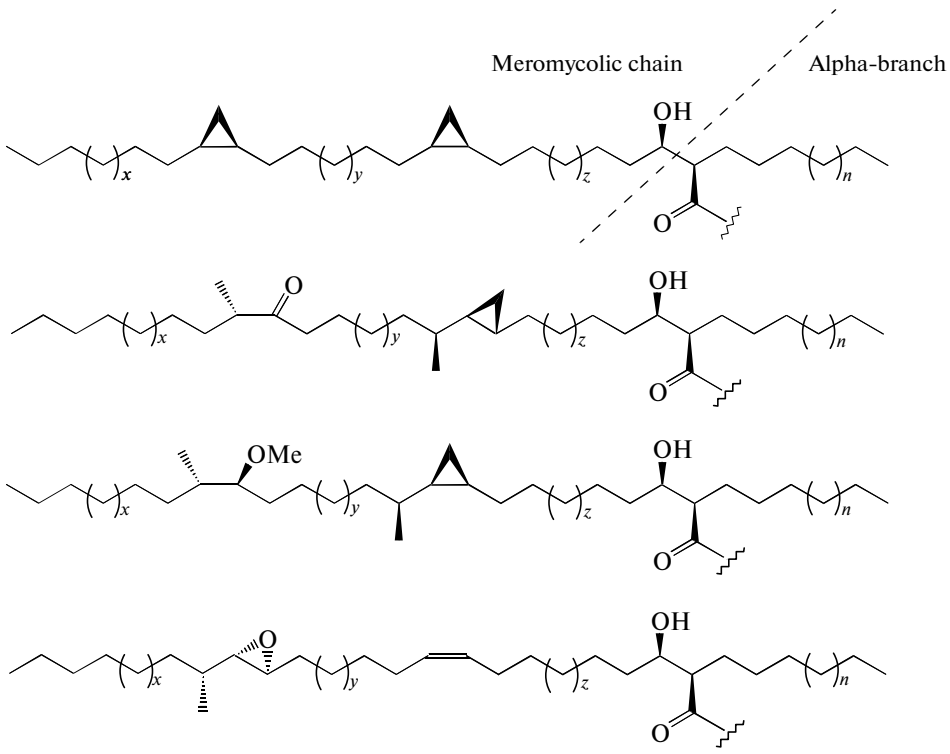


**Fig. 2.** Residues of mycolic acids in the structure of the cord factor (*x*, *y*, and *z* are the total lengths of $C_{60}$–$C_{90}$ chain, *n* = 13–19) [21].

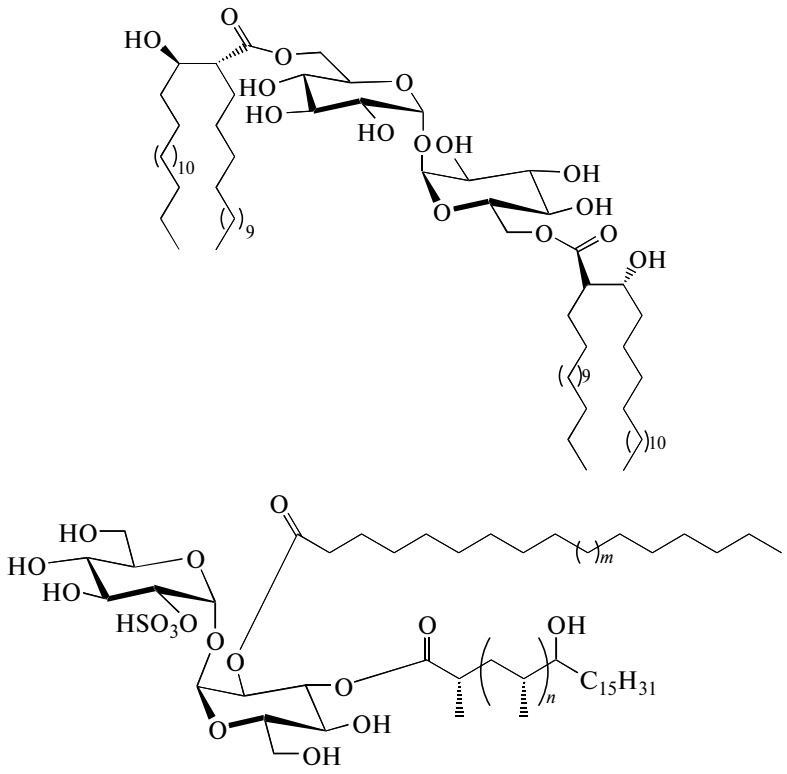


**Fig. 3.** Structure of trehalose 6,6'-dicorynomycolate (top) and diacylated trehalose 2-sulfates (bottom) (*m* = 1 or 3, *n* = 2–9) [21].

hydrolysis occurs under the effect of maltooligosyltrehalose trehalohydrolase (MTHase), resulting in trehalose cleavage and releasing the new maltooligosaccharide to participate in the next stage of the transformation [20, 30].

Multiple possibilities for various practical applications of trehalose led to the rise of a large series of works aimed to ensure availability of the disaccharide. Since chemical synthesis of α,α-trehalose is too complicated [31], biotechnological methods to produce it were developed. In principle, all of the above-mentioned biosynthetic pathways could be used for its biotechnological production. During the development of a biotechnological method, many additional factors are to be taken into account, such as the occurrence of the raw material, availability and stability of enzymes, reaction conditions, methods for the isolation of the product, etc.

## BIOLOGICAL FUNCTIONS OF TREHALOSE

### *Trehalose Functions in Plants*

In the past century, textbooks on plant physiology claimed that the disaccharide is normally not found in plants, but may appear under certain conditions and evidences forthcoming death of a plant [32]. The presence of trehalose in plants is presently undoubted, since it had been revealed in representatives of the angiosperms *Myrothamnus flabellifolius* (family *Myrothamnaceae*) and in herblike spore plants *Selaginella lepidophylla*. Later, trehalose was detected in rice and tobacco plants, although at very low concentrations, 10 μg/g [33], and the role of this disaccharide remained not fully clear [34]. It is supposed that trehalose plays a protective role during abiotic stress and protects some plants from a number of pathogens. At the same time, trehalose is a molecule of two faces [34]; for example, it may destabilize starch metabolism, therefore its role in the processes associated with stress in plants requires more thorough investigation.

Most sugars, as for example sucrose, the most abundant sugar in plants, act as signal molecules [35]. Currently, the data which would allow for a conclusion that trehalose also plays a role in signaling are insufficient. The difficulty is that this disaccharide interacts with the phospholipids of the lipid bilayer of the membranes and with the proteins that are parts of the cellular signal transduction systems. It should be taken into account that, apart from sucrose and trehalose, arbutin, methylarbutin, and amygdalin, which may also play protective function under stress conditions, are present in anhydrobiotic plants. Moreover, vitrification or transition into an amorphous state when a supercooled fluid with extremely low viscosity (the glassy state) is formed is an additional mechanism of membrane protection from dehydration in plants. A very important factor in surviving the anabiotic state is the condition of membranes, with phosphatidylcholine and sphingomyelin, which contain saturated fatty acids as their acyl chains in the membrane, promoting recovery of the organism from anabiosis. To conclude, it should be emphasized that investigation of the role of trehalose and trehalose-6-phosphate in both the processes of information transduction in cells (glycocode) and abiotic and anabiotic stresses started rather recently, and the data obtained require further confirmation [34].

### *Trehalose Functions in Bacteria*

The set of mutually compatible osmoprotectors in bacteria includes polyols (mainly, glycerol), sugars (sucrose, trehalose), amino acids (proline, glutamic acid), and quaternary amines (glycine betaine, ectoine, hydroxyectoine) [36]. The most common osmoprotectors are ectoine and glycine betaine in haloalkaliphilic bacteria and trehalose, L-proline, and glycine betaine in other prokaryotes. However, each protector has its unique function. The role of trehalose is very important upon bacteria dehydration. For example, this disaccharide protects the membranes of *Escherichia coli* and promotes settling of their glassy state upon drying, with the process not involving glycine betaine [37]. Trehalose synthesis is induced upon *E. coli* incubation at low temperature, and trehalose is necessary for the bacteria to survive cold [38]. At the same time, glycine betaine, in contrast to trehalose, does not provide for the thermoprotective effect under heat shock, but protects proteins upon thermal denaturing, acting as a stabilizer. A similar effect is produced by choline [39].

### *Trehalose Functions in Fungi*

**Dynamics of trehalose content in fungi during growth and in a quiescent state.** The life cycle of fungi (as of many prokaryotes) may be arbitrarily divided into two stages: (1) the stage when active growth occurs and primary metabolism products are formed, and (2) the stage when growth slows down, secondary metabolic products appear, and quiescent cells start to form. Both these stages involve the process of cytodifferentiation; we should pay special attention to the modern definition of the term. Cytodifferentiation is often viewed as a process leading to specialization of cell metabolism and functions. Differentiation is not necessarily a transition from a simple organization to a complex one; some properties may be lost by an organism, and some gained. Currently, differential gene expression is considered the main mechanism of cytodifferentiation. A specific feature of cell differentiation is that it irreversibly leads to a certain type of cells; this process, proceeding under genetic control, is termed determination. In the course of cytodifferentiation, the genes act at different time points, which may be seen as transcription of different mRNAs, that

is, the genes are being repressed or derepressed. A special role in the process belongs to mRNAs, while tRNA and rRNA also influence the process of cytodifferentiation, although at a lesser extent. The templates regulating such cytodifferentiation as the sporulation process are long-living, are regulated at the level of transcription, may be synthesized at the early stages of development of an organism, and remain in an inactive state for a long time, until a special signal associated with the activation of trehalose degradation arrives from the cytoplasm.

Differentiation and determination are supposed to be based on the special cell signals [40]; a tight connection between co-evolution and multispecies communication may be followed through in the case of fungal communication, and the process should be viewed in the context of fundamental biological functions, such as growth, morphogenesis, sexual interactions, etc.

Cytodifferentiation is often a result of stress (medium exhaustion, etc.) and its effect differs, depending on the developmental stage of an organism. Undoubtedly, it is useful to distinguish between the programmed and non-programmed stress [2]. A considerable difference between the two types of stress lies in the fact that in the former case the organism is already prepared for transition to a quiescent state and the processes of secondary metabolism and cytodifferentiation, including morphological cytodifferentiation, i.e., formation of quiescent cells (spores). In the case of non-programmed stress, the process of cytodifferentiation of the mycelium is hardly pronounced, the bilayer membrane has a different lipid composition, and the terminal goal of the antistress mechanisms is transition to homeostasis, rather than to quiescence. Interestingly, while trehalose is synthesized upon the effect of both types of stress, the fate of this metabolite clearly depend on the type of stress.

Using nuclear magnetic resonance (NMR) and $^{13}C$-labeled trehalose, it was found that in the process of yeast asci germination up to 80% of accumulated trehalose is transformed into glucose, which is not, however, used for growth purposes [41]. The remaining 20% of trehalose form a special pool contained in special cell compartments. Addition of labeled glucose revealed that it, rather than trehalose, was used in the processes of germination of dormant cells. Moreover, this disaccharide is actively removed from the cells during spore germination; under the effect of trehalase, trehalose is being cleaved to glucose and then is transformed to glycerol, which is rapidly excreted from the cells. At the same time, glucose obtained from trehalose by the action of trehalase is used as a carbon reserve in stored spores in the state of exogenous quiescence. These data lead to a conclusion that, in the course of growth processes, particularly during spore germination, accumulated trehalose is not used, but is actively removed from a cell in the form of glycerol. At the same time, during the storage of spore material, trehalose content gradually decreases as it is used to maintain the state of exogenous quiescence. Many issues remain incompletely clear, particularly, the reason for a cell to retain a pool of trehalose and or for glucose formed of trehalose upon spore germination to go unutilized; but the main idea is that high content of trehalose in the cell is incompatible with growth processes (it is not without reason that trehalose is often called a quiescence sugar).

Under non-programmed stress, as a rule, trehalose is formed at a high rate, since this disaccharide is an antagonist of growth processes and under any type of stress an organism needs to stop any processes of growth and proliferation. In this case, the simplest way is to remove glucose by transforming it to trehalose. However, the data evidencing that trehalose accumulation is not always a stress response have appeared lately [42]. For example, in a mutant strain *Saccharomycopsis fibuligera* A11 with high content of trehalose neither activation of trehalose phosphate synthetase nor changes in trehalose content were revealed under various stress impacts. These data evidence that much remains unknown concerning the functional importance of trehalose. The investigation of thermophilic fungi are of interest, for in these fungi active synthesis of trehalose coincides with the activation of growth processes [43], rather than with the idiophase.

**Effect of trehalose on the virulence and dimorphism in fungi.** In a number of works, the effect of exogenous trehalose on the manner of growth (dimorphism) of mucoraceous fungi has been demonstrated [44, 45]. Under conditions of cultivation favoring yeastlike growth, development of fungi exclusively via the mycelial type accompanied by an increase in trehalase activity [44] and increased level of polyunsaturated fatty acids (γ-linolenic and arachidonic acids) [45] was observed in the presence of trehalose, which evidences the effect of trehalose on the realization of morphogenetic programs in fungi.

Biosynthesis of trehalose affects not only the fungal development and stress response, but their virulence. However, in this case no uniform picture is observed. Thus, although trehalose biosynthesis is required for many processes, including the manifestation of virulence in *Cryptococcus gattii* (*TPS1* and *TPS2* genes) [46] and *Candida albicans* (*TPS1* gene) [47], impairments in the Δ*tpsAB* (trehalose synthase) gene, on the contrary, were accompanied by an increase in virulence in *Aspergillus fumigatus*, a pathogen causing invasive and often fatal lung infections [48].

In a model of mouse systemic candidiasis, the ability to form hyphae and pseudohyphae and, consequently, the virulence decreased in *Candida albicans atc1*Δ mutants (the gene codes for acidic trehalase bound to the cell wall), which were unable to grow on exogenous trehalose [49].

**Role of trehalose in stabilization and protection of proteins and membranes upon dehydration.** The state of anabiosis is characteristic of many organisms and in

most cases is associated with water losses. Some species, for example, rotifers, tardigrades, and nematodes, were found to be able to lose up to 93% water, while the vital functions of these organisms were completely restored in the presence of water. Many organisms were found to survive significant water losses (worms, up to 73%, turtles, 53%, domestic mice, 30%, etc.), with a corresponding decrease in the time they can stay in anabiosis [6]. Upon dehydration of microorganisms, in particular, of yeasts, as in the case of any stress, rapid synthesis of trehalose was noted [13, 50–54]. This process is probably universal, since it has been observed even in archaea [55]. An exception is, however, known: plants in such a situation preferably produce another disaccharide, sucrose, although trehalose has a lower capacity for crystal formation, the process which is dangerous for organisms upon dehydration and temperature decrease.

In the course of dehydration, trehalose stabilizes proteins and phospholipids in the lipid bilayer. In the process, the effect of trehalose on microviscosity—an integral value associated, among other things, with the rate of unsaturation of acyl chains of the lipids—is of particular importance. Microviscosity was found to increase in parallel with the concentration of trehalose in the cells; this affects protein folding and enzyme activity [56]. The mechanism of biomolecular stabilization was studied by Fourier-transformed infrared spectroscopy (FTIR) to characterize the interactions between trehalose, proteins, and sugars. Infrared and Raman spectroscopy were subsequently used to elucidate the importance of trehalose molecules, concentrating the remaining water close to proteins and thus stabilizing their structure [26].

The role of trehalose in stabilization of phospholipids is different. It interacts with lipids through formation of hydrogen bonds between OH groups of the disaccharide (replacing 10–12 water molecules) and the polar head groups of phospholipids, thus substituting the water removed upon dehydration and preserving the membrane [12, 57]. It should be noted that upon freezing and dehydration the rate of unsaturation of the lipid acyl chains increases considerably, which makes them more susceptible to peroxidation. In this case, the role of trehalose is more important for stabilization of the lipid bilayer than that of its close analogue, sucrose. Besides, water loss upon dehydration leads to changes in the organization of bilayer structure of membranes, that is, to an increase in the temperature of the gel–liquid crystal phase transition ($T_m$), and for some types of phospholipids, to formation of non-bilayer structures, when normal functioning of the membranes is impossible. In this case, trehalose efficiently prevents dehydration-induced increase of $T_m$ by the above-mentioned interaction with the polar head groups of phospholipids [58].

*Protective Polyols and Trehalose upon Stress in Fungi*

Apart from trehalose, a number of compounds capable of protective functions under stress are presently known; they include polyols glycerol, glycosylglycerol, arabitol, mannitol, and sorbitol, as well as amino acids glutamate, glycine, glycine betaine, ectoine, gamma-aminobutyric acid, proline, and betaine. In recent years, new types of protective compounds, such as trimethylamine-*N*-oxide, attract interest. In fungi, the effects of polyols, especially of mannitol and glycerol, are most studied [59].

Biochemical adaptation of fungi to temperature stress provided by carbohydrate protectors varies depending on taxonomy [59]. In representatives of the class *Zygomycetes*, *Cunninghamella japonica* and *Absidia coerulea*, a single biochemical mechanism based on glucose transformation into trehalose is realized. Trehalose stabilizes the membrane lipids and is a depot of such a highly active substrate as glucose. Interestingly, only under conditions of deep cooling (to 10°C), traces of glycerol appear in representatives of these species, and in a (–) *Blakeslea trispora* strain the stylospores obtained under hyperthermal conditions contain another polyol, inositol.

In fungi belonging to the class *Ascomycetes*, particularly *Aspergillus japonicus* and *Myceliophthora thermophila*, the set of cytosol carbohydrates is more representative. In addition to trehalose, it includes a number of polyols, also acting as protectors. A tendency to form trehalose and inositol under conditions of hyperthermia, and mannitol and glycerol, under hypothermia, is observed. An interesting feature associated with the optimal temperature of growth is observed. In a thermophilic *M. thermophila*, with optimal growth at 41–42°C, glycerol is not formed under low-temperature shock conditions, but this polyol is observed in a mesophilic *A. japonicus* with a lower optimal growth temperature.

The cytosol of basidiomycetes contains an even wider set of carbohydrate protectors. In these fungi, adaptation to temperature proceeds via other biochemical mechanisms. In *Lentinula edodes*, arabitol, and not mannitol as in ascomycetes, acts as a cryoprotector under hypothermia, although, similar to the latter ones, glycerol is detected.

Taking into account the optimum temperatures for fructification of fungi under natural conditions, several suppositions on the functional role of cytosol carbohydrates in basidiomycetes may be proposed. Additional valuable information was provided by the experiments on the effect of cold shock on *L. edodes* fungi. For this purpose, *L. edodes* mycelium grown on a solid medium at 26–27°C was subjected to cold shock (36 h at 8–10°C). A decrease in cultivation temperature initiated formation of primordia, in which arabitol became the dominating carbohydrate, with its content increasing from 30 to 65%. The level of mannitol decreased insignificantly, and the amount of trehalose

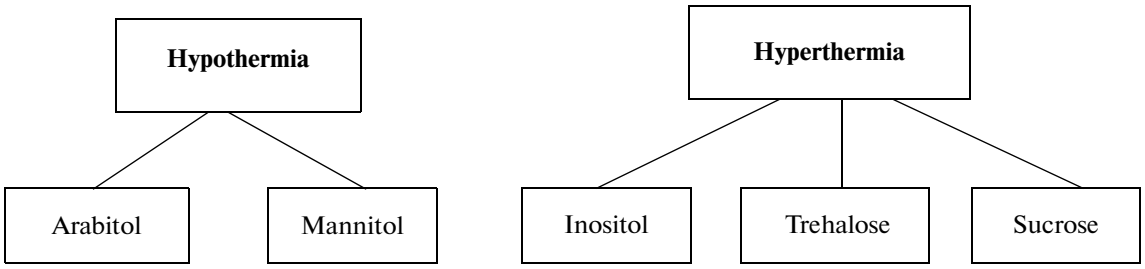


**Fig. 4.** Protective polyols of basidial fungi under conditions of hypo- and hyperthermia.

decreased almost threefold. In a basidiomycete *Pleurotus ostreatus* under hyperthermia, sucrose level increased sharply, and arabitol disappeared. Attention should be paid to the fact that in the presence of sucrose, differences in temperature-dependent trehalose accumulation were observed. In all fungi studied, the amount of trehalose decreases upon decrease in the cultivation temperature, which may be explained by the fact that trehalose may be quickly converted to glycerol in cold, which leads to a sharp decrease in the amount of the disaccharide. In *P. ostreatus* this biosynthetic mechanism is probably absent and adaptation to temperature stress involves the synergetic effect of trehalose and sucrose, with their ratio determined by an increase or decrease in temperature. Consequently, appearance of sucrose among the cytosol carbohydrates changes the biochemical mechanisms regulating the adaptation to hypo- and hyperthermia in basidiomycetes. One may propose a scheme of temperature dependence of carbohydrate synthesis in basidiomycetes presented on Fig. 4.

The fact that cytosol sugars in fungi perform certain functions is confirmed by other data. Very interesting is the composition of the brown film of *L. edodes* which appears prior to fructification and differs morphologically from the mycelium. In this case, similar to the hyphae of the surface mycelium, glucose predominates, but two new carbohydrates appear, sucrose and an unidentified disaccharide. The spores of *L. edodes* lack sucrose but contain arabitol, mannitol, and trehalose at practically equal amounts, so that the spore is protected from the effect of any temperature changes by protective compounds. Basidiospores of *Pleurotus ostreatus* also contain sucrose and inositol.

These data allow for a hypothesis that the evolution of fungi followed the route of synthesis of more and more protective carbohydrates with deeper specialization of their protective functions. While in the spores of lower fungi, for example, mucoraceous fungi, only trehalose is present (trehalose accounts for 90–95% total sugars in *Cunninghamella japonicus*), ascomycetes gain polyols—erythritol, mannitol, and inositol—as the main cryoprotectors, and in basidiomycetes, as well as in plants, sucrose is detected at certain stages of ontogenesis. The latter sugar may replace trehalose and probably performs some other function in stabilization of the lipid bilayer of the membranes [59].

### *Trehalose as an Antioxidant*

Exogenous synthetic antioxidants, such as 4,4-dicumyldiphenyl-*N*-oxide, as well as the natural ones, such as α-tocopherol and ubiquinone $Q_9$, including the fungal inherent antioxidants, were found to have common biological effects, stimulate fungi growth, change the lipid composition, increase the degree of unsaturation of acyl chains in the lipids, and increase phosphatidylserine content [60] against the background increase in trehalose content. The role of trehalose as an antioxidant was demonstrated in the experiments on *Saccharomyces cerevisiae*, when the yeast cells were subjected to heat shock or to the effect of proteasome inhibitor (MG 132). This induced accumulation of trehalose and an increase in viability of the yeast cells [61]. Ethanol, being a toxic compound, is known to induce dehydration and free-radical damage to the lipids in cell structures. Increased ethanol concentration in the medium is accompanied by an increase in trehalose levels [51, 53, 54]. Earlier, the process of cell division in the cell and tissue cultures of higher eukaryotes was shown to depend not only on the antioxidant content, but on the composition of the membrane lipids, the degree of their unsaturation, and their antioxidant activity. These tendencies were confirmed in experiments with prokaryotes and lower eukaryotes, including mycelial fungi [62]. In these studies, a correlation between the unsaturation degree of the lipid acyl chains, microviscosity of the lipid bilayer of the membranes, and the rate of trehalose accumulation in the fungal mycelium was established. These data indicated the antioxidant properties of trehalose.

There is evidence that trehalose acts as a fatty acid protector in model systems [63]. The effect is achieved by prevention of (1) degradation of unsaturated linoleic and α-linolenic acids with aldehyde formation and (2) autooxidation of unsaturated acids with peroxide formation. On the basis of these data, direct interaction of trehalose with acyl chains and stabilization of

fatty acid structure were concluded [63]. The mechanism of trehalose protection against free-radical oxidation of lipids was confirmed in 2003. Analysis of the $^{1}H$ and $^{13}C$ NMR spectra made it possible to evaluate the OH···π and CH···O interactions between trehalose and fatty acids. Trehalose considerably decreased the effect of free-radical oxidation on unsaturated acyls of the lipids via weak binding with the double bonds in acyl chains [64].

## INDUSTRIAL PRODUCTION AND PRACTICAL APPLICATION OF TREHALOSE

For preparative isolation of moderate amounts of trehalose, a method of extraction from the biomass of baker's yeast, containing ~20% by weight trehalose, was proposed [18]. The conditions of yeast storage and the process of fermentation had been elaborated by a research group in Riga at the end by the 20th century [6]. Yeast cells with high trehalose content (10–16%) were shown to preserve viability during long-term storage.

Large-scale industrial production of this disaccharide is performed by using the biotechnologies based on enzymatic transformations of starch [19]. The industrial process developed in Japan utilizes starch from maize or tapioca pretreated with α-amylase, isoamylase, and a number of other enzymes to yield a mixture of unbranched maltooligosaccharides, which is then further converted to trehalose by a combined effect of MTSase and MTHase. By 2005, the production unit yielded about 30000 ton 98% pure trehalose dihydrate yearly [65]. Further propositions on improvement of the method include application of new sources, new trehalose purification techniques, and utilization of side products, that is bioethanol and gluconic acid [66, 67]. New methods of enzymatic biotransformation based on the three major trehalose synthesis pathways—phosphorylase system found in mycelial fungi and yeasts, glycosyl transferase–hydrolase system characteristic of mesophilic bacteria *Arthrobacter* sp., and trehalose synthesis from maltose catalyzed by intramolecular transglycosylation (*Thermus* ssp.) [26]—are presently considered the most advanced. While 1 kg trehalose cost almost $700 in 1990, modern technologies have reduced the price of the disaccharide considerably.

Trehalose is most frequently used for storage of foodstuffs, biomaterials, and vaccines at room temperature, for prevention of enzyme degradation, and for protection of the cells, including mammalian cells, undergoing low-temperature dehydration [68]. Trehalose is used in cosmetics to stabilize liposomes and in food industry, where it is added together with sweeteners. When stabilizing sweeteners, trehalose, unlike other sugars, does not interact with amino acids and proteins and does not lead to rise of brown coloration. This is due to the fact that trehalose lacks free aldehyde groups, and the glycoside bond joining its two α-D-glucopyranose residues is characterized by increased stability compared to sucrose, in which the β-D-fructose is in a furanose form.

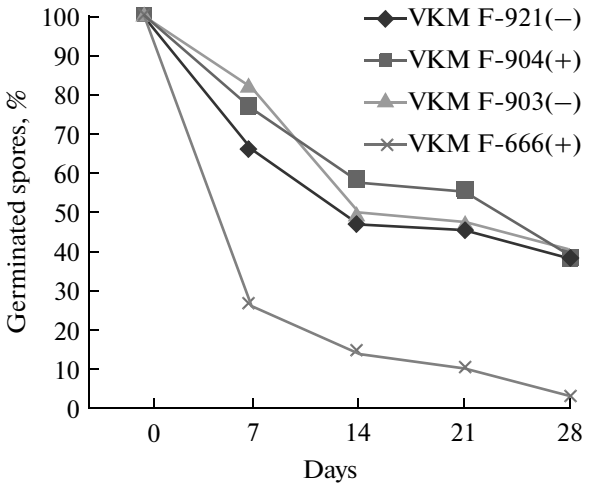


**Fig. 5.** Germination of *B. trispora* spores upon storage in water.

In some cases, trehalose may be a more efficient cryoprotector for storage of collection and industrials cultures of microorganism than glycerol, which is used traditionally.

According to our data presented in Figs. 5 and 6, spores of three out of four studied industrial carotene producer strains of *Blakeslea trispora* retained high viability in the course of storage in water at room temperature for a month. By the end of the experiment, in the case of only one strain, VKM F-666(+), the viability decreased tenfold compared to other strains. To store fungal cultures at negative temperatures, various protectors are used, glycerol being the most common one. As follows from Fig. 6a, after storage at –12°C, a sharp decrease in spore germination occurred under experimental conditions (potato–glucose medium, 5 h, 25°C) independently of the protector used. However, when trehalose was used as a cryoprotector, after three days, mycelial growth and formation of sporogenic apparatus were noted in petri dishes where the spores had been incubated to evaluate germination. When glycerol was used, the cells lost their viability completely. Assessment of the degree of viability retention by the spores after storage at –70°C demonstrated that in this case trehalose was also preferable to glycerol as a cryoprotector for *B. trispora* cultures. After 60 days of storage with trehalose, the degree of spore germination was 1.5–3 times higher than after storage in the presence of glycerol, especially in the case of the strain VKM F-666(+).

Due to its unique protective properties, trehalose becomes more and more frequently used in medicine. Currently, several preparations are known: Avastin and Herceptin (Roche); Advate (Baxter); and Lucentis

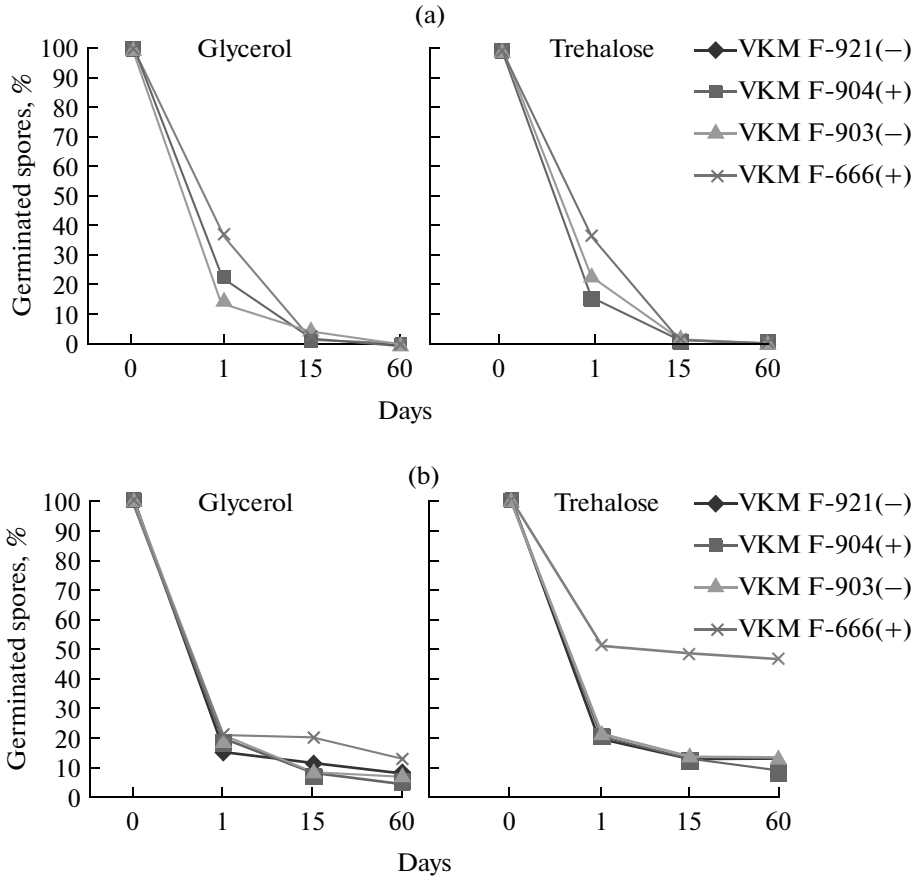


**Fig. 6.** Germination of *B. trispora* spores upon storage with protectors at –12°C (a) and –70°C (b).

(Novartis), which confirms the necessity to use trehalose as an excipient in drugs. Trehalose is used as a component of the Kyoto solution for the preservation of pancreatic gland tissue in transplantation. Trehalose is more efficient for protection of eyes from dehydration during prolonged reading than solutions of hydroxycellulose or hyaluronan. In this case, trehalose protects the corneal epithelial cells from the dry eye syndrome and from the effect of free radicals (oxidative occlusion of eye) [69]. In recent years, application of trehalose in ophthalmology has been growing [3]. Positive effect from trehalose application was reported for treatment of many diseases, including Huntington's and Alzheimer's disease. Relatively recently, trehalose and products of its metabolism have found application in crop research. For example, the gene coding for trehalose phosphorylase (TP) obtained from *Pleurotus sajor-caju* was used in the works on tobacco plant modification. The genetically modified plant accumulated trehalose and acquired higher resistance to water deficiency than the wild type plants. This direction of plant bioengineering, that is utilization of trehalose under conditions of abiotic stress, gains growing popularity [70].

To conclude, nature has created a molecule unique in its chemical composition and biological properties with the role to preserve life under virtually any conditions.

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