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REVIEW

Fungal Lycopene: the Biotechnology of Its Production and Prospects for Its Application in Medicine

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Abstract—This article deals with the lycopene of mycelial fungi. It pays special attention to its physical and chemical properties, occurrence in nature, biological functions, and the biotechnology of lycopene production. Data are presented concerning the medically important properties of lycopene and the drug Mycolycopene prepared on its basis. Its prospective use in the therapy of prostate cancers is discussed.

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The 21st century is considered the Age of Biotechnology by the international community. Mycelial fungi take a prominent place among microbial producers of biotechnologically important substances for the following reasons: (i) their physiological and biochemical properties enable us to use them for producing biologically active substances (BAS) formerly obtained from plants, animals, and bacteria, e.g., gibberellins, alkaloids, abscisin, ergosterol, and essential fatty acids, and (ii) the wide medical use of fungi over the course of the last decade has resulted in the initiation of a new medical subfield known as mycological pharmacology.

Owing to these developments, mycologists have recently changed their attitude towards fungi. Apart from sources of foodstuffs, especially protein, they have been increasingly regarded as natural, ready-to-use medicines. Mycelia and fruiting bodies are currently valued for the BAS they contain, in addition to their protein, carbohydrate, and lipid content and amino acid composition. Fungal BAS prevent the development of diseases that are widespread in the 21st century, including cardiovascular problems, malignant tumors, and diabetes.

To obtain drugs from fungi, purely empirical studies were conducted a decade ago. More recently, scientific criteria have been developed regarding the drug preparation technology.

To provide scientific foundations for goal-directed drug preparation from fungi, three areas of research have been developed: (i) detailed studies of the chemical composition of fungal fruiting bodies/mycelia; (ii) a special subfield of natural compound chemistry, which has been rapidly advancing recently; and (iii) novel analytical tools that have contributed considerably to our knowledge of new BAS contained in mycelial fungi. Among these natural substances, the most important place has been recently taken by carotenoids.

Lycopene is the carotenoid exhibiting the highest antioxidant activity. It is an acyclic carotenoid containing 11 conjugated double bonds that are characterized by a linear arrangement in the *all-trans* form. The *all-trans* isomer of lycopene occurs in an overwhelming majority of natural sources. Low amounts of the *cis* isomer, i. e., (7, 9, 7', 9'-*cis*) lycopene, are contained in certain tomato breeds along with lycopene derivatives such as neolycopene. This isomer is distinguished by a number of chemical properties including a less bright color, a comparatively low extinction coefficient, a low melting point, and peculiar absorption maximums in the UV range. Lycopene also differs from β-carotene in its physical and chemical characteristics [1]. The most important peculiarities of lycopene are its very high singlet oxygen-quenching activity and a manifest capacity to suppress the proliferation of MSF-7 tumor cells. The main difference between lycopene and β-carotene is that lycopene lacks provitamin A activity.

Lycopene is not as widespread in nature as, e. g., β-carotene. It is mainly obtained from tomatoes. Lycopene also occurs in watermelons, rosehips, pink grapefruits, guavas, papayas, and apricots [2]. Animals, including insects, are unable to synthesize carotenoids, which they obtain from food. However, it has recently been demonstrated that the American cockroach *Periplaneta americana* can synthesize carotenoids

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from their precursor, mevalonic acid pyrophosphate [3]. A large number of other organisms such as bacteria, plants, and fungi also contain carotenoids including lycopene. Most heterotrophs intensely synthesize carotenoids during the second growth phase (the idiophase). Carotenoid formation is triggered by depletion of the cultivation medium of nitrogen sources while sufficient carbon content is maintained.

The biological function of lycopene in fungi is virtually unknown. However, the experimental data accumulated up to now enable us to make a suggestion on this account. Lycopene synthesis occurs if the enzymes (cyclases) involved in the formation of β-ionone rings are inhibited in the carotenoid synthesis pathway. In this case, no β-carotene forms.

Lycopene is the strongest natural antioxidant among carotenoids. Such compounds are required for protecting the membrane lipid bilayer against the destructive effects of peroxidation under stress. In addition, active lycopene synthesis is accompanied by the cessation of reproduction processes, due to the inhibition of the formation of carotene, the precursor of sex hormones (trisporic acids) and sporopollenin. Sporopollenin is the product of oxidative polymerization of β-carotene. It forms part of the cell walls of the zygospores and sporangiospores of mucorous fungi. Accordingly, lycopene formation results in the interruption of sexual reproduction processes under stress. Owing to its high antioxidative activity, lycopene is more efficient in maintaining the integrity of membranes than other carotenoids. The fact that lycopene synthesis increases under stress supports the idea that this polyene forms simultaneously with trehalose (known as the "stress sugar"), which provides for the biochemical adaptation of cells under deleterious conditions. Hence, the onset of lycopene synthesis and the cessation of β-carotene synthesis mark the termination of reproduction (i.e., of the formation of zygospores, sex cells). It cannot proceed under stress while the organism involved is making the transition to a new level of biochemical adaptation.

Fungi of the order *Mucorales* form the largest amounts of carotenoids. However, β-, γ-, and α-carotenoids, and not lycopene, predominate under conventional cultivation conditions. Fungi of the genera *Phycomyces* and *Blakeslea* are potential lycopene producers. Spontaneous and inducible mutants as well as autoselected strains are used for this purpose. To obtain lycopene from *P. blakesleeanus*, blue-light-illuminated *carR* mutants are used [5]. However, the lycopene yield remains low even in this system. It does not conform to the commercial requirements to be met by the final product of a biotechnological process. Therefore, fungi of the genus *Blakeslea* (see below) are currently preferred in carotenoid production processes.

The biotechnology of producing carotenoids including lycopene has been intensely developed since the mid-20th century for the following reasons. Since people in Western countries prefer "refined" food containing saturated fats and purified carbohydrates and consume small amounts of vegetables and herbs, the suggestion was made that food ingredients made of plants decrease the risk of tumors including prostate hyperplasia and cancer [2, 6]. A study conducted in the late 20th century revealed some patterns characteristic of these diseases. It was established that the highest mortality rate related to these diseases occurs in Northern Europe and the USA. The most favorable situation was found in China, where only rare cases were detected [7].

In this period, the medical community gave special attention to plant carotenoid pigments with coupled double bonds and high antioxidant activity. They were inspired by the ideas of Academician N.M. Emmanuel' [8] concerning the involvement of free-radical oxidation in the formation and development of malignant tumors and the important role of antioxidants in maintaining the immune status of an organism. Intense research on plant antioxidants was initiated. It was shown that lycopene possesses the highest antioxidant activity among carotenoids. Lycopene also stimulates intercellular interactions and is involved in cell growth coordination. Unlike β-carotene, it is not converted to vitamin A and, therefore, remains in the blood plasma as the main carotenoid component. The high antioxidant activity of vitamin A is linked to its immunonodulating effect [9]. Therefore, lycopene is of potential clinical interest as an antitumor preparation.

The clinical importance of lycopene considerably increased after carotenoids were detected in the prostate, with lycopene as the dominant carotenoid species [10]. Of particular interest was the fact that the prostate contains 10 to 20 *cis* lycopene isomers and derivatives. However, the *all-trans* isomer accounts for almost 90% of the lycopene contained in food. According to the results of a study conducted with lycopene, it possesses unique properties distinguishing it from other carotenoids. β- and α-carotenes inhibit tumor cell growth to a significantly lesser extent than lycopene [11]. This finding is consistent with the data on lycopene concentrations in the prostate tissues of patients whose diet included tomato juice. The lycopene content of their prostate increased almost threefold in comparison to the control group, while the blood serum content of PSA and 8-OhdG leucocytes decreased. These data provide strong evidence of a relationship between the food lycopene content and the lycopene level of prostate tissues. This is also in accordance with in vitro and in vivo studies of tumor growth conducted in the last decade and demonstrating a protective effect of lycopene on patients with prostate cancer [11]. A hypothesis was suggested that the anticancer activity of lycopene is due to its capacity to discontinue the cell cycle at the G_0/G_1 stage by inhibiting the oncogen-induced phosphorylation of regulatory antioncogen proteins p53 or Rb [12].

Interestingly, lycopene appears to play a more important role than just decreasing the cancer risk. Data were presented in Finland in 2001 that a low lycopene level in the blood plasma correlates with the development of such diseases as arteriosclerosis and cerebral thrombosis [13]. It seems pertinent that lycopene occurs in the liver, testicles, adrenals, kidneys, prostate, lungs, blood, etc., while accumulating in three organs (the prostate, testicles, and adrenals) only [14]. This points to an important role of this carotenoid in the functioning of these organs. It was also established that a high lycopene level in cells (20 pmol/10^6 cells) decreases lipid oxidation by 86% and reduces 8-oxo-7,8-dihydro-2'-deoxyguanosine formation under the influence of the iron nitrile acetate/ascorbate system by 77%, thereby preventing cancer development caused by oxidative DNA destruction [15]. In addition, a decrease in the lycopene level may be associated with gastrointestinal diseases [16].

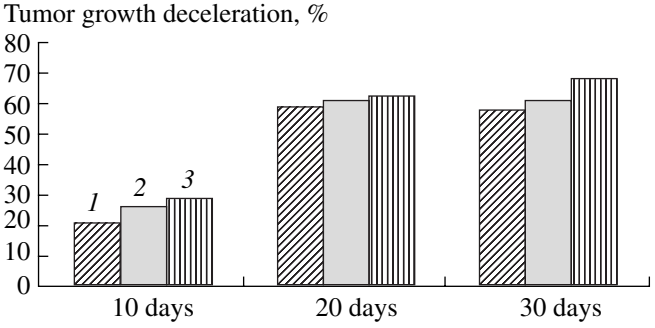
Owing to its high antioxidant activity and putative anticancer effect, lycopene has been extensively used as a food additive since 1990. For example, Betatene, a 4% lipid suspension obtained from tomatoes was advertised in 1996 as a potential anticancer preparation. Lycopene accounts for 70% of the total lipids in Betatene. The LycoRed Company (Israel) produces several preparations, including Lyc-O-Mato, with 6, 7, 10, and 15% of lycopene, and Lyc-O-Mato Powder with 0.8% of lycopene from tomatoes with unmodified genome. The Tomatol preparation obtained from tomato paste by extracting lycopene with solvents was developed by the Invest Research and Development Company in Russia [17]. Tomatol is intended to be used as a preventive and therapeutic drug, but tomato paste is not a reliable and competitive source of raw materials. Crystalline lycopene obtained from tomatoes by companies outside of Russia (Hoffmann La Roche and BASF) is currently available in the market; these companies also produce β-carotene. Recently, a German company has made considerable progress in producing synthetic lycopene, but its cost is higher than that of the already quite expensive natural lycopene from tomatoes.

The high cost of tomato lycopene is due to the fact that its production is season-dependent and threatened by the phytopathogen *Phytophthora infestans*, which can destroy the whole harvest. Additional factors that slow down the production process include the large areas required for cultivating tomatoes, pesticide use, and, above all, a labor-intensive chemical purification process, which is due to tomatoes' peculiar carotenoid composition and the presence of undesirable lycopene-like pigments including neolycopene.

However, the lycopene content in tomatoes does not exceed 30 mg per kg. Taking into account the indisputable potential value of lycopene and its current extensive use, albeit presently confined to food additives, the question arises of how to obtain the carotenoid by microbiological methods providing for its inexpensive production on a large scale.

Importantly, no industrial biotechnological technique for obtaining lycopene from fungi had been developed before we started our research. We believed that the mucorous fungus *Blakeslea trispora* that yields record carotenoid amounts (up to 4–5 g/l of β-carotene) was a particularly promising candidate. The inhibition of the cyclases that convert lycopene to β-carotene was required in order to change the carotenoid biosynthesis pathway and obtain lycopene as the main product. Changing the cultivation conditions can inhibit the lycopene cyclization stage. For instance, a neutral or weakly alkaline medium provides for the preferential formation of lycopene. In addition, lycopene cyclization was known to be blocked by substituted amines (triethylamine and 2-(4-chlorophenylthiotriethylamine)) and nitrogen-containing heterocyclic compounds (azines). Nicotine and its derivatives also stimulate lycopene synthesis in fungi that are capable of β-carotene formation [18]. These compounds stimulate lycopene synthesis not only in fungi but in plants and prokaryotes, as well; (2-chloroethyl)-trimethyl ammonium chloride is a particularly efficient agent. However, such stimulators have not yet been used for practical purposes, due to their high cost, relative toxigenicity, and the low yield of the final product. It has recently been suggested that carotenoids, including lycopene, should be obtained by modifying the metabolic pathways and applying recombinant DNA technology. For this purpose, a number of microorganisms that use the mevalonate metabolic pathway but fail to synthesize lycopene (*Escherichia coli, Candia utilis, Saccharomyces cerevisiae*, and *Zymomonas mobilis*) have been used [19]. However, a deficit in the necessary precursors and a low carotenoid yield still preclude the use of these producers for obtaining the required amounts of the end products using microbial synthesis.

We have suggested a new stimulator of lycopene synthesis, MAP, that is an azine and almost completely inhibits lycopene cyclization without producing any toxic effect when added at a concentration of 0.005% to the fermentation medium [20]. Testing the conventional method used for extracting lycopene from tomatoes revealed that this method is not suitable for *B. trispora* mycelium. Therefore, we developed a new method of obtaining lycopene [21]. However, scaling up the process of lycopene production from fungi under industrial conditions and determining the cost of the final product demonstrated that the method of extracting the carotenoid should be modified. Its main shortcomings include a low yield of the lycopene obtained by vegetable oil extraction and significant losses associated with its subsequent recrystallization. Further studies revealed that this extraction method also results in the loss of a number of valuable biologically active compounds that can be used as medicines. These are neutral lipids whose biological value, apart from the vital linoleic acid ($C_{18:2}$) accounting for over 50% of their



Deceleration of the growth of prostate tumors in ACI rats by Mycolycopene (*1*), surfagon (*2*), and their combination (*3*).

fatty acids, is due to the presence of palmitoleic acid ($C_{16:1}$) that protects membranes against stress effects. In addition to lycopene, the lipid globules of *B. trispora* mycelium contain ubiquinones and other natural antioxidants.

Therefore, it was expedient to retain these compounds in the lycopene-containing extract from mycelium biomass and to make the lycopene production method less expensive and more efficient. These goals were attained by developing Mycolycopene, a new medicine [22] on the basis of fungal lycopene. This novel biologically active substance is produced in the form of gelatin capsules that contain a 2% lycopene suspension and the above-mentioned BAS (ubiquinol, essential fatty acids, etc.) in sunflower oil. The tests conducted at the Laboratory for Developing Nontoxic Immunomodulators of the N.N. Blokhin Russian Oncology Center (Russian Academy of Medical Sciences) revealed that, apart from antioxidant activity, Mycolycopene possesses antimutagenic, radioprotective, and immunomodulating properties. These properties manifest themselves to a greater extent with Mycolycopene than with a 2% suspension of crystalline fungal lycopene (with a purity level of 95%) in sunflower oil. For example, the radioprotective effect of Mycolycopene is two times stronger.

We also tested the antitumor effect of Mycolycopene on male rats of the ACI line with an inoculated prostate tumor obtained from inducible tumor tissues according to the procedure developed in the Laboratory for Experimental Tumor Endocrine Therapy of the N.N. Blokhin Russian Oncology Center (Russian Academy of Medical Sciences). For comparison, we used the peptide hormone surfagon, an analogue of luliberine (the gonadotropin super-releasing factor). The rats were subdivided into four groups with ten rats in each. The test took 30 days, and the preparations were administered to them daily. The rats of the first group served as controls and received 0.2 ml of sunflower oil per diem. The rats of the second group were given Mycolycopene, starting from the second day after inoculating the tumor (2.5 mg/kg). The rats of the third group received 10 μg/kg of surfagon following tumor inoculation. The rats of the fourth group received surfagon and Mycolycopene, starting from the second day after inoculating the tumor.

The data obtained are shown in the Figure. They indicate that an antitumor effect occurred in all groups except the control. The tumor growth deceleration (TGD) level with Mycolycopene (58%) after 20 days was close to that with surfagon (60%). The highest TGD values were obtained with the Mycolycopene–surfagon combination (68%). Importantly, the combined effect of these preparations resulted in a better physical state of the rats involved (they had a smooth fur and were highly active) than the effect of surfagon alone. Accordingly, Mycolycopene is of indisputable interest in terms of prostate cancer therapy, based on its TGD value.

Tomatoes contain 0.05–0.1% of lycopene in their dry biomass, whereas our biotechnology enables us to produce up to 3.0–4.0% of lycopene in fungal biomass. The lycopene yield is 1.3–1.5 g/l during fermentation. Based on these findings, the above clinical tests with Mycolycopene, and the advantageous biotechnology of its production, studies aimed at using Mycolycopene in medicine are should be considered a promising area of research. The data obtained on the antitumor effect of Mycolycopene open up new possibilities for employing this drug in a complex approach to prostate cancer therapy.

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