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Articles and Statements

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Can the First Organic Forms Of Life Originate in Hot Mineral Water with HDO?

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Abstract

The isotopic composition, the temperature and the pH value of water were analyzed in experiments with modelling the primary hydrosphere and hydrological conditions for origin of first organic forms in hot mineral water with HDO. For this aim we performed experiments with hot mineral water and seawater from Bulgaria and water with varying content of deuterium using IR- and DNES-spectroscopy. As model systems were used cactus juice of *Echinopsis pachanoi* and Mediterranean jellyfish *Cotylorhiza tuberculata*. The reactions of condensation–dehydration occurring in alkaline aqueous solutions at t = 65–95 °C and pH = 9–10, resulting in synthesis from unorganic molecules the larger organic molecules as polymers and short polipeptides, were discussed, as well as the possible mechanisms of the deuterium accumulation in form of HDO in hot water. It was shown that hot alkaline mineral water with temperature from +65 °C to +95 °C and the pH value from 9 to 11 is more suitable for the origination of life and living matter than other analyzed water samples. In hot mineral waters the local maximums in IR-spectra are more manifested compared to the local maximums obtained in IR-spectra of the same water at a lower temperature. The difference in the local maximums from +20 °C to +95 °C at each +5 °C according to the Student *t*-criterion makes up $p < 0,05$.

Keywords: deuterium, hydrosphere, evolution, origin of life, IR spectroscopy, DNES.

Introduction

The previous biological experiments with D_2O and structural-conformational studies with deuterated macromolecules, performed by us, enable to modeling conditions under which the first living forms of life might be evolved [1–3]. The content of deuterium in hot mineral water may be increased due to the physical-chemical processes of the deuterium accumulation. It can be presumed that primary water might contain more deuterium at early stages of evolution of first

living structures, and deuterium was distributed non-uniformly in the hydrosphere and atmosphere [4]. The primary reductive atmosphere of the Earth consisted basically of gas mixture CO, H_2, N_2, NH_3, CH_4, lacked O_2–O_3 layer protecting the Earth surface from rigid short-wave solar radiation carrying huge energy capable to cause radiolysis and photolysis of water. The processes accompanying accumulation of deuterium in the hydrosphere are solar radiation, volcanic geothermal processes and electric discharges in the atmosphere. These natural processes could lead to the enrichment of the hydrosphere by deuterium in the form of HDO which evaporates more slowly then H_2O, and condenses faster. If this is true, this is a significant fact regarding thermal stability of deuterated macromolecules in the preservation of life under thermal conditions, because chemical bonds with participation of deuterium are stronger than those ones formed of hydrogen.

The natural prevalence of deuterium makes up approximately 0,015–0,020 atom%, and depends strongly on the uniformity of substance and the total amount of matter formed in the course of early Galaxy evolution [5]. The average ratio of $D/^1H$ in nature makes up approximately 1:5700. In natural waters, the deuterium is distributed irregularly: from 0,02–0,03 mol.% for river water and sea water, to 0,015 mol.% for water of Antarctic ice – the most purified from deuterium natural water containing in 1,5 times less deuterium than that of seawater. According to the international SMOW standard isotopic shifts for D and ^{18}O in sea water: $D/^1H = (155{,}76\pm0{,}05)\cdot10^{-6}$ (155,76 ppm) and $^{18}O/^{16}O = (2005{,}20\pm0{,}45)\cdot10^{-6}$ (2005 ppm). For the SLAP standard isotopic shifts for D and ^{18}O in seawater make up $D/^1H = 89\cdot10^{-6}$ (89 ppm) and for a pair of $^{18}O/^{16}O = 1894\cdot10^{-6}$ (1894 ppm). In surface waters, the ratio $D/^1H = \sim(1{,}32–1{,}51)\cdot10^{-4}$, while in the coastal seawater – $\sim(1{,}55–1{,}56)\cdot10^{-4}$. The natural waters of CIS countries are characterized by negative deviations from SMOW standard to $(1{,}0–1{,}5)\cdot10^{-5}$, in some places up to $(6{,}0–6{,}7)\cdot10^{-5}$, but however there are also observed positive deviations at $2{,}0\cdot10^{-5}$.

The constant sources of deuterium are explosions of nova stars and thermonuclear processes frequently occurring inside the stars. Probably, it could explain a known fact, why the amount of deuterium is slightly increased during the global changes of climate in warming conditions. The gravitational field of the Earth is insufficiently strong for the retaining of lighter hydrogen, and our planet is gradually losing hydrogen as a result of its dissociation into interplanetary space. Hydrogen evaporates faster than heavy deuterium, which can be collected by the hydrosphere. Therefore, as a result of this natural process of fractionation of H/D isotopes throughout the process of Earth evolution there should be an accumulation of deuterium in the hydrosphere and surface waters, while in the atmosphere and in water vapour deuterium content tends to be low. Thus, on the planet there occurs a natural process of separation of H and D isotopes, playing an essential role in the maintenance of life on the planet.

The second point regards the influence of temperature on the biochemical processes in living matter. Our recent studies have shown that the most favorable for the origin of life and living matter seem to be hot alkaline mineral waters interacting with $CaCO_3$ [6, 7]. According to the law for conservation of energy the process of self-organization of primary organic forms in water solutions may be supported by the thermal energy of magma, volcanic activity and solar radiation. According to J. Szostak, the accumulation of organic compounds in small isolated lakes is more possible compared to the ocean [8]. It is most likely that life originated near a hydrothermal vent: an underwater spout of hot water. The geothermal activity gives more opportunities for the origination of life. In 2009 A. Mulkidjanian and M. Galperin demonstrate that the cell cytoplasm contains potassium, zinc, manganese and phosphate ions, which are not particularly widespread in the sea aquatorium [9]. J. Trevors and G. Pollack proposed in 2005 that the first cells on the Earth assembled in a hydrogel environment [10]. Gel environments are capable of retaining water, oily hydrocarbons, solutes, and gas bubbles, and are capable of carrying out many functions, even in the absence of a membrane. Hydrocarbons are an organic compounds consisting entirely of hydrogen and carbon. The data presented in this paper show that the origination of living matter most probably occurred in hot mineral water. This may occurred in ponds and hydrothermal vents in seawater or hot mineral water. An indisputable proof of this is the presence of stromatolites fossils. They lived in warm and hot water in zones of volcanic activity, which could be heated by magma and seem to be more stable than other first sea organisms [11].

Therefore, the purpose of the research was studying the hydrological conditions of primary hydrosphere (temperature, pH, isotopic composition) for possible processes for origin of first organic forms in hot mineral water with HDO. Various samples of water from Bulgaria, as well water with varying deuterium content were studied within the frames of the research.

Material and Methods

Chemicals

For preparation of water with varying content of deuterium (HDO) was used D_2O (99,9 atom.%) from the Russian Research Centre "Isotope" (St. Petersburg, Russian Federation). D_2O was preliminary distilled over $KMnO_4$ with the subsequent control of isotope enrichment by ^1H-NMR-spectroscopy on a Brucker WM-250 device ("Brucker", Germany) (working frequency: 70 MHz, internal standard: Me_4Si).

Biological Objects

The objects of the study were used the cactus juice of *Echinopsis pachanoi* and the Mediterranean jellyfish *Cotylorhiza tuberculata* (Chalkida, Greece, Aegean Sea).

Water Samples

The samples of water were taken from various water springs of Bulgaria:
1 – Mineral water (Rupite, Bulgaria);
2 – Seawater (Varna resort, Bulgaria);
3 – Mountain water (Teteven, Bulgaria);
5 – Deionized water (the control).
6 – Water with varrying deuterium content (HDO).

IR-Spectroscopy

IR-spectra of water samples were registered on Brucker Vertex ("Brucker", Germany) Fourier-IR spectrometer (spectral range: average IR – 370–7800 cm^{-1}; visible – 2500–8000 cm^{-1}; permission – 0.5 cm^{-1}; accuracy of wave number – 0.1 cm^{-1} on 2000 cm^{-1}) and on Thermo Nicolet Avatar 360 Fourier-transform IR (M. Chakarova).

DNES-Spectral Analysis

The research was made with the method of differential non-equilibrium spectrum (DNES). The device measures the angle of evaporation of water drops from 72 ° to 0 °. As the main estimation criterion was used the average energy ($\Delta E_{H...O}$) of hydrogen O...H-bonds between H_2O molecules in water's samples. The spectra of water were measured in the range of energy of hydrogen bonds 0,08–0,1387 eV with using a specially designed computer program.

High-Frequency Coronal Electric Discharge Experiments

A device for high-frequency coronal electric discharge was used in this study, constructed by I. Ignatov and Ch. Stoyanov. The frequency of the applied saw-tooth electric voltage was 15 kHz, and the electric voltage – 15 кV. The electric discharge was obtained using a transparent firm polymer electrode on which a liquid sample of water (2–3 mm) was placed. The spectral range of the photons released upon electric discharge was from $\lambda = 400$ to $\lambda = 490$ nm and from $\lambda = 560$ to $\lambda = 700$ nm.

Results and Discussion

Studying Various Water Samples on the Feasibility for Origin of Life

We have carried out the research of various samples of mineral water obtained from mineral springs and seawater from Bulgaria (Fig. 1, curves *1–5*). For this aim we employed IR-spectrometry and the DNES method relative to the control – deionized water.

For calculation of the function *f(E)* represented the energy spectrum of water, the experimental dependence between the wetting angle (θ) and the energy of hydrogen bonds (E) is established:

$$f(E) = \frac{14{,}33 f(\theta)}{[1-(1+bE)^2]^2}, \qquad (1)$$

where b = 14,33 eV^{-1}

The relation between the wetting angle (θ) and the energy (E) of the hydrogen bonds between H_2O molecules is calculated by the formula:

$$\theta = arcos\ (-1 - 14{,}33E) \qquad (2)$$

Cactus juice was also investigated by the DNES method (Fig. 1, *curve 1*). The cactus was selected as a model system because this plant contains approximately 90 % of water. The closest to the spectrum of the cactus juice was the spectrum of mineral water contacting with Ca^{2+} and HCO_3^- ions (Fig. 1, *curve 2*). DNES-spectra of cactus juice and mineral water have magnitudes of local maximums (E, eV) at –0,1112; -0,1187; -0,1262; -0,1287 and –0,1387 eV. The similar local maximums in the DNES-spectrum between the cactus juice and seawater were detected at -0,1362 eV. The DNES-spectrum of the control sample of deionized water (Fig. 1, *curve 5*) was substantially different from DNES-spectra of seawater and mineral water.

Another important parameter was measured by the DNES method – the average energy ($\Delta E_{H...O}$) of hydrogen H...O-bonds among the individual molecules H_2O, which makes up -0,1067±0,0011 eV. When the water temperature is changed, the average energy of hydrogen H...O-bonds alternates. This testified about the restructuring of average energies of hydrogen H...O-bonds among individual H_2O molecules with a statistically reliable increase of local maximums in DNES-spectra.

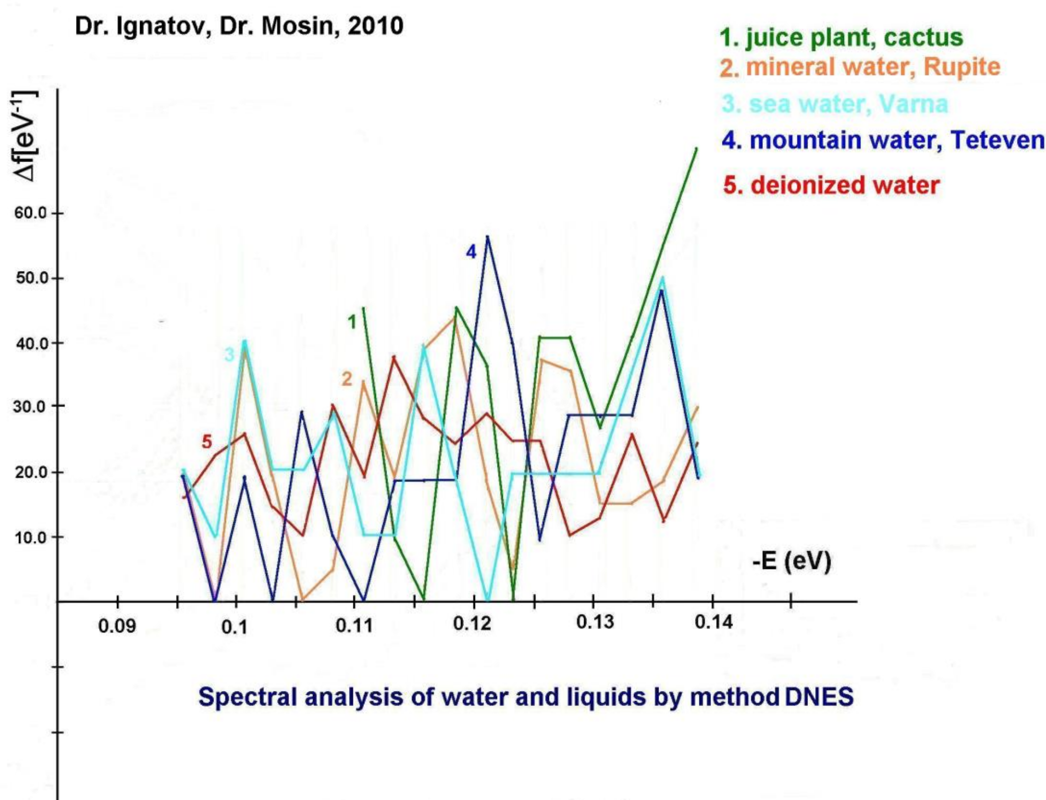


Figure 1. DNES-spectra of water samples of various origin: 1 – the cactus juice; 2 – mineral water from Rupite village (Bulgaria); 3 – seawater (Varna, Bulgaria); 4 – mountain water (Teteven, Bulgaria); 5 – deionized water (the control)

As it was shown from these experimental data, the closest to the IR-spectrum of the cactus juice was mineral water from Rupite Village (Bulgaria), which the DNES and IR spectrum are shown in Fig. 2 and Fig. 3 (Thermo Nicolet Avatar 360 Fourier-transform IR). IR-spectra of cactus juice and mineral water containing HCO_3^- (1320–1488 mg/l), Ca^{2+} (29–36 mg/l), pH (6,85–7,19), have local maximums at λ = 8,95; 9,67; 9,81; 10,47 and 11,12 μm (Fourier-IR spectrometer Brucker Vertex). The common local maximums in the IR-spectrum between the cactus juice and seawater are detected at λ = 9,10 μm. The local maximums obtained with the IR method at λ = 9,81 μm (k = 1019 cm^{-1}) and λ = 8,95 μm (k = 1117 cm^{-1}) (Thermo Nicolet Avatar 360 Fourier-transform IR) are located on the spectral curve of the local maximum at λ = 9,7 μm (k = 1031 cm^{-1}) (Fig. 3). With the DNES method, the results of which are shown in Table 1, were obtained the following results – 8,95; 9,10; 9,64; 9,83; 10,45 and 11,15 μm (wave length λ, μm), or – 897; 957; 1017; 1037; 1099 and 1117 cm^{-1} (wave numbers, k, cm^{-1}).

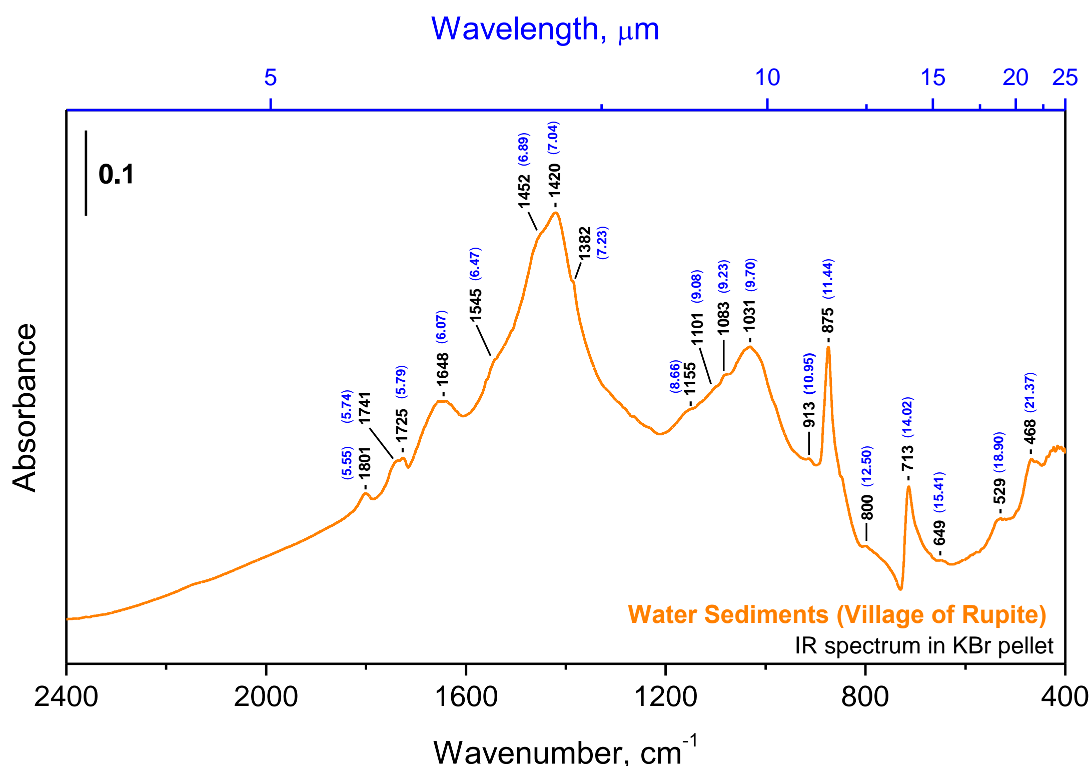


Figure 2. IR-spectrum of water sediments obtained from Rupite Village (Bulgaria)

Table 1: Characteristics of spectra of water of various origin obtained by the DNES-method*

-E, eV			λ, μm	k, cm^{-1}
Cactus juice	**Mineral water from Rupite Village (Bulgaria)**	**Seawater**		
0,1112	0,1112	–	11,15	897
0,1187	0,1187	–	10,45	957
0,1262	0,1262	–	9,83	1017
0,1287	0,1287	–	9,64	1037
0,1362	–	0,1362	9,10	1099
0,1387	0,1387	–	8,95	1117

Notes:

*The function of the distribution of energies Δf was measured in reciprocal electron volts (eV^{-1}). It is shown at which values of the spectrum -E (eV) were observed the biggest local maximums of this function; λ – wave length; k – wave number.

The results with the Mediterranean jellyfish *Cotylorhiza tuberculata* indicated that the jellyfish has local maximums in IR-spectra at λ = 8,98 and λ = 10,18 μm (Fig. 3). Before measurements the jellyfish was kept in seawater for several days. On comparison the seawater has a local maximum at λ = 8,93 μm in IR-spectra. These results were obtained with Thermo Nicolet Avatar 360 Fourier-transform IR. With the DNES method the local maximums in spectra for jellyfish are detected at λ = 8,95 and 10,21 μm, and for seawater – at λ = 9,10 μm. A differential spectrum was recorded between jellyfish and seawater by using the Thermo Nicolet Avatar 360 Fourier-transform IR method. In the IR-spectrum of jellyfish are observed the more pronouncedly expressed local maximums, detected by Thermo Nicolet Avatar 360 Fourier-transform IR and the DNES method. The measurements demonstrate that two common local maximums are observed in IR-spectra of jellyfish and seawater. These maximums are not observed in the IR-spectrum of the cactus juice and mineral water from Rupite (Bulgaria). Jellyfish contains approximately 97 (w/w) % of water and is more unstable living organism compared to those ones formed the stromatolites. The explanation for this is the smaller concentration of salts in jellyfish and, therefore, the smaller number of local maximums in the IR-spectrum in relation to seawater.

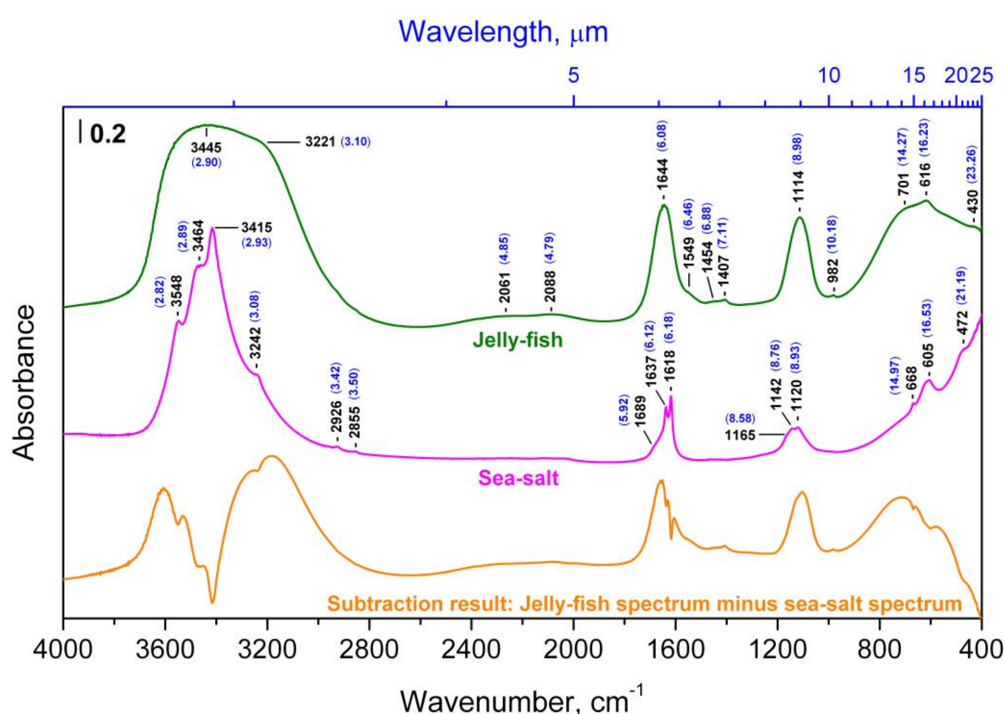
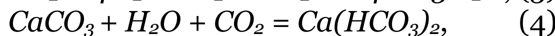


Figure 3. IR-spectrum of seawater obtained from Varna (Bulgaria) and jellyfish *Cotylorhiza tuberculata*, Chalkida (Greece), Aegean Sea

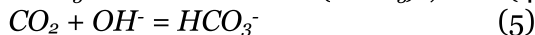
Such a character of IR- and DNES-spectra and distribution of local maximums may prove that hot mineral alkaline water is preferable for origin and maintenance of life compared to other types of water analyzed by these methods. Thus, in hot mineral waters the local maximums in the IR-spectrum are more manifested compared to the local maximums obtained in IR-spectrum of the same water at a lower temperature. The difference in the local maximums from t = +20 °C to t = +95 °C at each +5 °C according to the Student *t*-criterion makes up $p < 0,05$. These data indicate that the origination of life and living matter depends on the structure and physical chemical properties of water, as well as its temperature and the pH value. The most closed to the IR- and the DNES-spectrum of water, which contains bicarbonates and calcium ions typical for the formation of stromatolites, is the IR-spectrum of the cactus juice. For this reason cactus juice was applied as a model system. The most closed to local maximums in the IR-spectrum of the cactus juice are local maximums in IR-spectra of alkaline mineral water interacting with $CaCO_3$ and then seawater.

In connection with these data the following reactions participating with $CaCO_3$ in aqueous solutions are important:

$$CO_2 + 4H_2S + O_2 = CH_2O + 4S + 3H_2O, \quad (3)$$



$$CaCO_3 + H_2O + CO_2 = Ca(HCO_3)_2, \quad (4)$$



$$CO_2 + OH^- = HCO_3^- \quad (5)$$

$$2HCO_3^- + Ca^{2+} = CaCO_3 + CO_2 + H_2O \quad (6)$$

The equation (3) shows how some chemosynthetic bacteria use energy from the oxidation of H_2S and CO_2 to S and formaldehyde (CH_2O). The equation (4) is related to one of the most common processes in nature: in the presence of H_2O and CO_2, $CaCO_3$ transforms into $Ca(HCO_3)_2$. In the presence of hydroxyl OH^- ions, CO_2 transforms into HCO_3^- (equation (5)). Equation (6) is valid for the process of formation of the stromatolites – the dolomite layered acretionary structures formed in shallow seawater by colonies of cyanobacteria. In 2010 D. Ward described fossilized stromatolites in the Glacier National Park (USA) [13]. The stromatolites aged 3,5 billion years had lived in warm and hot water in zones of volcanic activity, which could be heated by magma. This suggests that the first living forms evidently evolved in hot geysers [14]. It is known that water in geysers is rich in carbonates, while the temperature is ranged from +100 °C to +150 °C. In 2011 a team of Japanese scientists under the leadership of T. Sugawara showed that life originated in warm or, more likely, in hot water [15]. From an aqueous solution of organic molecules, DNA and synthetic enzymes were created proto cells. Under experimental conditions the initial solution was heated to a temperature close to the water's boiling point +95 °C. Then the temperature was lowered to +65 °C with formation of proto cells with primitive membrane. These experiments are excellent confirmation of the possibility that first organic forms of life originated in hot water.

The above-mentioned data can predict a possible transition from synthesis of small organic molecules under the temperatures +70–100 °C to more complex organic molecules as proteins. There are reactions of condensation-dehydration of amino acids into separate blocks of peptides that occur under alkaline conditions, with pH = 9–11. The important factor in reaction of condensation of two amino acid molecules into dipeptide is allocation of H_2O molecule when a peptide chain is formed. Because the reaction of polycondensation of amino acids is accompanied by dehydration, the H_2O removal from the reaction mixture speeds up the reaction rates. This testifies that formation of early organic forms may have occured nearby active volcanoes, because at early periods of geological history volcanic activity occurred more actively than during subsequent geological times. However, the dehydratation accompanies not only amino acid polymerization, but also association of other small blocks into larger organic molecules, and also polymerization of nucleotides into nucleic acids. Such association is connected with the reaction of condensation, at which from one block a proton is removed, and from another – a hydroxyl group with the formation of H_2O molecule.

In 1969 the possibility of existence of condensation-dehydration reactions under conditions of primary hydrosphere was proven by M. Calvin [16]. From most chemical substances hydrocyanic acid (HCN) and its derivatives – cyanoamid (CH_2N_2) and dicyanoamid ($HN(CN)_2$) possess the dehydration ability and the ability to catalyze the process of linkage of H_2O from primary hydrosphere [17]. The presence of HCN in primary hydrosphere was proven by S. Miller's early experiments [18]. The chemical reactions with HCN and its derivatives are complex with a chemical point of view; in the presence of HCN, CH_2N_2 and $HN(CN)_2$ the condensation of separate blocks of amino acids accompanied by the dehydration, can proceed at normal temperatures in strongly diluted H_2O-solutions. These reactions show the results of synthesis from separate smaller molecules to larger organic molecules of polymers, e.g. proteins, polycarboxydrates, lipids and ribonucleic acids. Furthermore, the polycondensation reactions catalyzed by HCN and its derivatives depend on acidity of water solutions in which they proceed [19]. In acid aqueous solutions with pH = 4–6 these reactions do not occur, whereas alkaline conditions with pH = 9–10 promote their course. There has not been unequivocal opinion, whether primary water was alkaline, but it is probable that such the pH value possessed mineral waters adjoining with basalts, i.e. these reactions could occur at the contact of water with basalt rocks, that testifies our hypothesis.

It should be noted, that the geothermal sources might be used for synthesis of various organic molecules. Thus, amino acids were detected in solutions of formaldehyde CH_2O with

hydroxylamine NH_2OH, formaldehyde with hydrazine (N_2H_4) in water solutions with HCN, after the heating of a reactionary mixture to +95 °C [20]. In model experiments the reaction products were polymerized into peptide chains that are the important stage towards inorganic synthesis of protein. In a reactionary mixture with a $HCN–NH_3$ solution in water were formed purines and pyrimidines. In other experiments amino acid mixtures were subjected to influence of temperatures from +60 °C up to +170 °C with formation of short protein-like molecules resembling early evolutionary forms of proteins subsequently designated as thermal proteinoids. They consisted of 18 amino acids usually occurring in protein hydrolyzates. The synthesized proteinoids are similar to natural proteins on a number of other important properties, e. g. on linkage by nucleobases and ability to cause the reactions similar to those catalyzed by enzymes in living organisms as decarboxylation, amination, deamination, and oxidoreduction. Proteinoids are capable to catalytically decompose glucose [21] and to have an effect similar to the action of α-melanocyte-stimulating hormone [22].

Under certain conditions (temperature, pH) in hot mixture of thermal proteinoids in water solutions are formed elementary structures like proteinoid microspheres with diameter 5–10 μm [23]. The best results on polycondensation were achieved with the mixes of amino acids containing aspartic and glutamic acids, which are essential amino acids occurring in all modern living organisms. By morphological features the proteinoid microspheres with a diameter ~5–10 μm resemble cell membrane, which in certain conditions (pH = 4–5) may be double. The catalyst for their formation could serve sulfur and its derivatives which were found in ancient rocks in the form of grains of sulfides, as well as pyrite sands. Synthesis of protenoid microspheres from a mixture of thermal proteinoids is important because it provides material for the next stage of the evolution of life. This is the stage from disparate organic molecules to organized proteinoid molecules having organized structure and separated from the surrounding environment by the primitive membrane.

In further experiments was used the gas electric discharge analogous to S. Miller's experiments [24]. The first experiments on the modeling of non-equilibrium conditions with gas electric discharge simulating primary atmosphere and electrosynthesis of organic substances from anorganic ones under the energy of the electric field in a primary oxygen-free atmosphere were held in 1953 by S. Miller (USA) [24]. For this aim the mixture of water and gases consisted of hydrogen (H_2), methane (CH_4), ammonia (NH_3) and carbon monoxide (CO) was placed into a closed glass container being exposed by the pulsating electrical spark discharges at the temperature of boiling water; oxygen was not allowed into the unit. After the processing the reaction mixure by the electric discharge it was found that from the initial inorganic substance was synthesized the organic compounds – aldehydes and amino acids. Experiments found that approximately ~10–15 % of carbon was transferred into an organic form. However, about ~2 % of carbon was detected in the amino acids, the most common of which was glycine. The initial analysis showed the presence in the reaction mixture obtained after the processing by spark electric discharge 5 amino acids. A more complete analysis carried out in 2008 [25], showed the formation by electrosynthesis in the reaction mixture 22 amino acids having from 5 to 20 carbon atoms in the molecule. Interestingly is that along with the amino acids in the reaction mixture after the treatment with electric spark discharges were detected trace amounts of nucleic acid precursors – nucleosides.

It should be noted that in the implementation of the gas discharge effect as well as in experiments of S. Miller are modeled extreme non-equilibrium conditions with gas electric discharge, resulting that in a thin layer of air gap with thickness ~100 μm are formed reactive radicals reacting with each other to form new compounds (electrosynthesis). Such extreme conditions are thought to have occurred in the primary oxygen-free atmosphere of the Earth, which supposedly consisted of a mixture of water and gases – H_2, CH_4, NH_3 and CO, subjected to spark electrical discharges (lightning) under the conditions of high solar (UV) and geothermal activity.

The analogous experiment was conducted by the authors under laboratory conditions. According to our previous experiments, the first living structures may have evolved in warm and hot mineral water with a high content of bicarbonate (HCO_3^-) anions, cations of alkali metals (Na^+, Ca^{2+}, Mg^{2+}, Zn^{2+}) and deuterium in the form of HDO [26]. There occurred gas electric discharge (lightning) in the primordial atmosphere close to the water surface. In the course of experiment was used the similar gas electric discharge on water drops placed on the electrode of the device for gas electric discharge formation. The similar composition and water temperature were modeled on

the electrode of the gas discharge device made of hostafan, with electric voltage – 15 kV, electric impulse duration – 10 μs; electric current frequency – 15 kHz, wherein the air gap layer on the boundary with water sample was formed the electrical discharge, similar to plasma phenomena (lightning) and the electrostatic discharge on the surface of organic and inorganic samples of various kinds. Water drops were heated up to the boiling point in an electric field of high frequency and the electric discharge was applied, analogious as that in the primordial atmosphere. As a result, an organized structure with a size of 1,2–1,4 mm was formed in interelectrode space (Fig. 4). It was formed as a result of accretion of smaller elementary structures sized up 5–10 μm into the biggest structure having the size 1,2–1,4 mm and concentrated in the spase where the electric field is applied.

It should be noted that no structure was organized in a control sample of water being placed on the electrode. Before its placement on the electrode, the water was heated to boiling point and then cooled down. The structure organization increased with the increase of the duration of the gas electric discharge. Moreover, in experiments was observed the formation of small structures and their further "adjoining" to the larger structure. The large structure was preserved the original size for some time in the absence of the electric discharge.

Figure 4. The organized structure in water sample subjected to the temperature +100 °C in the electric field of high voltage and frequency (I. Ignatov, 2014). The material of the electrode – hostafan; the electric voltage – 15 kV, the electric impulse duration – 10 μs; the electric current frequency – 15 kHz.

This experiment shows that self-organization in water under certain external termal conditions may take place. In natural conditions water is heated up to +100 °C by the magma. The structure formed from heated water was evidently a result of self-organization. Living organisms are complex self-organizing systems. Thermodynamically they belong to the open systems because they constantly exchange substances and energy with the environment. The changes in the open systems are relatively stable in time. The stable correlation between components in an open system is called a dissipative structure. According to I. Prigozhin, the formation of dissipative structures and the elaboration to living cells is related to changes in entropy [27].

Taking into account these views it may be concluded that the initial stage of evolution, apparently, was connected with formation at high temperature of the mixtures of amino acids and nitrogenous substances – analogues of nucleic acids. Such synthesis is possible in aqueous

solutions under thermal conditions in the presence of H_3PO_4. The next stage is the polycondensation of amino acids into thermal proteinoids at temperatures 65–95 °C. After that in a mix of thermal proteinoids in hot water solutions were formed membrane like structures.

Our data are confirmed by experiments of T. Sugawara (Japan), who in 2011 created the membrane like proto cells from an aqueous solution of organic molecules, DNA and synthetic enzymes under the temperature close to the water boiling point +95 °C [28]. This data confirm the possibility that first organic forms of life might have been originated in hot mineral water.

Physical-chemical Properties of D_2O-solutions

The numerous studies carried out by us with various biological objects in D_2O, proved that when biological objects being exposed to water with different deuterium content, their reaction varies depending on the isotopic composition of water (the content of deuterium in water) and magnitude of isotope effects determined by the difference of constants of chemical reactions rates k_H/k_D in H_2O and D_2O. The maximum kinetic isotopic effect observed at ordinary temperatures in chemical reactions leading to rupture of bonds involving hydrogen and deuterium atoms lies in the range k_H/k_D = 5–8 for C–H versus C–D, N–D versus N–D, and O–D versus bonds [29].

The chemical structure of D_2O molecule is analogous to that one for H_2O, with small differences in the length of the covalent H–O-bonds and the angles between them. The difference in nuclear masses stipulates the isotopic effects, which may be sufficiently essential for the $^1H/D$ pair. As a result, physical-chemical properties of D_2O differ from H_2O: D_2O boils at t = +101,44 °C, freezes at t = +3,82 °C, has maximal density at t =+11,2 °C (1,106 g/cm^3) [30]. In mixtures of ^2H_2O with H_2O the isotopic exchange occurs with high speed with the formation of semi-heavy water (^1HDO): $D_2O + H_2O = {}^1HDO$. For this reason deuterium presents in smaller content in aqueous solutions in form of ^1HDO, while in the higher content – in form of D_2O. The chemical reactions in D_2O are somehow slower compared to H_2O. D_2O is less ionized, the dissociation constant of D_2O is smaller, and the solubility of the organic and inorganic substances in D_2O is smaller compared to these ones in H_2O. Due to isotopic effects the hydrogen bonds with the participation of deuterium are slightly stronger than those ones formed of hydrogen.

The comparative analysis of IR-spectra of H_2O solutions and its deuterated analogues (D_2O, HDO) is of considerable interest for biophysical studies, because at changing of the atomic mass of hydrogen by deuterium atoms in H_2O molecule their interaction will also change, although the electronic structure of the molecule and its ability to form H-bonds, however, remains the same. The IR spectra of water usually contain three absorption bands, which can be identified as 1 – absorption band of the stretching vibration of OH^- group; 2 – absorption band of the first overtone of the bending vibration of the molecule HDO; 3 – absorption band of stretching vibration of OD^- group. OH^- group is able to absorb much infrared radiation in the infrared region of the IR-spectrum. Because of its polarity, these groups typically react with each other or with other polar groups to form intra-and intermolecular hydrogen bonds. The hydroxyl groups not involved in formation of hydrogen bonds are usually given the narrow bands in IR spectrum and the associated groups – broad intense absorption bands at lower frequencies. The magnitude of the frequency shift is determined by the strength of the hydrogen bond. Complication of the IR spectrum in the area of OH^- stretching vibrations can be explained by the existence of different types of associations, a manifestation of overtones and combination frequencies of OH^- groups in hydrogen bonding, as well as the proton tunneling effect (on the relay mechanism. Such complexity makes it difficult to interpret the IR spectrum and partly explains the discrepancy in the literature available on this subject.

The local maximums in IR-spectra reflect vibrational-rotational transitions in the ground electronic state; the substitution with deuterium changes the vibrational-rotational transitions in H_2O molecule that is why it appears other local maximums in IR-spectra. In the water vapor state, the vibrations involve combinations of symmetric stretch (v_1), asymmetric stretch (v_3) and bending (v_2) of the covalent bonds with absorption intensity (H_2O) $v_1;v_2;v_3$ = 2671; 1178,4; 2787,7 cm^{-1}. For liquid water the absorption bands are observed in other regions of the IR-spectrum, the most intense of which are located at 2100 cm^{-1} and 710–645 cm^{-1}. For D_2O molecule these ratio compiles 2723,7; 1403,5 and 3707,5 cm^{-1}, while for HDO molecule – 2671,6; 1178,4 and 2787,7 cm^{-1}. HDO (50 mole% H_2O + 50 mole% ^2H_2O; ~50 % HDO, ~25 % H_2O, ~25 % D_2O) has local maxima in IR-

spectra at 3415 cm^{-1}, 2495 cm^{-1} 1850 cm^{-1} and 1450 cm^{-1} assigned to OH^- -stretch, OD^- -stretch, as well as combination of bending and libration and HDO bending respectively.

In the IR-spectrum of liquid water absorbance band considerably broadened and shifted relative to the corresponding bands in the spectrum of water vapor. Their position depends on the temperature [31]. The temperature dependence of individual spectral bands of liquid water is very complex. Furthermore, the complexity of the IR-spectrum in the area of OH^- stretching vibration can be explained by the existence of different types of H_2O associations, manifestation of overtones and composite frequencies of OH^- groups in the hydrogen bonds, and the tunneling effect of the proton (for relay mechanism). Such complexity makes it difficult to interpret the spectrum and partly explains the discrepancy in the literature available on this subject.

In liquid water and ice the IR-spectra are far more complex than those ones of the vapor due to the vibrational overtones and combinations with librations (restricted rotations, i.g. rocking motions). These librations are due to the restrictions imposed by hydrogen bonding (minor L_1 band at 395,5 cm^{-1}; major L_2 band at 686,3 cm^{-1}; for liquid water at 0 ^oC, the absorbance of L_1 increasing with increasing temperature, while L_2 absorbance decreases but broadens with reduced wave number with increasing temperature [32]. The IR spectra of liquid water usually contain three absorbance bands, which can be identified on absorption band of the stretching vibration of OH^- group; an absorption band of the first overtone of the bending vibration of the molecule HDO and absorption band of stretching vibration of OD^- group. Hydroxyl group OH^- is able to absorb much infrared radiation in the infrared region of the IR-spectrum. Because of its polarity, these groups typically react with each other or with other polar groups to form intra- and inter-molecular hydrogen bonds. The hydroxyl groups, which are not involved in formation of hydrogen bonds, usually produce the narrow bands in IR spectrum, while the associated groups – broad intense absorbance bands at lower frequencies. The magnitude of the frequency shift is determined by the strength of the hydrogen bond. The complication of the IR spectrum in the area of OH^- stretching vibrations can be explained by the existence of different types of associations of H_2O molecules, a manifestation of overtones and combination frequencies of OH^- groups in hydrogen bonding, as well as the proton tunneling effect (on the relay mechanism).

The assignment of main absorbtion bands in the IR-spectrum of liquid water is given in Table 2. The IR spectrum of H_2O molecule was examined in detail from the microwave till the middle (4–17500 cm^{-1}) visible region and the ultraviolet region – from 200 nm^{-1} to ionization limit at 98 nm^{-1} [33]. In the middle visible region at 4–7500 cm^{-1} are located the rotational spectrum and the bands corresponding to the vibrational-rotational transitions in the ground electronic state. In the ultraviolet region (from 200 nm^{-1} to 98 nm^{-1}) are located the bands corresponding to transitions from the excited electronic states close to the ionization limit in the electronic ground state. The intermediate region of the IR-spectrum – from 570 nm to 200 nm corresponds to transitions to higher vibrational levels of the ground electronic state.

Table 2: The assignment of main frequencies in IR-spectra of liquid water H_2O and D_2O

Main vibrations of liquid H_2O and ^2H_2O				
Vibration(s)	**H_2O (t = +25 ^oC)**		**D_2O (t = +25 ^oC)**	
	ν, cm^{-1}	**E_0, M^{-1} cm^{-1}**	**ν, cm^{-1}**	**E_0, M^{-1} cm^{-1}**
Spinning ν_1 + deformation ν_2	780–1645	21,65	1210	17,10
Composite $\nu_1 + \nu_2$	2150	3,46	1555	1,88
Valence symmetrical ν_1, valence asymmetrical ν_3, and overtone $2\nu_2$	3290–3450	100,65	2510	69,70

The results of IR-spectroscopy with Infra Spec VFA-IR device show that at λ = 4,1 μm, even at low concentrations of deuterium of 0,35 and 0,71%, there is observed a decline in the local maximums relative to the local maximum of 100% pure water (the local maximums in IR-spectra reflect vibrational-rotational transitions in the ground electronic state because at changing the

atomic mass of hydrogen and deuterium atoms in the water molecule their interaction will also change, although the electronic structure of the molecule and its ability to form H-bonds, however, remains the same; with the substitution with deuterium the vibrational-rotational transitions are changed, that is why it appears other local maximums in IR-spectra. The result is reliable regarding the content of deuterium in natural waters from 0,015–0,03%.

At further transition from H_2O monomers to H_4O_2 dimmer and H_6O_3 trimmer absorption maximum of valent stretching vibrations of the O–H bond is shifted toward lower frequencies (v_3 = 3490 cm^{-1} and v_1 = 3280 cm^{-1}) [34] and the bending frequency increased (v_2 = 1644 cm^{-1}) because of the hydrogen bonding. The increased strength of hydrogen bonding typically shifts the stretch vibration to lower frequencies (red-shift) with greatly increased intensity in the infrared due to the increased dipoles. In contrast, for the deformation vibrations of the H–O–H, it is observed a shift towards higher frequencies. The absorption bands at 3546 and 3691 cm^{-1} were attributed to the stretching modes of the dimmer [$(H_2O)_2$]. These frequencies are significantly lower than the valence modes of v_1 and v_3 vibrations of isolated H_2O molecules at 3657 and 3756 cm^{-1} respectively). The absorption band at 3250 cm^{-1} represents overtones of deformation vibrations. Among the frequencies between 3250 and 3420 cm^{-1} it is possible the Fermi resonance (this resonance is a single substitution of intensity of one fluctuation by another fluctuation when they accidentally overlap each other). The absorption band at 1620 cm^{-1} is attributed to the deformation mode of the dimmer. This frequency is slightly higher than the deformation mode of the isolated H_2O molecule (1596 cm^{-1}). A shift of the band of deformation vibration of water in the direction of high frequencies at the transition from a liquid to a solid state is attributed by the appearance of additional force, preventing O–H bond bending. The deformation absorption band in IR-spectrum of water has a frequency at 1645 cm^{-1} and very weak temperature dependence. It changes little in the transition to the individual H_2O molecule at a frequency of 1595 cm^{-1}. This frequency is found to be sufficiently stable, while all other frequencies are greatly affected by temperature changes, the dissolution of the salts and phase transitions. It is believed that the persistence of deformation oscillations is stipulated by processes of intermolecular interactions, e.g. by the change in bond angle as a result of interaction of H_2O molecules with each other, as well as with cations and anions.

Thus, the study of the characteristics of the IR spectrum of D_2O-solutions allows to answer the question not only on the physical parameters of the molecule and the covalent bonds at isotopic substitution with deuterium, but also to make a certain conclusion on associative environment in water. The latter fact is important in the study of structural and functional properties of water associates and its isotopomers at the isotopic substitution with deuterium. The substitution of H with D affects the stability and geometry of hydrogen bonds in an apparently rather complex way and may through the changes in the hydrogen bond zero-point vibration energies, alter the conformational dynamics of hydrogen (deuterium)-bonded structures of macromolecules as DNA and proteins in D_2O.

Conclusions

The experimental data testify that the origination of first organic forms of life depends on physical-chemical properties of water and external factors – temperature, pH, electric discharges and isotopic composition. Hot mineral alkaline water interacting with $CaCO_3$ is most closed to these conditions. Next in line with regard to its quality is seawater. For chemical reaction of dehydration-condensation to occure in hot mineral water, water is required to be alkaline with the pH ranged 9–11. In warm and hot mineral waters the local maximums in IR-spectra from 8 to 14 μm were more expressed in comparison with the local maximums measured in the same water samples with lower temperature. The content of deuterium in hot mineral water may be increased due to the physical chemical processes of the deuterium accumulation stipulated by the solar radiation, volcanic geothermal processes and electric discharges in the atmosphere. These natural processes could lead to the enrichment of the hydrosphere by deuterium in the form of HDO which evaporates more slowly than H_2O, but condenses faster. If the primary hydrosphere really contained HDO, that this may explain the thermal stability of the first organic life forms in the hot mineral water, as the thermal stability of deuterated macromolecules like DNA and proteins in D_2O solutions is somewhat higher than their protonated forms due to isotopic effects of deuterium.

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References:

1. Ignatov I., Mosin O.V. Color coronal (Kirlian) spectral analysis in modeling of nonequilibrioum conditions with the gas electric discharges simulating primary atmosphere. S. Miller's experiments // Naukovedenie. 2013. № 3(16). P. 1–15 [in Russian] [Online] Available: URL: http://naukovedenie.ru/PDF/05tvn313.pdf (May 10, 2013).

2. Ignatov I., Mosin O.V. Origin of life and living matter in hot mineral water // *Advances in Physics Theories and Applications*. 2015. V. 39. P. 1–22.

3. Ignatov I., Mosin O.V. // Modeling of possible conditions for origin of first organic forms in hot mineral water // *European Journal of Molecular Biotechnology*. 2014. V. 6. № 4. P. 169–179.

4. Ignatov I., Mosin O.V. Possible processes for origin of life and living matter with modeling of physiological processes of bacterium *Basillus subtilis* as model system in heavy water // Journal *of Natural Sciences Research*. 2013. V. 3. № 9. P. 65–76.

5. Linsky J.L. *D/H and nearby interstellar cloud structures* / Ed. J.I. Linsky. In: Space Science Reviews. – NY: Springer Science, Business Media. 2007. V. 130. 367 p.

6. Ignatov I., Mosin O.V. Isotopic composition of water and its temperature in modeling of primordial hydrosphere experiments / *VIII Int. Conference Perspectives of the Development of Science and Technique, Biochemistry and Biophysics*. 2012. V. 15. P. 41–49.

7. Ignatov I., Mosin O.V. Modeling of possible processes for origin of life and living matter in hot mineral and seawater with deuterium // *Journal of Environment and Earth Science*. 2013. V. 3. № 14. P. 103–118.

8. Szostak J.W. An optimal degree of physical and chemical heterogeneity for the origin of life? // *Philos. Trans. Royal Soc. Lond. Biol. Sci.* 2011. V. 366. № 1580. P. 2894–901.

9. Mulkidjanian A.Y., Galperin M.Y. On the origin of life in the Zinc world. Validation of the hypothesis on the photosynthesizing zinc sulfide edifices as cradles of life on Earth // *Biology Direct*. 2009. V. 4. P. 26.

10. Trevors J.I., Pollack G.H. Hypothesis: origin of live in hydrogel environment // *Progress in Biophysics and Molecular Biology*. 2005. V. 89. № 1. P. 1–8.

11. Ignatov I. Origin of life and living matter in hot mineral water / *7th Ann. Conference on the Physics, Chemistry and Biology of Water*. 12–16 October 2012. – NY: Vermont Photonics, USA. 2012. P. 67.

12. Ignatov I., Mosin O.V. Color coronal Kirlian spectral analysis in modelling nonequilibrium conditions with the gas electric disharges, simulating primary atmosphere // *Biomedical Radio Electronics*. 2014. №. 2. P. 38–47 [in Russian].

13. Schirber M. First fossil-makers in hot water // *Astrobiology Magazine*. 2010 [Online] Available: URL: http://www.astrobio.net/exclusive/3418/first-fossil-makers-in-hot-water (January 3, 2010).

14. Ponsa M.L., Quitte G., Fujii T., Rosingc M.T., Reynarda B., Moynierd F., Doucheta Ch., Albaredea F. Early archean serpentine mud volcanoes at Isua, Greenland, as a niche for early life // *Proc. Natl. Acad. Sci. U.S.* 2011. V. 108. P. 17639–17643.

15. Kurihara K., Tamura M., Shohda K., Toyota T., Suzuki K., Sugawara T. Self-Reproduction of supramolecular giant vesicles combined with the amplification of encapsulated DNA // *Nature Chemistry*. 2011. V. 4. № 10. P. 775–781.

16. Calvin M. *Chemical evolution* / Ed. M. Calvin. – Oxford: Clarendon. 1969. 278 p.

17. Mathews C.N. Peptide synthesis from hydrogen-cyanide and water / C.N. Mathews, R. Moser // *Nature*. 1968. V. 215. P. 1230–1234.

18. Miller S.L. A production of amino acids under possible primitive Earth conditions / S.L. Miller // *Science*. 1953. V. 117. № 3046. P. 528–529.

19. Abelson P. Chemical events on the "primitive" earth / P. Abelson // *Proc. Natl. Acad. Sci. U.S.* 1966. V. 55. P. 1365–1372.

20. Harada I. Thermal synthesis of natural amino acids from a postulated primitive terrestrial atmosphere / I. Harada, S.W. Fox // *Nature*. 1964. V. 201. P. 335–336.

21. Fox S.W. Catalytic decomposition of glucose in aqueous solution by thermal proteinoids / S.W. Fox, G. Krampitz // *Nature*. 1964. V. 203. P. 1362–1364.

22. Fox S.W. Melanocytestimulating hormone: Activity in thermal polymers of alpha-ammo acids / S.W. Fox, C.T. Wang // *Science*. 1968. V. 160. P. 547–548.

23. Nakashima T. Metabolism of proteinoid microspheres / Ed. T. Nakashima. In: *Origins of life and evolution of biospheres*. 1987. V. 20. № (3–4). P. 269–277.

24. Miller S.L. A production of amino acids under possible primitive Earth conditions / S.L. Miller // *Science*. 1953. V. 117. № 3046. P. 528–529.

25. Johnson A.P. The Miller volcanic spark discharge experiment / A.P. Johnson, H.J. Cleaves, J.P. Dworkin, D.P. Glavin, A. Lazcano, J.L. Bada // *Science*. 2008. V. 322. № 5900. P. 404–412.

26. Ignatov I. Microorganisms in the presence of D_2O and IR spectra in hot mineral water with more deuterium atoms for origin of life and living matter / I. Ignatov, O.V. Mosin // *Journal of Health, Medicine and Nursing*. 2014. V. 5. P. 80–93.

27. Nikolis P., Prigozhin I. *Self-organization in non-equilibrium systems* / Ed. P. Nikolis, I. Prigozhin. – Moscow: Mir. 1979. 512 p. [in Russian].

28. Sugawara T. Self-reproduction of supramolecular giant vesicles combined with the amplification of encapsulated DNA // *Nature Chemistry*. 2011. V. 1127. P. 775–780.

29. Mosin O.V. Studying of isotopic effects of heavy water in biological systems on example of prokaryotic and eukaryotic cells / O.V. Mosin, I. Ignatov // *Biomedicine*, Moscow. 2013. V. 1, № 1–3. P. 31–50 [in Russian].

30. Mosin O.V. Studying of methods of biotechnological preparation of proteins, amino acids and nucleosides, labeled with stable isotopes ^2H and ^{13}C with high levels of isotopic enrichment / O.V. Mosin. Autoref. disser. thesis PhD. – Moscow: M.V. Lomonosov State Academy of Fine Chemical Technology, 1996. 26 p.

31. Ignatov I. Possible processes for origin of first chemoheterotrophic microorganisms with modeling of physiological processes of bacterium *Bacillus subtilis* as a model system in ^2H_2O / I. Ignatov, O.V. Mosin. // *European Journal of Molecular Biotechnology*. 2015. V. 9. № 3. P. 131–155.

32. Zelsmann H.R. Temperature dependence of the optical constants for liquid H_2O and D_2O in the far IR region // *J. Mol. Struct*. 1995. V. 350. P. 95–114.

33. Yakhnevitch G.B. *Infrared spectroscopy of water* / G.B. Yakhnevitch, ed. – Moscow: Nauka, 1973. 207 p. [in Russian].

34. Ignatov I. Structural mathematical models describing water clusters / I. Ignatov, O.V. Mosin // *Journal of Mathematical Theory and Modeling*. 2013. V. 3. № 11. P. 72–87.

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Могли ли первые органические формы жизни возникнуть в горячей минеральной воде с HDO?

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Аннотация. Изучен состав воды, температура и значение pH в экспериментах по моделированию первичной гидросферы и возможных условий возникновения органических форм жизни в горячей минеральной воде с HDO. Для этой цели проведены исследования горячей минеральной и морской воды из Болгарских источников методами ИК- и ДНЭС-спектроскопии. В качестве модельных систем использовали сок кактуса *Echinopsis pachanoi* и средиземноморскую медузу *Cotylorhiza tuberculata*. Рассмотрены реакции конденсации-дегидратации в щелочных водных растворах со значением pH = 9–10, результатом которых является синтез из мелких молекул более крупных органических молекул полимеров как короткие полипептиды, а также механизмы аккумуляции дейтерия в форме HDO в горячей минеральной воде. Показано, что горячие минеральные воды с температурой от +65 °C до +95 °C и значением pH от 9 до 11 более пригодны для возникновения жизни, чем другие исследованные образцы воды. В горячей минеральной воде локальные максимумы в ИК-спектре проявлялись больше всего, по сравнению с локальными максимумами в ИК-спектре той же воды при более низкой температуре. Разница в локальных максимумах от +20 °C до +95 °C при увеличении температуры на каждый +5 °C составила в соответствии с *t*-критерием Стьюдента $p < 0{,}05$.

Ключевые слова: дейтерий, гидросфера, эволюция, возникновение жизни, ИК-спектроскопия, ДНЭС.

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Individual Sensitivity of the Peripheral Blood Lymphocytes to the *in Vitro* Action of Mitomycin C in Children with Juvenile Rheumatoid Arthritis

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Abstract

The study was designed to estimate the levels of spontaneous and induced chromosome aberrations in the peripheral blood lymphocytes (PBL) of patients with juvenile rheumatoid arthritis (JRA) and their healthy peers. An increased spontaneous level of chromosomal aberrations has been found in children and adolescents with JRA in comparison with healthy age-mates. It has been established that the effect of the mitomycin C model mutagen on the PBL *in vitro* causes a multiple increase in the levels of chromosomal aberrations in both groups under investigation.

Keywords: chromosomal instability, cytogenetic analysis, juvenile rheumatoid arthritis, mutagenesis, peripheral blood lymphocytes, mitomycin C.

Introduction

A significant deterioration of the environment is being observed over the last years due to the latest achievements of scientific and technological progress, namely: the development of industry, the use of pesticides, herbicides and insecticides in agriculture, habitation of human beings in ecologically unfavorable areas, the use of drugs, bad habits, etc. [6]. All of these factors may have a negative impact on the human organism and cause disorders in the integrity of the genome. Undoubtedly, this is reflected on the function of the cell, its viability, i.e. on the availability of sound tissues and subsequently on the human health status. The probability of transformation of some aberrant cells into oncogenic cells has also been proved. It is widely accepted that chromosomal breaks and alterations, absence of chromosome disjunction in metaphase, endoreduplication and nuclear fusion form the basis of the initial stage of carcinogenesis [1, 8]. Therefore, it is necessary to conduct a combined assessment and monitoring of the cellular genome status in the people, belonging to the risk groups: the Chernobyl disaster liquidators and their descendants, workers of dangerous industrial branches, residents of contaminated areas, and patients with various multifactorial diseases. The most sensitive and simple method for bioindication of the mutagenic effect on living organisms is determining the spontaneous level of chromosomal aberrations. This method is recommended by the WHO and IAEA and is intended for the assessment of chromosomal and genomic disorders in the peripheral blood lymphocytes (PBL) *in vitro* [11].

To determine an individual hypersensitivity of a human being to the influence of mutagenic factors an additional mutagenic load on the PBL *in vitro* is used in the G2-phase of the cell cycle [16]. This method enables the authors to estimate a potential possibility of the genomic instability occurrence, when an organism gets in unfavorable environmental conditions [15]. Ionizing radiation is commonly used as a mutagen-provocateur, but over the last years specialists began to use chemical mutagens, and most of all bleomycin, antitumor antibiotic, which, being a radiomimetic, is able to induce a strong cytogenetic effect in the PBL *in vitro* [8, 15]. Some other mutagens, namely: dimatif [14], dimethoate, an insecticide, and mitomycin C, an antitumor antibiotic are used along with bleomycin to determine the sensitivity of the chromosomes to the effects of the chemical mutagenic load *in vitro* [4, 8].

Hence, determination of individual hypersensitivity of the PBL *in vitro* to a test-effect of mutagenic load is an extremely relevant and timely task. At present, the assessment of spontaneous and induced types of mutagenesis is carried out in various non-communicable diseases [7, 9]. Taking into consideration that a significant increase has been obtained in our previous studies in the level of spontaneous chromosome aberrations in probands with juvenile rheumatoid arthritis (JRA), in comparison with their healthy peers, the necessity to study the hidden chromosomal instability of the PBL *in vitro* arises in patients with JRA [3, 12].

Our study is aimed at determining the hypersensitivity of the PBL to the *in vitro* effect of mitomycin C model mutagen in children and adolescents with JRA.

Material and methods

Cytogenetic analysis was carried out in the State Institution “Institute for Children and Adolescents Health Care of the National Academy of Medical Sciences of Ukraine” in 30 children and adolescents of both sexes with JRA (main group) and 30 healthy children (control group), aged 5–17 years. To determine the sensitivity of chromosomes in patients with the JRA to the genotoxic effect of mitomycin C *in vitro* we have studied the levels of spontaneous and induced chromosome aberrations in chromosome preparations, obtained from the PBL culture.

The PBL cultivation was carried out according to the standard technique [19], for 72 hours at +37 °C, using PHA ("Sigma", Germany), RPMI 1640 medium and fetal calve serum. As a mutagen-provocateur we used mitomycin C, antitumor antibiotic, which was added to the culture at the 67-th hour of incubation at a final concentration of 3 μcg/ml. Stops of mitoses were performed at the 70-th hour of cultivation by addition of colchicine at the concentration of 0.1 μg/ml. After hypotonic treatment with KCl (0.075 M) for 12 minutes, the cells were fixed with a mixture of ethanol and glacial acetic acid (3 : 1). The cell suspension was pipetted on chilled wet slides, and ready preparations were dyed with Giemsa stain.

100 metaphase plates were analyzed to assess the frequency and types of chromosomal abnormalities. Disorders in the chromatid, chromosome and genome types were taken into consideration. Metaphase plates were examined using «Leica Galen III», «Ergoval» and «Leica CME» binocular microscopes, 10×18 eyepiece, 100×lens, and 1.25×binocular head.

Statistical calculations were performed on a PC. The coefficient of hidden chromosomal instability (C_{hci}) was calculated to reveal individuals with hypersensitivity to the action of mitomycin C [15]:

$$C_{hci} = M_{hci} / M,$$

where:

M_{hci} – individual values of the chromosomal aberrations frequency under the test-effect of mitomycin C at the concentration of 3 μg/ml;

M – average group values of the chromosomal aberrations frequency under the impact of mitomycin C at the same concentration.

According to Pilinskaya (2010), an induced cytogenetic effect exceeds the average group level of chromosomal aberrations in hypersensitive persons, so C_{hci} in them will always be > 1. The Student's t test was used to reveal the significance of differences between the compared parameters [2].

Results and Discussion

Average group level of spontaneous mutagenesis in the PBL of patients with JRA

corresponded to 4.35 per 100 cells and by 2.3 times exceeded the frequency of chromosomal abnormalities in their healthy peers (1.85 per 100 cells; $p < 0.001$). In the group of patients with JRA individual values of chromosomal instability ranged from 0 to 14 anomalies per 100 metaphases, moreover, the final values were recorded in isolated cases. In 16 patients (40 %) the level of chromosomal aberrations was within the limits of population norm (from 0.0 % to 3.0 %) [5]. Most frequent the individual values of spontaneous mutagenesis were within the limits of 4–6 disorders per 100 cells (45 % of the examined persons), which corresponded to the average finding in the group. A high level of chromosomal aberrations (from 7 to 14 per 100 cells) was revealed in 15 % of children and adolescents with JRA.

Addition of the mitomycin C model mutagen into the culture of the PBL resulted in a significant increase in the level of chromosomal aberrations in the main group (17.3, $p < 0.001$) and to 14.6 anomalies per 100 metaphase plates in the control group ($p < 0.001$).

Individual values of the induced mutagenesis in the study group ranged from 2 to 36 anomalies per 100 cells, and from 0 to 36 per 100 cells in the control group. To analyze individual hypersensitivity of chromosomes in the PBL *in vitro* in children and adolescents with JRA and in their healthy peers to the mutagenic load, we determined the difference between spontaneous and induced mutagenesis (supra-spontaneous cytogenetic effect) for each child. In both groups the distribution of supra-spontaneous level of chromosomal aberrations is approximately the same.

To determine the coefficient of hidden chromosomal instability (C_{hci}) the authors calculated the average group values of the chromosomal aberrations frequency (*M*) under the test-effect of mitomycin C. In the main group, this index came to 17.3 aberrations per 100 metaphases, and in the control group it was 14.6. In 14 patients (46.6 %) with JRA individual values of the induced mutagenesis exceeded the average group level of chromosomal abnormalities; in healthy age-matched children they were registered in 16 (53.3%) individuals. In addition, both groups were divided into two subgroups depending on the level of spontaneous chromosomal aberrations: main group ≤ 3.0 % (11 people) and ≥ 4.0 % (19 people); control group ≤ 3.0 % (22 people) and ≥ 4.0 % (8 people).

16.6 % of patients with JRA and normal levels of spontaneous mutagenesis had a hidden chromosomal instability. From 30 tested individuals 20.0 % had a low level of spontaneous mutagenesis, and they turned out to be resistant to the effects of the chemically-induced mutagenesis. An increased sensitivity of the PBL to the mutagenic load has been established in 33.3 % of probands with an elevated level of spontaneous mutagenesis (Fig. 1). The probands were most sensitive to the effects of mutagenes or they were debilitated patients with a high risk for the cells oncogenic transformation.

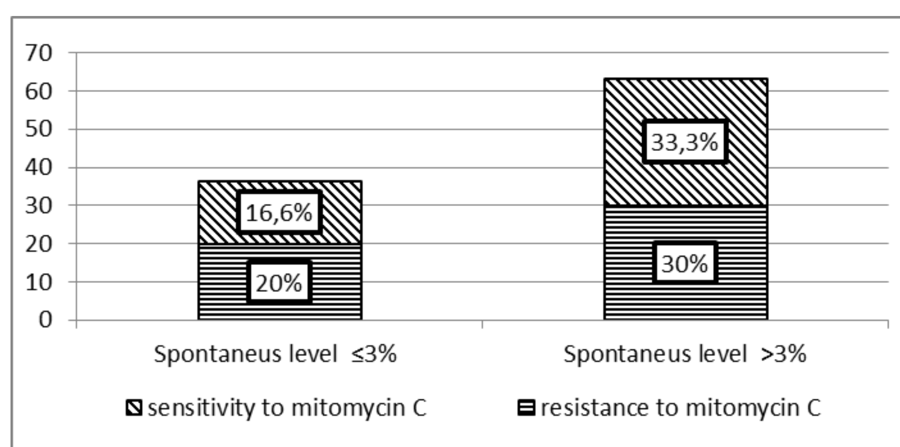

Figure 1. Distribution of sensitive and resistant to the mutagenic load patients with JRA and different levels of spontaneous mutagenesis

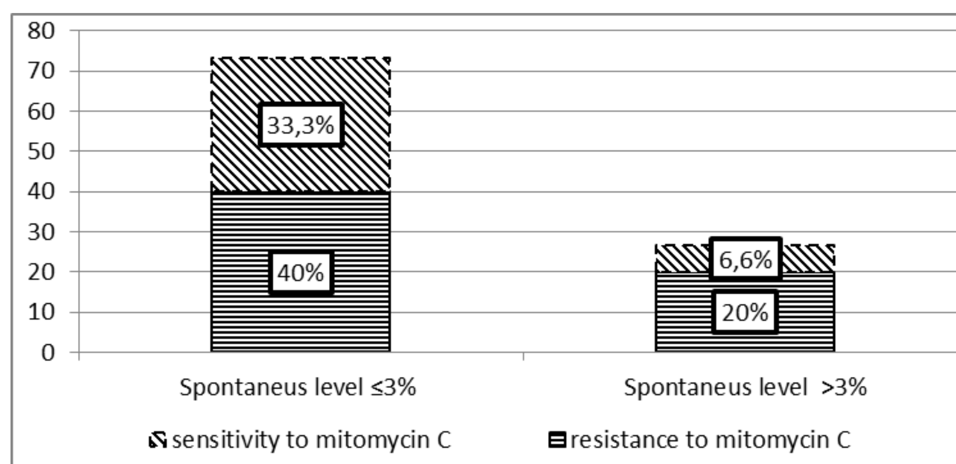

Figure 2. Distribution of sensitive and resistant to the mutagenic load persons among healthy peers with different levels of spontaneous mutagenesis

Sensitivity to the mutagenic load has been determined in 33.3 % of children from the control group with normal levels of spontaneous mutagenesis. In healthy children and adolescents with an elevated level of spontaneous mutagenesis the sensitivity of the PBL to the effects of model mutagen has been registered only in 6.6 % (Fig. 2).

Thus, we have determined an increase in the average group level of chromosomal aberrations in children and adolescents with JRA as compared with healthy peers. Analysis of the individual values of spontaneous mutagenesis in the represented groups has revealed the prevalence of individuals with an elevated level of chromosomal abnormalities in the main group. Moreover, the impact of the mitomycin C model mutagen on the PBL *in vitro* causes a multiple increase in the chromosomal abnormalities in the groups under investigation.

According to the data of literature [16], obtained at the determination of chromosomal radiosensitivity in healthy subjects, no relationship has been revealed between the individual findings of spontaneous mutagenesis and the value of the PBL individual radiosensitivity *in vitro*. The phenomenon testifies to the fact that not all the persons with high levels of spontaneous mutagenesis have an increased PBL sensitivity to the effects of the mutagen *in vitro*. The results obtained are consistent with the published data, as an increased individual chromosomal sensibility to the effects of the model mutagen is observed both at the elevated and normal levels of spontaneous mutagenesis in the two groups in approximately equal proportions (45.0 % – patients with JRA and 40.0 % – healthy peers). The existence of predisposing and protective to the effects of mutagens genotypes has been established owing to the work of some researchers [10, 13]. Perhaps this is a cause of approximately the same average group values of the induced mutagenesis in both groups.

According to our study, only 10 patients (33.3 %) among 30 children and adolescents with JRA have the increased levels of spontaneous and induced chromosomal abnormalities. These patients have the greatest risk of the pathological process complications and require constant monitoring of the chromosome apparatus state, as well as full control over the use of dietary or pharmacological antimutagens [6, 17, 18].

Conclusion

A multiple increase in chromosomal abnormalities has been established in the groups under investigation after addition of the mitomycin C model mutagen in the PBL culture. Distribution of supra-spontaneous levels of chromosomal aberrations is approximately the same in both groups which may be caused by the existence of the predisposing and protective genotypes, regarding the effects of mutagens.

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References:

1. Andreev S.G., Adelman Y.A. Ways exchange interactions damage leading to intrachromosomal aberrations depending on the structure of interphase chromosomes. *Radiation Biology. Radioecology* 41 (2001) 469-474.
2. Atramentova L.A., Utevskaya O.M. Statistical Methods in Biology. Gorlovka: Vidavnitstvo Lihtar. (2008) 248 p.
3. Bagatska N.V., Nefidova V.E., Medzianovska O.V. Evaluation of spontaneous and induced mutagenesis in peripheral blood lymphocytes of patients with juvenile rheumatoid arthritis. *Factors in experimental evolution of organisms* 10 (2012) 272-276.
4. Boltina IV, Kravchouk OP, Glavatsky OYa Chromosomal aberration in peripheral blood lymphocytes of patients with glial tumors in encephalon. *Oncology* 3 (2001) 23-25.
5. Boltina IV The use of the parameter «frequency of chromosome aberrations» in formation of risk groups regarding oncological diseases. *Cytology and Genetics* 41 (2007) 66-74.
6. Dvornic AS, Pererva TP, Kunakh VA Antimutagenesis as protection system of an organism against damaging factors of endogenous and exogenous origin. *Cytology and Genetics* 38 (2004) 62-71.
7. Gainetdinova D.D., Gaisina L.Z. Phenomenon of cell genome instability in the pathogenesis of children cerebral palsy. *Journal of Neurology and Psychiatry* 112 (2012) 49-51.
8. Hsu TC, Johnston DA, Cherry LM Sensitivity to genotoxic effects of bleomycin in humans: Possible relationship to environmental carcinogenesis. *International J. of Cancer* 43 (2006) 403-409.
9. Kinne R.W., Liehr T, Beensen V, Kunisch E, Zimmermann T, Holland H, Pfeiffer R, Stahl HD, Lungershausen W, Hein G, Roth A, Emmrich F, Claussen U and Froster UG Mosaic chromosomal aberrations in synovial fibroblasts of patients with rheumatoid arthritis, osteoarthritis, and other inflammatory joint diseases. *Arthritis Res* 5 (2001) 319-330.
10. Lin J, Swan GE, Shields PG, Benowitz NL, Gu J, Amos CI, Andrade M, Spitz MR and Wu X Mutagen sensitivity and genetic variants in nucleotide excision repair pathway: genotype-phenotype correlation. *Cancer Research* 16 (2007) 89-96.
11. Maznik NA, Vinnikov VA The dynamics of the cytogenetic effects in the peripheral blood lymphocytes of those who worked in the cleanup of the aftermath of the accident at Chernobyl Atomic Electric Power Station. Cytology and Genetics 31 (1997) 41-47.
12. Medzianovska O.V. Determination of the level of spontaneous chromosome abnormalities of the children who are down with juvenile rheumatoid arthritis. *The Journal of V.N.Karazin Kharkiv National University* 1079 (2013) 76-80.
13. Oliveira AL, Rodrigues FF, Santos RE, Aoki T, Rocha MN, Longui CA, Melo MB GSTT1, GSTM1, and GSTP1 polymorphisms and chemotherapy response in locally advanced breast cancer. *Genet Mol Res* 9 (2010) 1045-1053.
14. Pilinskaya M.A., Dybskiy S.S., Pedan L.R. (1993). Modification by dimatyph of cytogenetic damages in blood lymphocytes of children exposed to constant low-rate irradiation. *Cytology and Genetics* 27: 87-90.
15. Pilinskaya M.A., Dybskiy S.S., Dybskaya Y.E., Pedan L.R. (2010). Radiation induced modification of human somatic cells chromosomes sensitivity to the testing mutagenic exposure of bleomycin. *Cytology and Genetics* 44: 58-64.
16. Ryabchenko N.M., Dyomina E.A. (2008). Significance of spontaneous and radiation induced levels of chromosomal aberrations in estimation of human individual radiation sensitivity. *Vavilov Society of Geneticists and Breeders of Ukraine* , 6, 125-130.
17. Sloczynska K., Powroznik B., Pekala E., Waszkielewicz A.M. (2014). Antimutagenic compounds and their possible mechanisms of action. *J Appl Genetics* 55, 273-285.
18. Zasukhina G.D., Vasilyeva I.M., Shagirova Zh.M., Mikhajlov V.F., Vedernikov A.I., Gromov S.P. and Alfimov M.V. (2011). The effect of natural and synthetic antimutagens in human radiosensitive cells differing in genetic polymorphism *Doklady Biological Sciences* 440, 306-308.
19. Zerova-Lubimova T.E., Gorovenko N.G. (2003). Cytogenetic methodological investigation of human chromosomes: method. instruction. P. Shupyk KMAPE. 23 p.

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Индивидуальная чувствительность лимфоцитов периферической крови детей, больных ювенильным ревматоидным артритом, к тестирующему действию митомицина С *in vitro*

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Аннотация. Изучили уровень спонтанных и индуцированных хромосомных нарушений в лимфоцитах периферической крови больных ювенильным ревматоидным артритом (ЮРА) и здоровых сверстников. Определили повышение спонтанного уровня хромосомных нарушений у детей и подростков, больных ЮРА, в сравнении со здоровыми сверстниками. Установлено, что воздействие модельного мутагена митомицина С на ЛПК *in vitro* вызывает многократное повышение уровня хромосомных нарушений в обеих исследуемых группах.

Ключевые слова: хромосомная нестабильность, цитогенетический анализ, ювенильный ревматоидный артрит, мутагенез, лимфоциты периферической крови, митомицин С.

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Metabolism and Physiology of Methylotrophs

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Abstract

Methylotrophs are aerobic chemoheterotrophic microorganisms represented by cocci and bacilli mobile forms, are inhabitants of reservoirs and soils of various type, where there are going on various processes of decomposition of organic substances with formation of the one-carbon C_1-compounds and some C_2- and C_3-compounds, capable to be assimilated by methylotrophs. These microorganisms assimilating carbon on ribuloso-5-monophospate and serine pathways are allocated from soil ground, the sewage containing decomposing vegetative remainss, from ruminant paunch and other sources. Methylotrophic bacteria recently draw the increasing attention of biotechnology as feasible sources of natural biologically active compounds – fodder fibers and irreplaceable amino acids, carotenoid pigments, lipids and policarbohydrates. For preparation of these compounds are used genetically marked strains of methylotrophic bacteria, obtained *via* genetic engineering approaches and selection. The recently developed gene-engineering methods of manipulation with the methylotrophic genom allow create on the basis of microbic DNA of methylotrophs the expression vectors of eukaryotic proteins for medical and veterinary purposes as human insulins. In this review article there are submitted data including the results of the authors' own research on metabolism and physiology of methylotrophic bacteria.

Keywords: methylotrophs, taxonomy, physiology, metabolism, evolution.

Introduction

Methylotrophs is a taxonomic heterogeneous group of microorganisms represented by chemoheterotrophic obligate and facultative methylotrophic bacteria and yeasts capable of assimilating carbon *via* ribulose-5-monophosphate and serine pathways of assimilation of more reduced than CO_2-carbon C_1-compounds – formaldehyde (HCOH), formic acid (HCOOH), and compounds containing either a methyl group (CH_3) or two or more methyl groups that are not directly connected with each other, as dimethyl ether CH_3–O–CH_3 [1]. In nature this class of compounds is most widely occurred in natural gas methane (CH_4), which is found in deposits of

coal, oil and synthesized in large amounts by methane-forming bacteria under anaerobic conditions. The decay of pectins and other natural organic substances containing methyl esters producing methanol (CH_3OH) and ethanol (CH_3CH_2OH), which is also a substrate for the growth of methylotrophic bacteria. The tissues of plants and animals contain other substrates for methylotrophic bacteria – methyl formate (CH_3COOH), methylamines (CH_3NH_2), dimethylamines $(CH_3)_2NH$, trimethylamines $(CH_3)_3N$) and their oxides.

Assimilation of C_1-compounds by microbial cell is almost accomplished with breathing and, therefore is realized by strict aerobes. The only exception is the assimilation of methanol by methane-producing bacteria under anaerobic conditions. The ability to oxidize methane belongs mostly to prokaryotes. Among anaerobes such ability has methane-producing archaea, sulfate-reducing eubacteria, and some chemotrophic and phototrophic eubacteria. Methanol can also be used as a substrate for some methylotrophic yeasts.

Methylotrophs family comprises obligate and facultative aerobic eubacteria, which possess the ability to use one-carbon compounds as a source of carbon and energy. Further enumeration of such microorganisms is wide. They are represented by various Gram-positive and Gram-negative forms – representatives of the families of *Pseudomonas*, *Bacillus*, *Hyphomicrobium*, *Protaminobacter*, *Arthrobacter*, *Nocardia* and others. [2]. At present time methylotrophs are found among representatives of the following taxonomic genera: *Acidomonas, Afipia, Albibacter, Aminobacter, Amycolatopsis, Ancylobacter, Angulomicrobium, Arthrobacter, Bacillus, Burkholderia, Flavobacteria, Granulibacter, Hansschlegelia, Hyphomicrobium, Methylarcula, Methylibium, Methylobacillus, Methylobacterium, Methylohalomonas, Methyloligella, Methylonatrum, Methylophaga, Methylophilus, Methylopila, Methylorhabdus, Methylorosula, Methylotenera, Methyloversatilis, Methylovirgula, Methylovorus, Mycobacterium, Paracoccus, Pseudomonas, Xanthobacter*.

True methylotrophic bacteria belong to the family of *Methylococcaceae*, including a genera of *Methylococcus*, represented by vegetative cells resembling cocci and coccobacilli (0,7–1,5×1,0–1,5 μm) and *Methylomonas*, submitted by monads and rod-shaped cells (0,5–1,0×0,7–2,0 μm). The main taxonomic character while allocating this family is the morphological characteristics of the cells and the ability to use methane as a sole source of carbon and energy under aerobic conditions [3]. Methylotrophs, assigned to the family of *Methylococcaceae*, are gram-negative eubacteria with different morphology and cell's size, which form moving or still forms. Some members of this taxonomic family are able to form cysts.

The practical interest towards the study of methylotrophs is associated not only with the characteristics of their metabolism, but also with the prospects of their practical use in biotechnology as producers of high-grade feed protein and essential amino acids, as leucine and phenylalanine [4], and their isotopically labeled analogues as well [5]. The digestibility of biomass of methylotrophic bacteria amounts 85–98 %, while the productivity measured by a conversion of methanol makes up 37,6–67,5 % [6] (Table 1). As we demonstrated early, owing to strong growth in minimal salt media with methanol, high yields of biomass and bioconversion of methanol into the components of the cellular biomass (with conversion efficiency of 15,5–17,3 dry biomass per 1 g of consumed substrate) methylotrophic bacteria are considered as a cheap source of deuterated protein and amino acids [7]. The traditional approach for this is the growth of methylotrophic bacteria on minimal growth M9-media with 2 % (v/v) $C^2H_3O^2H$ and 98 % (v/v) ^2H_2O (Table 2). The profitability for obtaining the microbial protein is determined for methylotrophs, mainly by the cost of such inexpensive and available substrate, as is methanol.

Table 1: Growth parameters of different methylotrophic bacteria [6]

Bacterial strains	**The molar yield of dry biomass, g/mol of methanol**	**The specific growth rate, h^{-1}**	**The level of carbon conversion of methanol, %**	**The quantity of consumed nitrogen,%**
Ribulose-5-monophosphate pathway of carbon assimilation				
Pseudomonas C.	17,3	0,49	67,5	13,2
Pseudomonas methanolica	17,0	0,63	66,5	11,0
Methylomonas methanolica	15,7	0,52	62,0	11,7
Serine pathway of carbon assimilation				
Pseudomonas 1	12,1	0,17	47,5	11,37
Pseudomonas 135	12,1	0,14	47,5	11,48
Pseudomonas AM1	9,8	0,09	37,6	11,20
Pseudomonas M-27	13,1	0,11	51,0	9,40
Pseudomonas roseus	13,1	0,15	51,0	10,60

Table 2: Amino acid composition of the protein hydrolyzate of facultative methylotrophic bacteria *Brevibacterium methylicum 5652* obtained from maximally deuterated M9-medium with 2 % (v/v) $C^2H_3O^2H$ and 98 % (v/v) ^2H_2O and levels deuteration molecules [7]

Amino acid	**Yield, % of dry weight of 1 kg of biomass of**		**Number of deuterium atoms incorporated into the carbon skeleton of the molecule***	**The level of deuteration of molecules, % of the total number of hydrogen atoms****
	Protonated sample (control)	**The sample obtained in 98 % (v/v) ^2H_2O**		
Gly	8,03	9,69	2	90,0
Ala	12,95	13,98	4	97,5
Val	3,54	3,74	4	50,0
Leu	8,62	7,33	5	49,0
Ileu	4,14	3,64	5	49,0
Phe	3,88	3,94	8	95,0
Tyr	1,56	1,83	7	92,8
Ser	4,18	4,90	3	86,6
Thr	4,81	5,51	ND	ND
Met	4,94	2,25	ND	ND
Asp	7,88	9,59	2	66,6
Glu	11,68	10,38	4	70,0
Lys	4,34	3,98	5	58,9
Arg	4,63	5,28	ND	ND
His	3,43	3,73	ND	ND

** When calculating the level of deuteration protons (deuterons) at COOH- and NH_2-groups of amino acid molecules were not considered due to their easy dissociation and isotopic (^1H–^2H) exchange in $H_2O/^2H_2O$.

*** ND – no data.

The industrial value has also biotransformation carried out by methylotrophs: immobilized bacteria, cell extracts and purified enzymes for C_1-oxidation compounds, especially methanol dehydrogenase, catalyzing the oxidation of organic compounds with short chain aromatic and acyclic hydrocarbons, phenols, alcohols, and heterocyclic hydrocarbons may be used for biotransformation [8]. Biotransformation results in obtaining a product having commercial value, for example, for obtaining propylene oxide from propylene and the substrate for the synthesis of synthetic polymers. Under certain conditions of growing the methylotrophic bacteria up to 60 % of their biomas makes up of poly-β-hydroxybutyrate – a biopolymer having big commercial value as a substitute for plastics [9].

The content of phospholipids – cardiolipin, phosphatidylcholine and phosphatidylethanolamine in cell membranes of methylotrophic bacteria reaches up 8–10 % (w/w) by weight of dry biomass, which makes it possible to use methylotrophic bacteria as inexpensive sources of phospholipids for the cosmetics industry, medicine and diagnostic purposes [10] Furthermore, some methylotrophs are sources of cytochrome C that allows to replace the precious drug used in cardiology – cytochrome C, the source of which is the mammalian cardiac muscle [11].

Methylotrophs can also serve as a basis for creating genetically engineered producer strains of eukaryotic proteins for medical and veterinary usage. Thus the levels of expression of some eukaryotic genes, e.g., interferon α_1 and α_f in methylotrophic cells are higher than those ones in *E. coli* [12].

In addition, methylotrophs are capable of synthesizing the various natural pigments (melanins, carotenoids, prodiginines) carrying out the various protective functions in the cell [13]. Pigments of methylotrophic bacteria are of interest in terms of their practical use: carotenoids are used as pro-vitamins as part of feed additives and natural food colors. Melanogenny methylotrophs can be used to produce dihydroxy-phenylalanine, anticancer, radioprotective and humic substances. Currently, interest for the using of methylotrophic bacteria and components of their cell biomass in biotechnology is increasing due to the development of new technologies for chemical synthesis of methanol.

The purpose of this paper was to examine the data on the metabolism and physiology of methylotrophic microorhanisms.

Taxonomy of methylotrophs

Obligate methylotrophs

On the ability to utilize carbon methylotrophs are divided into two major taxonomic sub-groups of microorganisms – obligate and facultative methylotrophs [14]. Obligate methylotrophs are able to grow only on methane and C_1-compounds; of other substrates their growth can maintain only methanol and dimethyl ether. On the contrary, facultative methylotrophs are able to grow not only on methanol and methylamine, but on methane and some polycarbon C_n-compounds. Often they also grow on formic acid and on a small number of simple C_2- and C_4-compounds (see Table 3).

Table 3: Substrates obligate and facultative methylotrophs (for example, typical representatives of each group) [14]

Substrates		Obligate methylotrophs (*Methylomonas*)	Facultative methylotrophs (*Hyphomicrobium*)
C_1-compounds	Methan (CH_4)	+	–
	Dimethyl ether (CH_3–O–CH_3)	+	–
	Methanol (CH_3OH)	+	+
	Formic acid (HCOOH)	–	+
C_2- compounds	Ethanol (C_2H_5OH)	–	+
	Acetic acid (CH_3COOH)	–	+
C_4- compounds	β-hydroxybutyric acid (CH_3CH (OH) CH_2COOH)	–	+

The first studied obligate methylotrophic bacterium of *Methylomonas methanica* – the Gram-negative rods with polar flagella, was described almost 100 years ago, and for several decades had been the only known bacterium capable of oxidizing methane (Figure 1). The further development and improvement of the methods of accumulation and isolation of methane oxidizing bacteria in selective nutrient media recently led to the discovery of a large number of the novel microorganisms, which are similar in properties, but different in structure. Today all obligate methylotrophic bacteria are classified into the genera: *Methylococcus*, *Methylomonas*, *Methylosinus*, *Methylocystis*, *Methylobacillus*, *Methylophilus*, *Methylophaga*, *Methylovorus* and *Methylobacterium*.

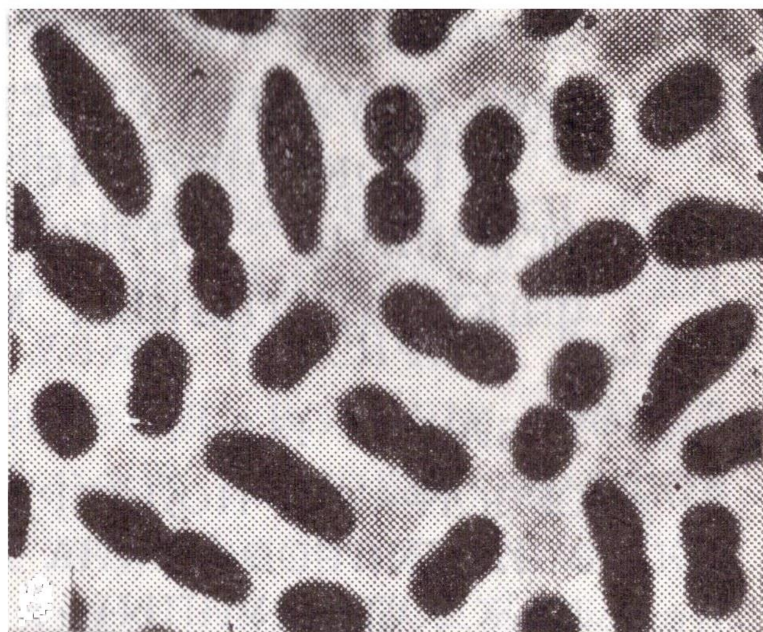


Figure 1. Electron micrograph of rod-shaped obligate methylotrophic bacterium *Methylomonas methanica* [15]

A characteristic feature of methylotrophs is the presence in their cells the developed system of intracytoplasmic membranes, which are divided into 2 types: the intra-cytoplasmic membrane of type I and the intra-cytoplasmic membrane of type II (Figure 2). The intra-cytoplasmic membrane of type I is presented by stacks of tightly packed vesicular discs distributed throughout the cytoplasm, while the intra-cytoplasmic membrane of type II has the form of lamellae – a system of intra-cytoplasmic membranes, derived from the cytoplasmic membrane and kept a distinct relationship with it. These membranes have the form of individual bubbles, tubes or plates (lamellae) arranged around the periphery of the cell cytoplasm [16]. On the topology and structure these membrane systems are reminiscent of the intra-cytoplasmic membrane of some nitrifying bacteria.

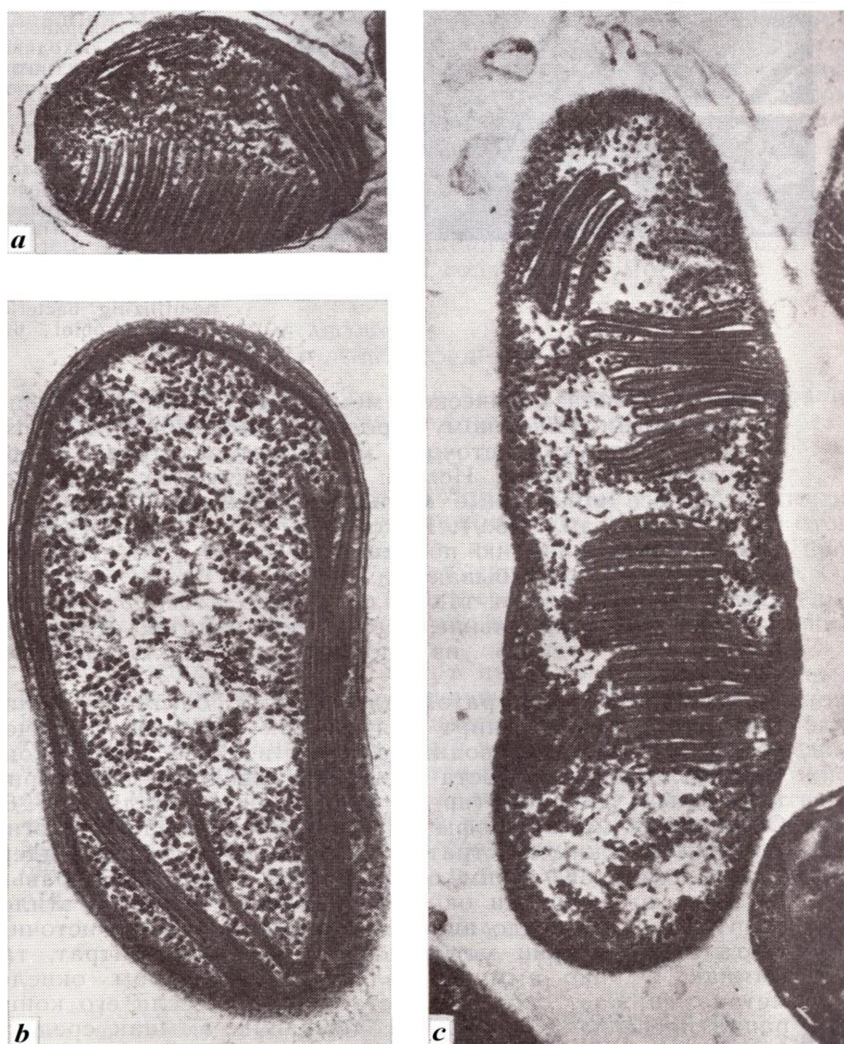


Figure 2. Electron micrographs of thin sections of cells of three obligate methylotrophs with intra-cytoplasmic membrane systems of two types: *a* – *Methylococcus*, the membrane system of type I; *b* – *Methylosinus*, the membrane system of type II; *c* – *Methylomonas*, the membrane system of type I [16]

Being based on the structural features of intra-cytoplasmic membranes, all obligate methylotrophic bacteria can be divided into two main groups – rod-shaped and coccoid bacteria [17]. Some of them form stable to drying resting cells, which in their structure resemble cysts similar to nitrogen-fixing bacteria *Azotobacter* and exospores, which are small spherical cells, spunning off from the poles of the parent cell (Figure 3).

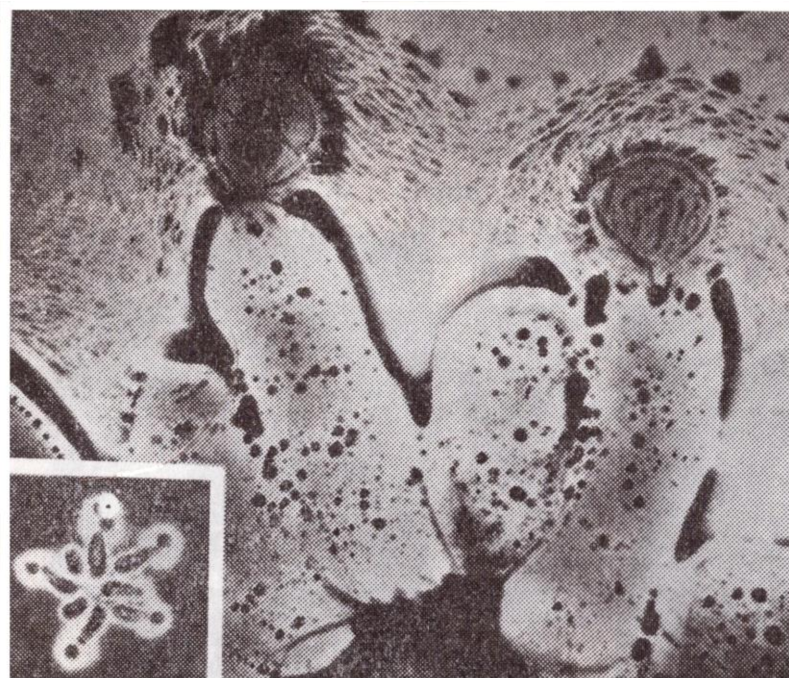


Figure 3. Electron micrograph of exospores of obligate methylotrophic bacterium *Methylosinus*. The inset at the left bottom shows the electron micrograph of budding exospores budding [17]

The best substrates for all obligate methylotrophic bacteria are methane and methanol. The rate of bacterial growth on methanol usually is not great. Obligate methylotrophs are able to oxidize only a few organic substrates that are unable to support their growth. Such compounds include formic acid (HCOOH), which they further oxidized to CO_2, ethylene (C_2H_4) and ethyl alcohol (CH_3CH_2OH), oxidized to acetaldehyde (CH_3CHO). As a nitrogen source, these bacteria can utilize both nitrate and ammonia. However, ammonia as being the methane oxidation inhibitor reduces the rate of bacterial growth when its concentration in growth medium is greater than 0,05 %. As a rule in nutrient media containing ammonia are formed trace amounts of nitrates. Thus, methane oxidizing bacteria are nitrifying bacteria, although scientific evidences that they can produce energy at such a small ratio of ammonium oxidation, are not numerous.

Facultative methylotrophs

Facultative methylotrophs are included some of the genera *Pseudomonas*, *Arthrobacter*, *Mycobacterium*, *Bacillus*, *Acetobacter*, *Achromobacter*, *Nocardia*, *Hyphomicrobium*, *Brevibacterium* and others. Despite the fact that obligate methylotrophs are able to grow only *via* assimilation of methanol as a sole carbon source, cumulative cultures at the utilization of the substrates are enriched with microorganisms of other types, so called facultative methylotrophs. These bacteria, unlike obligate methylotrophs are capable along with methane and methanol to assimilate as carbon sources some other one-carbon and poly-carbon compounds. The best studied of the facultative methylotrophs is the budding bacterium *Hyphomicrobium*. This bacterium is known as a powerful denitrifier, and can be isolated from enrichment cultures by incubation of microorganisms in a medium containing methanol and NH_4^+ ions under anaerobic conditions.

Facultative methylotrophic bacterium *Brevibacterium methylicum 5652*

We isolated from aerobic enrichment the cultures with methanol the leucine-dependent Gram-positive rod-shaped facultative methylotrophic aerobic bacteria *Brevibacterium methylicum 5652* implementing ribulose-5-monophosphate pathway of carbon assimilation, producer of phenylalanine and other metabolically related amino acids [18]. Unlike other traditional producers of *L*-phenylalanine, which do not have negative control of prephenate dehydratase (EC 4.2.1.51) or 3-deoxy-*D*-arabino-heptulosonate-7-phosphate synthase (EC 2.5.1.54), the uniqueness of this

strain consists in that it requires *L*-leucine for growth. The initial stage of biochemical research with this strain of methylotrophic bacteria was connected with obtaining auxotrophic mutants, which in majority cases are characterized by the limited spectrum of mutant phenotypes and, besides that the high level of reversions. The initial *L*-leucine dependent strain *B. methylicum*, producer of *L*-phenylalanine was obtained *via* selection at previous stage of research after processing of parental strain by nitrozoguanidin. Screening for resistant cell colonies was carried out by their stability to the analogue of phenylalanine – *meta*-fluoro-phenylalanine (50 μg/ml). The analogue resistant mutants allocated on selective media were able to convert methanol and accumulate up to 1 gram per 1 liter of *L*-phenylalanine into growth media, which is important for biotechnological use of this strain in the production of phenylalanine.

Further attempts were made to intensify the growth and biosynthetic parameters of this bacterium in order to grow on media M9 with higher concentration of deuterated substrates – $C^2H_3O^2H$ and ^2H_2O. For this, was applied deuterium enrichment technique *via* plating cell colonies on 2 % (w/v) agarose media M9 supplemented with 2 % (v/v) $C^2H_3O^2H$ with an increase in the ^2H_2O content from 0; 24,5; 49,0; 73,5 up to 98 % (v/v) ^2H_2O, combined with subsequent selection of cell colonies which were resistant to deuterium. The degree of cell survive on maximum deuterated medium was approx. 40 %. The data on the yield of biomass of initial and adapted *B. methylicum*, magnitude of lag-period and generation time on protonated and maximum deuterated M9 medium are shown in Figure 4. The yield of biomass for adapted methylotroph (*c*) was decreased approx. on 13 % in comparison with control conditions (*a*) at an increase in the time of generation up to 2,8 h and the lag-period up to 40 h (Figure 4). As is shown from these data, as compared with the adapted strain, the growth characteristics of initial strain on maximally deuterated medium were inhibited by deuterium.

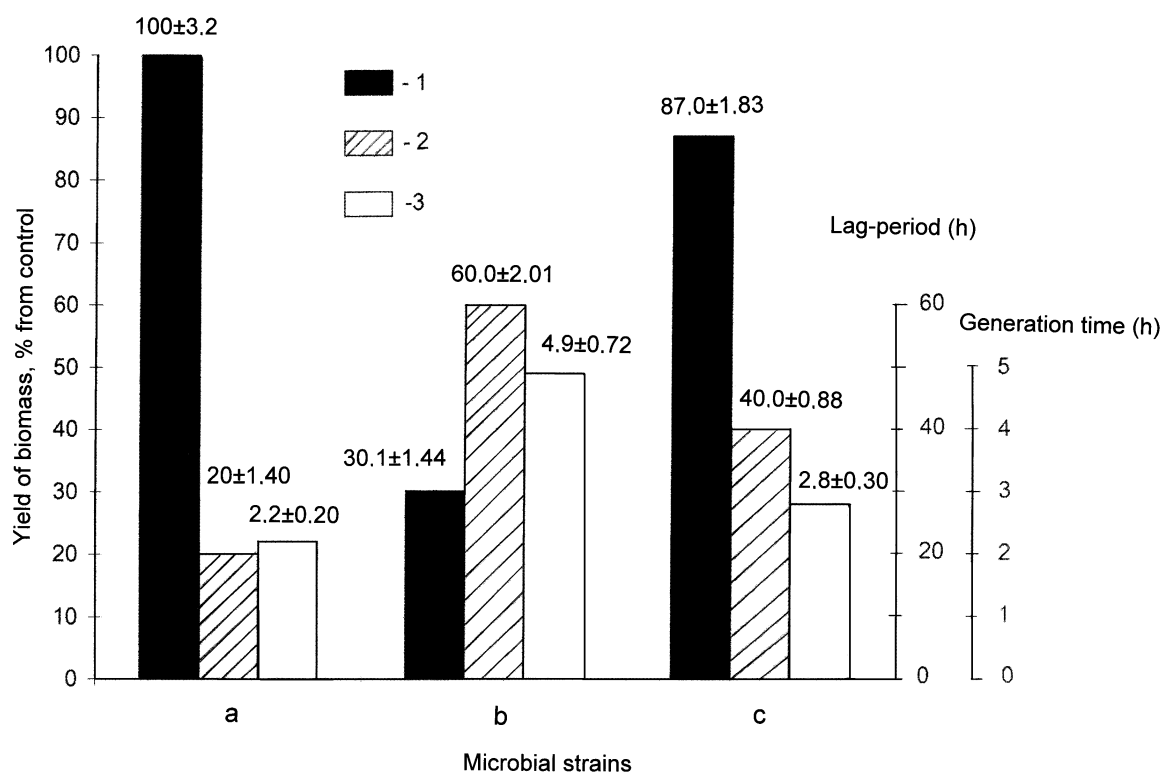


Figure 4. Yield of microbial biomass of *B. methylicum*, magnitude of lag-period and generation time in various experimental conditions: initial strain on protonated M9 medium (control) with water and methanol (*a*); initial strain on maximally deuterated M9 medium (*b*); adapted to deuterium strain on maximally deuterated M9 medium (*c*): 1 – yield of biomass, % from the control; 2 – duration of lag-period, h; 3 – generation time, h.

Experimental conditions are given in Table 4 (expts. 1–10) relative to the control (expt. 1) on protonated medium M9 and to the adapted bacterium (expt. 10'). Various compositions of $C^2H_3O^2H$ and ^2H_2O were added to growth media M9 as hydrogen/deuterium atoms could be assimilated both from $C^2H_3O^2H$ and ^2H_2O. The maximum deuterium content was under conditions (10) and (10') in which we used 98 % (v/v) ^2H_2O and 2 % (v/v) $C^2H_3O^2H$. The even numbers of experiment (Table 4, expts. 2, 4, 6, 8, 10) were chosen to investigate whether the replacement of CH_3OH by its deuterated analogue affected growth characteristics in presence of ^2H_2O. That caused small alterations in growth characteristics (Table 4, expts. 2, 4, 6, 8, 10) relative to experiments, where we used protonated methanol (Table 4, expts. 3, 5, 7, 9). The gradual increment in the concentration of ^2H_2O into the growth medium caused the proportional increase in lag-period and yields microbial biomass in all isotopic experiments. Thus, in the control (Table 4, expt. 1), the duration of lag-period did not exceed 20,2 h, the yield of microbial biomass (wet weight) and production of phenylalanine were 200,2 and 0,95 gram per 1 liter of growth medium. The results suggested, that below 49 % (v/v) ^2H_2O (Table 4, expts. 2–4) there was a small inhibition of bacterial growth compared with the control (Table 4, expt. 1). However, above 49 % (v/v) ^2H_2O (Table 4, expts. 5–8), growth was markedly reduced, while at the upper content of ^2H_2O (Table 4, expts. 9–10) growth got 3,3-fold reduced. With increasing content of ^2H_2O in growth media there was a simultaneous increase both of lag-period and generation time. Thus, on maximally deuterated growth medium (Table 4, expt. 10) with 98 % (v/v) ^2H_2O and 2 % (v/v) $C^2H_3O^2H$, lag-period was 3 fold higher with an increased generation time to 2,2 fold as compared to protonated growth medium with protonated water and methanol which serve as control (Table 4, expt. 1). While on comparing adapted bacterium on maximally deuterated growth medium (Table 4, expt. 10') containing 98 % (v/v) ^2H_2O and 2 % (v/v) $C^2H_3O^2H$ with non adapted bacterium at similar concentration showed 2,10 and 2,89 fold increase in terms of phenylalanine production and biomass yield due to deuterium enrichment technique, while, the lag phase as well as generation time also got reduced to 1,5 fold and 1,75 fold in case of adapted bacterium.

Table 4: Effect of variation in isotopic content (0–98 % ^2H_2O, v/v) in growth medium M9 on bacterial growth of *B. methylicum* and phenylalanine production

Exp. number	**Media components, % (v/v)**				**Lag-period (h)**	**Yield in terms of wet biomass (g/l)**	**Generation time (h)**	**Phenylalanine production (g/l)**
	H_2O	**^2H_2O**	**CH_3OH**	**$C^2H_3O^2H$**				
1(control)	98,0	0	2	0	20,2±1,40	200,2±3,2	2,2±0,20	0,95±0,12
2	98,0	0	0	2	20,3±1,44	184,6±2,78	2,4±0,23	0,92±0,10
3	73,5	24,5	2	0	20,5±0,91	181,2±1,89	2,4±0,25	0,90±0,10
4	73,5	24,5	0	2	34,6±0,89	171,8±1,81	2,6±0,23	0,90±0,08
5	49,0	49,0	2	0	40,1±0,90	140,2±1,96	3,0±0,32	0,86±0,10
6	49,0	49,0	0	2	44,2±1,38	121,0±1,83	3,2±0,36	0,81±0,09
7	24,5	73,5	2	0	45,4±1,41	112,8±1,19	3,5±0,27	0,69±0,08
8	24,5	73,5	0	2	49,3±0,91	94,4±1,74	3.8±0.25	0,67±0,08
9	98,0	0	2	0	58,5±1,94	65,8±1,13	4,4±0,70	0,37±0,06
10	98,0	0	0	2	60,1±2,01	60,2±1,44	4,9±0,72	0,39±0,05
10'	98,0	0	0	2	40,2±0,88	174,0±1,83	2,8±0,30	0,82±0,08

* The date in expts. 1–10 described the growth characteristics for non-adapted bacteria in growth media, containing 2 % (v/v) $CH_3OH/C^2H_3O^2H$ and specified amounts (%, v/v) of ^2H_2O.
** The date in expt. 10' described the growth characteristics for bacteria adapted to maximum content of deuterium in growth medium.
***As the control used exprt. 1 where used ordinary protonated water and methanol

The adapted *B. methylicum* eventually came back to normal growth at placing over in protonated growth medium after some lag-period that proves phenotypical nature of a phenomenon of adaptation that was observed for others adapted by us strains of methylotrophic bacteria and representatives of other taxonomic groups of microorganisms [19, 20]. The effect of reversion of growth in protonated/deuterated growth media proves that adaptation to ^2H_2O is rather a phenotypical phenomenon, although it is not excluded that a certain genotype determines the manifestation of the same phenotypic trait in the growth media with different isotopic compositions. On the whole, improved growth characteristics of adapted methylotroph essentially simplify the scheme of obtaining the deutero-biomass which optimum conditions are M9 growth medium with 98 % (v/v) ^2H_2O and 2 % (v/v) $C^2H_3O^2H$ with incubation period 3–4 days at temperature +35 °C.

Literature reports clearly reveal that the transfer of deuterated cells to protonated medium M9 eventually after some lag period results in normal growth that could be due to the phenomenon of adaptation wherein phenotypic variation was observed by the strain of methylotrophic bacteria. The effect of reversion of growth in protonated/deuterated growth media proves that adaptation to ^2H_2O is a phenotypical phenomenon, although it cannot be excluded that a certain genotype determined the manifestation of the same phenotypic attribute in media with high deuterium content. The improved growth characteristics of the adapted bacterium essentially simplify the obtaining of deutero-biomass in growth medium M9 with 98 % (v/v) ^2H_2O and 2 % (v/v) $C^2H_3O^2H$.

Adaptation, which conditions are shown in experiment 10' (Table 4) was observed by investigating of growth dynamics (expts. 1*a,* 1*b,* 1*c*) and accumulation of *L*-phenylalanine into growth media (expts. 2*a,* 2*b,* 2*c*) by initial (*a*) and adapted to deuterium (*c*) strain *B. methylicum* in maximum deuterated growth medium M9 (Figure 5, the control (*b*) is obtained on protonated growth medium M9). In the present study, the production of phenylalanine (Fig. 5, expts. 1b, 2b, 3b) was studied and was found to show a close linear extrapolation with respect to the time up to exponential growth dynamics (Fig. 5, expts. 1a, 2a, 3a). The level of phenylalanine production for non-adapted bacterium on maximally deuterated medium M9 was 0,39 g/liter after 80 hours of growth (Fig. 5, expt. 2b). The level of phenylalanine production by adapted bacterium under those growth conditions was 0,82 g/liter (Fig. 5, expt. 3b). Unlike to the adapted strain the growth of initial strain and production of phenylalanine in maximum deuterated growth medium were inhibited. The important feature of adapted to ^2H_2O strain *B. methylicum* was that it has kept its ability to synthesize and exogenously produce *L*-phenylalanine into growth medium. Thus, the use of adapted bacterium enabled to improve the level of phenylalanine production on maximally deuterated medium by 2,1 times with the reduction in the lag phase up to 20 h. This is an essential achievement for this strain of methylotrophic bacteria, because up till today there have not been any reports about production of phenylalanine by leucine auxotrophic methylotrophs with the NAD^+ dependent methanol dehydrogenase (EC 1.6.99.3) variant of the RuMP cycle of carbon assimilation. This makes this isolated strain unique for production of phenylalanine.

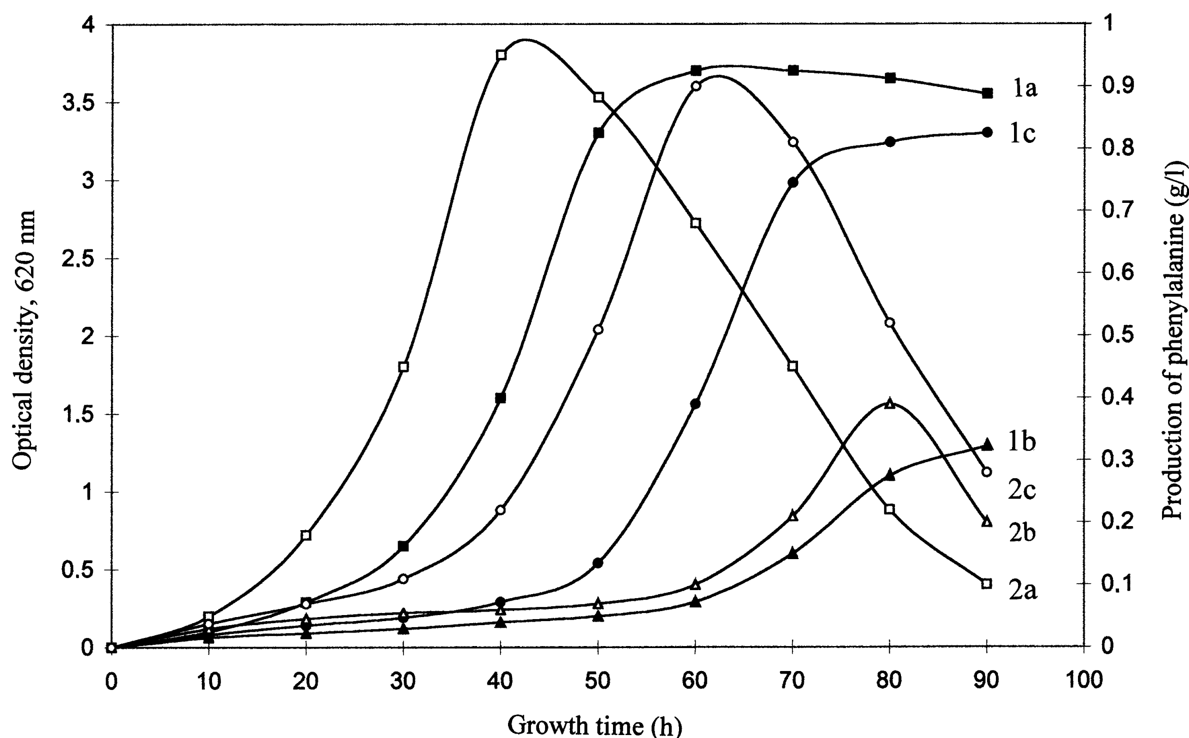


Figure 5. Growth dynamics of *B. methylicum* (1a, 2a, 3a) and production of phenylalanine (1b, 2b, 3b) on media M9 with various isotopic content: 1a, 1b – non-adapted bacterium on protonated medium (Table 1, expt. 1); 2a, 2b – non-adapted bacterium on maximally deuterated medium (Table 1, expt. 10); 3a, 3b – adapted bacterium on maximally deuterated medium (Table 1, expt. 10')

The general feature of phenylalanine biosynthesis in H_2O/^2H_2O-media was increase of its production at early exponential phase of growth when outputs of a microbial biomass were insignificant (Figure 5). In all the experiments it was observed that there was a decrease in phenylalanine accumulation in growth media at the late exponential phase of growth. Microscopic research of growing population of microorganisms showed that the character of phenylalanine accumulation in growth media did not correlate with morphological changes at various stages of the cellular growth. Most likely that phenylalanine, accumulated in growth media, inhibited enzymes of its biosynthetic pathways, or it later may be transformed into intermediate compounds of its biosynthesis, e.g. phenylpyruvate. Phenylalanine is synthesised in cells of microorganisms from prephenic acid, which through a formation stage of phenylpiruvate turns into phenylalanine under the influence of cellular transaminases. However, phenylalanine was not the only product of biosynthesis; other metabolically related amino acids (alanine, valine, and leucine/isoleucine) were also produced and accumulated into growth media in amounts of 5–6 μmol in addition to phenylalanine.

With increasing of ^2H_2O content in growth media, the levels of deuterium enrichment in [^{2}H]amino acid molecules were varried propotionaly. The similar result on proportional specific increase of levels of deuterium enrichment into [^{2}H]phenylalanine and other metabolically related [^{2}H]amino acids (alanine, valine and leucine/isoleucine) was observed in all isotopic experiments where used increasing concentration ^2H_2O in growth media (Table 5). Predictably, enrichment levels of [^{2}H]phenylalanine related to the family of aromatic amino acids synthesised from shikimic acid and metabolically related [^{2}H]amino acids of pyruvic acid family – alanine, valine and leucine at identical ^2H_2O concentration in growth media are correlated among themselves. Such result is fixed in all isotope experiments with ^2H_2O (Table 5). Unlike [^{2}H]phenylalanine, deuterium enrichment levels in accompanying [^{2}H]amino acids – Ala, Val and Leu/Ile keep a stable constancy within a wide interval of ^2H_2O concentration: from 49 % (v/v) to 98 % (v/v) ^2H_2O (Table 5).

Summarizing these data, it is possible to draw a conclusion on preservation of minor pathways of the metabolism connected with biosynthesis of leucine and metabolic related amino acids of pyruvic acid family – alanine and valine, which enrichment levels were in correlation within identical concentration of H_2O in growth media (phenylalanine is related to the family of aromatic amino acids synthesized from shikimic acid). Since leucine was added into growth media in protonated form, another explanation of this effect, taking into consideration the various biosynthetic pathways of Leu and Ileu (Ileu belongs to the family of aspartic acid, while Leu belongs to the pyruvic acid family), could be cell assimilation of protonated leucine from growth media. Since Leu and Ileu could not be clearly estimated by EI MS, nothing could be said about possible biosynthesis of [^{2}H]isoleucine. Evidently, higher levels of deuterium enrichment can be achieved by replacement of protonated leucine on its deuterated analogue, which may be isolated from hydrolysates of deuterated biomass of this methylotrophic bacterium.

Table 5: Effect of deuterium enrichment levels (atom%) in the molecules of [^{2}H]amino acids excreted by *B. methylicum**

[^{2}H]amino acid	Concentration of ^2H_2O in growth media, % (v/v)**			
	24,5	**49,0**	**73,5**	**98,0**
Ala	24,0±0,70	50,0±0,89	50,0±0,83	50,0±1,13
Val	20,0±0,72	50,0±0,88	50,0±0,72	62,5±1,40
Leu/Ileu	20,0±0,90	50,0±1,38	50,0±1,37	50,0±1,25
Phe	17,0±1,13	27,5±0,88	50,0±1,12	75,0±1,40

* At calculation of enrichment levels protons (deuterons) at COOH- and NH_2-groups of amino acids were not considered because of dissociation in H_2O (^2H_2O).
** The data on enrichment levels described bacteria grown on minimal growth media M9 containing 2 % (v/v) $C^2H_3O^2H$ and specified amounts (%, v/v) of ^2H_2O.

Metabolism of methyl compounds

The process of enzymatic oxidation of methane in the cells of methanotrophs can be represented schematically in Figure 6.

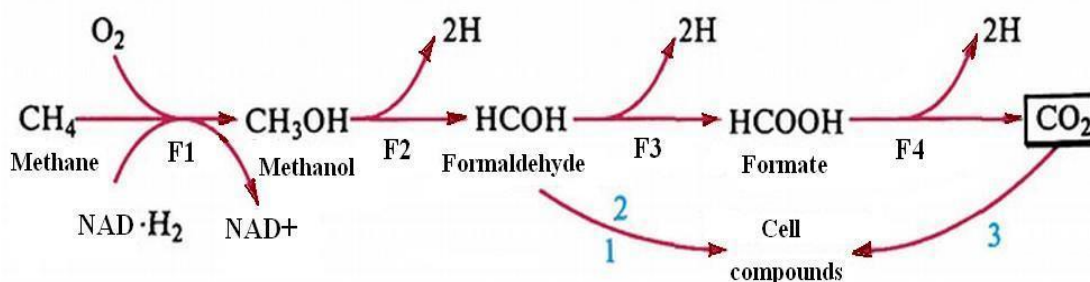


Figure 6. Diagram of the enzymatic oxidation of methane: F1 – methane monooxygenase; F2 – methanol dehydrogenase; F3 – formaldehyde dehydrogenase; F4 – formate dehydrogenase

The initial stage of the oxidation of methane to methanol is catalyzed by $NAD \cdot H_2$-dependent methane monooxygenase – a key enzyme of aerobic methanotrophs; other enzymes are present in other members of methylotrophs. The literature describes two forms of this enzyme: associated with intra-cytoplasmic membranes and soluble methane monooxygenase [21]. Electron donor for the first form of the enzyme can be the reduced cytochrome (Cyt) or $NAD \cdot H_2$, forming as a result of the reversed electron transport; for the second form of the enzyme – only $NAD(P) \cdot H_2$ or compounds which are oxidized with its formation. Carbon is fixed by the cell at the stage of carbon dioxide (3) or formaldehyde formation (2) (Figure 6).

Subsequent stages of the enzymatic oxidation of methanol are catalyzed by relevant dehydrogenases – CytC dependent methanol dehydrogenase, CytC-dependent formaldehyde dehydrogenase (or its function is performed by methanol dehydrogenase) and NAD^+-dependent

formate dehydrogenase, differing by the structure, the nature of electron acceptors, and other parameters.

The energy efficiency of the oxidation of C_1-compounds by relevant dehydrogenases is determined by the place of receipt of the electrons along the respiratory chain, which in composition of transporters and their localization on the membrane is similar to those ones typical for most aerobic eubacteria. In the oxidative metabolism of C_1-compounds are involved $NAD{\cdot}H^+$, flavins, quinones, cytochromes *a*, *b*, *c*. Oxidation of methanol to formaldehyde, catalyzed by methanol dehydrogenase containing as prosthetic group the residue of pyrrolo-quinoline quinone (coenzyme PQQ), is accompanied by the transfer of electrons in the mitochondrial respiratory chain at the level of cytochrome c. This process leads to the synthesis of ATP molecule (Figure 7).

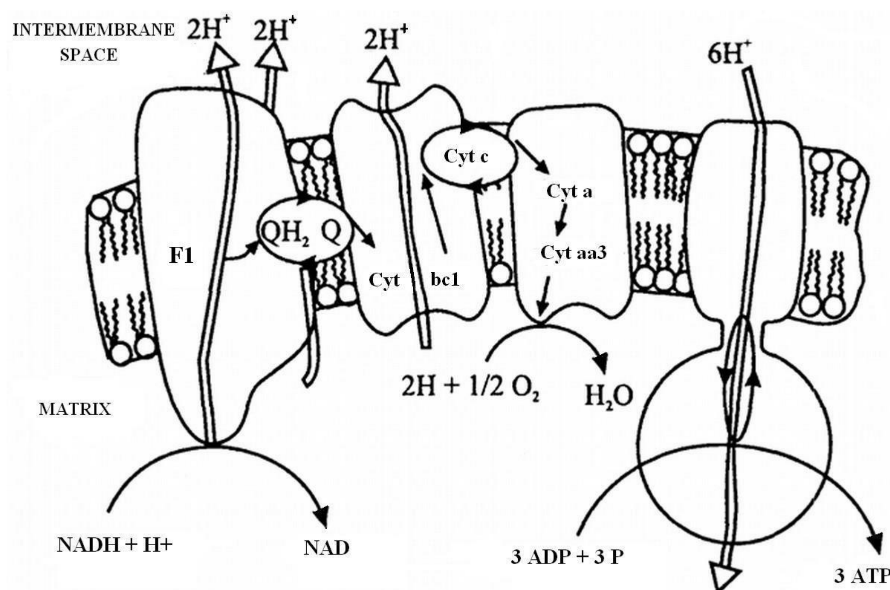
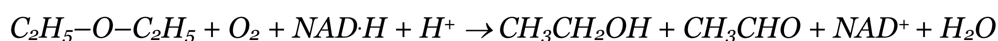


Figure 7. The respiratory electron transport chain: Cyt – cytochrome, Q – quinone, Fl – flavoprotein.

Formaldehyde in methylotrophs is a key metabolite, at which diverge the constructive and energy pathways [22]. Part amount of formaldehyde is converted into cell substances *via* the specific to methylotrophic bacteria assimilation cyclic pathways, while most part of formaldehyde is oxidized with NAD^+-dependent formaldehyde dehydrogenase to formate, which is further cleaved to CO_2 with using formate dehydrogenase.

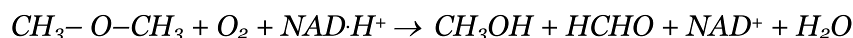
The NAD^+-dependent oxidation of formaldehyde and formate suggesting that the transfer of an electron pair can be linked to the transmembrane movement of protons involving ATP. The experimental data indicate, however, for smaller outputs of ATP. However, the question on the level at which the electrons are transferred from formaldehyde and formate into the respiratory chain is not entirely clear.

Some Gram-positive methylotrophic bacteria can utilize as a substrate for their growth diethyl ether (C_2H_5–O–C_2H_5). This compound is cleaved at oxidation by oxygenation, resulting in formation of ethanol (CH_3CH_2OH) and acetaldehyde (CH_3CHO):



$$C_2H_5\text{–}O\text{–}C_2H_5 + O_2 + NAD{\cdot}H + H^+ \rightarrow CH_3CH_2OH + CH_3CHO + NAD^+ + H_2O$$

A similar oxidation of dimethyl ether to methane in bacteria may result in formation of methanol and formaldehyde, although the mechanism of the reaction is not completely understood:



$$CH_3\text{–}\ O\text{–}CH_3 + O_2 + NAD{\cdot}H^+ \rightarrow\ CH_3OH + HCHO + NAD^+ + H_2O$$

In contrast to the oxidation of methanol occurring with the participation of methanol dehydrogenase, the oxidation of primary alcohols is carried out by pyridine-dependent dehydrogenases.

Experiments carried out by us on the study of the incorporation of deuterium into the components of the cellular biomass by assimilation of $C^2H_3O^2H$ at the growth of methylotrophic bacteria *Methylobacillus flagelatum KT* and *Brevibacterium methylicum 5652* in the growth media with 2 % (v/v) $C^2H_3O^2H$, showed a small amount of deuterium entering into the molecules with carbon of $C^2H_3O^2H$ (not more than 5 %) [23]. This result is explained by dilution of the deuterium label at the expense of biochemical processes associated with the decomposition of $C^2H_3O^2H$ at its assimilation by the cell, and the isotopic exchange reaction and dissociation in aqueous media. Thus, of four deuterium atoms in the molecule of $C^2H_3O^2H$, only one deuterium atom at the hydroxy group $–O^2H$ is most mobile and therefore readily dissociates in an aqueous medium to form C^2H_3OH. The three remaining deuterium atoms in the molecule of $C^2H_3O^2H$ are entered into the cycle of enzymatic oxidation of methanol that leads to loss of deuterium label due to the formation of compounds being more oxidized than methanol. In particular, such incorporation of deuterium into the components of the cellular biomass confirms the classical scheme of the enzymatic oxidation of methanol to formaldehyde in methylotrophs cells, which then utilized by ribulose-5-monophosphate or serine pathway of carbon assimilation.

Assimilation of carbon by methylotrophic bacteria

Methylotrophs are able to form carbon in cells from C_1-compounds, as from organic substrate, as well as due to the assimilation of CO_2, formed at oxidation of C_1-compounds in the reduced oxidative pentose phosphate pathway (Calvin cycle), resulting in fixation of carbon dioxide and formation from it the hexose molecule [24]. Experiments with using ^{13}C-labeled carbon substrates showed that the bulk of carbon in the cell was derived from oxidized substrate, instead of CO_2. The tricarboxylic acid cycle (TCA cycle) also plays no significant role in the catabolic pathways of methylotrophs, because TCA-cycle enzyme activity in the cells of methylotrophs is relatively low. In fact, the carbon source is an intermediate – formaldehyde, which in methylotrophs is a key metabolite, at which diverge structural and energy pathways leading to the two main pathways of assimilation of C_1-compounds by the cell – ribulose-5-monophosphate and serine pathways of carbon assimilation [25]. The enzymes which catalyze biochemical reactions are specific for each cycle.

The ribulose-5-monophosphate (RMP) pathway in many respects is similar to the Calvin cycle with CO_2 assimilation with the difference that in this cycle as an acceptor of CO_2 acts the pentose molecule. The key reaction of the RMP cycle is the addition of formaldehyde to ribulose-5-phosphate catalyzed by hexoso phosphate synthase with forming a phosphorylated sugar – hexulose-6-phosphate, which is then isomerized to fructose-6-phosphate with participation of phospho hexulose isomerase (Figure 8, *A*). Then fructose-6-phosphate further is subjected to phosphorylation with phosphofructokinase. The resulting fructose-1,6-diphosphate is splitted into two molecules of trioses: 3-phosphoglyceraldehyde (3-PGA) and phospho-dioxyacetone, which are used for further enzymatic reactions. 3-PGA and fructose-6-phosphate is involved in a series of reactions leading to the regeneration of the acceptor of formaldehyde – ribulose-5-phosphate. These reactions are similar to those ones for the reduced oxidative pentose phosphate pathway, in which there occurs the catalyzed by the ribulose diphosphate carboxylase the acceptance by the ribulose-1,5-bisphosphate a molecule of CO_2 and subsequent hydrolytic cleavage of the resulting hexoses into 2 molecules of 3-phosphoglyceric acid (3-PG), subjected to a series of sequential enzymatic reactions leading to the formation of a molecule of glucose. However, the reduced oxidative pentose phosphate pathway is not widespread in methylotrophs and is found only in their individual representatives, which are able to grow autotrophically as well as those ones which can utilize formic acid (HCOOH) due to CO_2 assimilation. A prerequisite for the growth of methylotrophs on formic acid is the ability to synthesize the two key enzymes of the pathway – phospho ribulokinase and ribulose diphosphate carboxylase.

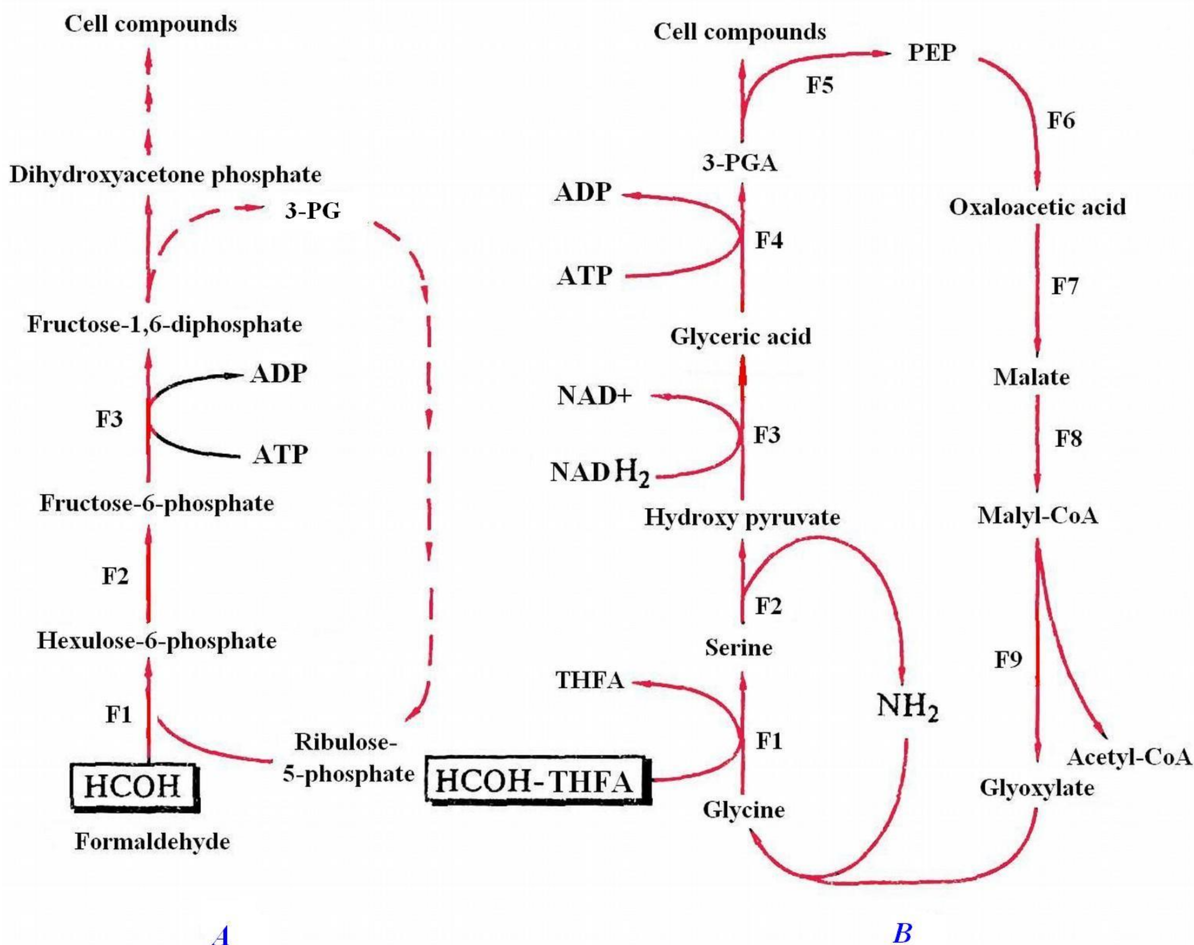
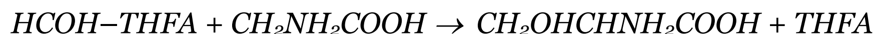


Figure 8. The pathways of assimilation of formaldehyde by methylotrophs:
A – The ribulose-5-monophosphate pathway: F1 – hexose phosphate synthase; F2 – phospho hexulose isomerase; F3 – phosphofructokinase; F4 – fructose-biphosphate aldolase; dashed line indicates the reactions of regeneration of ribulose-5-phosphate, similar to the corresponding reduced oxidative pentose phosphate pathway; *B* – The serine pathway: F1 – serine hydroxymethyltransferase; F2 – serine glyoxylate aminotransferase; F3 – hydroxy pyruvate reductase; F4 – glycerate kinase; F5 – enolase; F6 – PEP carboxylase; F7 – malate dehydrogenase; F8 – malate tiokinaze; F9 – malyl-CoA lyase

The serine pathway differs significantly from the RMP pathway by the nature of formed intermediates and enzymes (Figure 8, *B*). The key enzyme in this pathway is serine hydroxymethyltransferase which catalyzes the formation of serine from glycine and formaldehyde presented in the form of a derivative of tetrahydrofolic acid (THFA):



$$HCOH–THFA + CH_2NH_2COOH \rightarrow CH_2OHCHNH_2COOH + THFA$$

Then through a chain of consecutive reactions of transamination, the consistent recovery and phosphorylation of which leads to the formation of 3-phosphoglyceric acid (3-PGA) (Figure 8, *B*). One part of 3-PGA is used for regeneration of glycine, the primary acceptor of C_1; another portion of 3-PGA under the influence of the enzyme enolase is converted into phosphoenolpyruvic acid (PEP) and then participates in subsequent reactions. The subsequent carboxylation of PEP with PEP-carboxylase results in the synthesis of the molecule of oxaloacetic acid (OAA). This reaction is notable because at this stage CO_2 is introduced into the serine cycle. Then OAA is converted with involving the malate dehydrogenase to malate, which in turn under the influence of malil-CoA lyase splits into glyoxylic acid and acetyl-CoA. The subsequent series of reactions lead to regeneratation of glycine, and thus, the cycle is closed (Figure 8, *B*).

Investigation of the distribution of two cyclic pathways of carbon assimilation in facultative and obligate methylotrophs – RMP and serine pathways has led to the establishment of interesting features on the structure of the intra-cellular membrane [26]. Thus, the assimilation of formaldehyde through the RMP pathway is characteristic for methylotrophs with type I of membrane organization, and through the serine pathway – for methylotrophs with a system of intracytoplasmic membranes of type II. Another distinctive feature is that in the facultative methylotrophs the serine pathway is more common. Among the obligate methylotrophs the serine pathway functions only in those microorganisms which have the membrane system of type II (*Methylosinus*, *Methylocystis*), and the RMP pathway – in methylotrophs with a membrane system of type I (*Methylomonas*, *Methylobacter*, *Methylococcus*).

Evolution of methylotrophs

Since the nutritional needs of chemoautotrophs and methylotrophs are very simple, they were considered to be primitive organisms belonging perhaps to the earliest forms of life on Earth [27, 28]. Lately the submission of their place in the evolution of living organisms have changed. Biochemical apparatus of methylotrophic bacteria is just as complicated as that one for most of chemo-heterotrophs [29]. It is supposed that the first living organisms on Earth originated in anaerobic conditions, when primitive ocean was rich in organic matter formed at earlier stades of evolution. Oxygen-rich biosphere arose much later, about 2 billion years. ago. This important geochemical revolution in evolution is explained by photosynthesis. With such a character of the evolution the aerobic chemo-autotrophs and methylotrophs could appear only after oxygenic photosynthesis had been evolved. It can be assumed that chemo-autotrophs and methylotrophs could evolved from common prokaryotic microorganisms precursors that carry out photosynthesis, but lost the photosynthesis apparatus, and their electron transport chain functioning in photosynthesis, began to carry out a new feature of assimilation of C_1-compounds. Some contemporary representatives of the two major groups of prokaryotes, photosynthetic and non-photosynthetic ones, have very interesting properties. These ones include the existence of several complex characteristic of these types of systems of internal membranes; absence of a functioning of tricarboxylic acid cycle; availability of the Calvin cycle, or its analogue, the pentose phosphate cycle; localization in carboxysomes the key enzyme of the Calvin cycle (ribulose diphosphate carboxylase). Recent studies suggest the role of methylotrophs in the evolution of microorganisms. Methylotrophs play a crucial role in the circulation of methane and other C_1-compounds in the biosphere, which are maintained at a constant level, mainly due to the activity of methylotrophs.

The primary organisms (eobionts) were according to modern concepts heterotrophs, feeding by abiogenic organic substances [30]. In the process of life they emitted carbon dioxide, enriching the atmosphere. The atmosphere at that time was predominantly carbonic and did not contain oxygen. The first living organisms on Earth evidently originated in anaerobic conditions, when the primitive ocean was rich in organic matter formed at earlier stages of evolution [31]. Metabolic processes that occur with the participation of oxygen (primarily oxidative phosphorylation in breathing), and relatively few are evolutionarily later than anaerobic processes. In the absence of oxygen, it is impossible to complete combustion (oxidation) of the organic molecules of nutrients. However, as was demonstrated by the properties of the currently existing anaerobic cells, the essential for life energy is being obtained in the course of redox processes. In aerobic systems the final acceptor (oxidizing agent) of hydrogen serves oxygen, while in anaerobic – other substances. Oxidation without oxygen is implemented in two fermentation pathways – glycolysis and alcoholic fermentation. Glycolysis consists in splitting of multistage hexoses up to two molecules of pyruvate (pyruvic acid) containing three carbon atoms. In this way the two molecules of NAD reduced to NADH and two molecules of ADP phosphorylated to get two molecules of ATP. The wide occurrence of glycolysis in bacteria, when multistage hexoses are in splitting up to two molecules of pyruvate (pyruvic acid), indicates that it is one of the most ancient metabolic pathways [32]. The biochemical reactions of glycolysis and its parallel pathway, the pentose phosphate pathway, widespread in methylotrophs, occur metal-catalyzed under the oxygen-free conditions of the Archean ocean, and, probably, also in the absence of enzymes [33]. Glycolysis could thus have originated because of chemical limitations of the prebiotic world.

The main metabolic pathways as glycolysis and Krebs cycle are present in all living organisms and characterized to the universal common ancestor that was a prokaryote with combined amino

acid, nucleotide, carbohydrate and lipid metabolism [34–36]. The preservation of these ancient pathways in evolution may result from the fact that these reactions are optimal for solving specific problems with metabolism. Thus, the end products of glycolysis and the Krebs cycle are being formed with high efficiency and with a minimum number of steps. The first metabolic pathways based on enzymes might have been part of a purine nucleotide metabolism.

The oxygen-rich biosphere arose much later, about 2 billion years ago. This important geochemical revolution in evolution is explained by photosynthesis. With such a character of the evolution the aerobic chemo-autotrophs could appear only after oxygenic photosynthesis had been evolved. It can be assumed that chemo-autotrophs and chemo-heterotrophs could have evolved from common prokaryotic microorganisms precursors that carry out photosynthesis, but lost the photosynthesis apparatus, and their electron transport chain functioning in photosynthesis, began to carry out a new feature of assimilation of carbon compounds. Some contemporary representatives of the two major groups of prokaryotes, photosynthetic and non-photosynthetic ones, have very peculiar properties. These include the existence of several complex characteristic of these types of systems of internal membranes; absence of a functioning of tricarboxylic acid cycle; availability of the Calvin cycle, or its analogue, the pentose phosphate cycle; localization in carboxysomes the key enzyme of the Calvin cycle (ribulose diphosphate carboxylase) [37, 38]. Recent studies performed by us suggest the role of chemo-heterotrophs in the evolution of microorganisms [39]. Eukaryotic cells apparently arose only when there was oxygen in the atmosphere. All eukaryotes, with very few exceptions, are aerobic organisms. Prokaryotes occupy many different ecological niches. The development of various types of metabolism in prokaryotes was apparently due to a simple cell structure, highly regulation systems, a rapid growth and the presence of multiple gene transfer mechanisms. On the path of further evolution of prokaryotes there were insurmountable difficulties related primarily to the small size of the genome, its haploid state and the small size of the cells. The new environment with aerobic conditions allows to obtain more energy, but to its use it was needed larger cells, extensive structural differentiation and therefore on many times greater gene. Large and small biological molecules provide the biosynthesis, metabolism and bioenergetics. The wastes of primary protozoa were compounds such as lactic acid and ethanol. These compounds had much less energy consumption compared to carbohydrates, but they were able to release a large amount of energy if fully oxidized to CO_2 and H_2O. As a result of the evolution originated new living organisms capable to fix oxygen in the form of H_2O and CO_2, and in return to receive the energy of combustion of what was formerly their waste.

Conclusion

Methylotrophs is taxonomycally heterogeneous group of chemoheterotrophic microorganisms presented by obligate and facultative methylotrophic bacteria and yeasts, capable of assimilating carbon from more reduced than CO_2 C_1-compounds. The practical interest to this taxonomic group of methylotrophs is associated with the peculiarities of their metabolism and with the prospects of their practical use in biotechnology as producers of high-grade feed protein and essential amino acids, and other important natural biologically active compounds, such as pigments, carbohydrates and lipids. Digestibility of biomass of methylotrophic bacteria eukaryotes makes up 85–98 %, and the productivity, as measured by a conversion of methanol amountds 37,6–67,5 %. Due to good growth in minimal media with methanol, high yields of biomass and bioconversion level of methanol into the components of the cell biomass (with conversion efficiency 15,5–17,3 g dry biomass per 1 g of consumed substrate) methylotrophic bacteria are regarded as cheaper sources of protein and essential amino acids. The profitability for obtaining the microbial protein is determined for methylotrophs mainly by the cost of such inexpensive and available substrate, as is methanol.

References:

1. Gal'chenko V.F., Andreev A.V., Trocenko Y.A. Taxonomic identification of methylotrophic bacteria. – Pushchino ONTI NCBI USSR Academy of Sciences, 1986, P. 95.

2 Romanovskaya V.A., Mohamed el Said. Modern state of classification of methylotrophic bacteria // Microbiology. 1988. V. 48. № 2. P. 97–108.

3 Trocenko YA Biochemistry and physiology of methylotrophic microorganisms / Collection of scientific articles of USSR Academy of Sciences. – Pushchino: Institute of Biochemistry and Physiology of Microorganisms, 1987, P. 25.

4. Mosin O.V., Ignatov I., Skladnev D.A., Shvets V.I. A strain of Gram-positive facultative methylotrophic bacteria *Brevibavcterium methylicum* – producer of [^{2}H]phenylalanine // Drugs development and registration. 2014. № 1(6). P. 58–67.

5. Karnaukhova E.N., Mosin O.V., Reshetova O.S. Biosynthetic production of stable isotope labeled amino acids using methylotroph *Methylobacillus flagellatum* //Amino Acids. 1993. V. 5, № 1. P. 125.

6. Mosin O.V., Shvets V.I., Skladnev D.I., Ignatov I. Microbial synthesis of ^{2}H-labelled L-phenylalanine with different levels of isotopic enrichment by a facultive methylotrophic bacterium *Brevibacterium methylicum* with RuMP assimilation of carbon // Biochemistry (Moscow) Supplement Series B: Biomedical Chemistry. 2013. V. 7, № 3. P. 249–260.

7. Mosin O.V., Ignatov I. Microbiological synthesis of ^{2}H-labeled phenylalanine, alanine, valine, and leucine/isoleucine with different degrees of deuterium enrichment by the Gram-positive facultative methylotrophic bacterium *Brevibacterium methylicum* // International Journal of Biomedicine. 2013. V. 3, № 2. P. 132–138.

8. Gal'chenko V.F. Microbial growth on C_1-compounds. – Pushchino ONTI NC BI USSR Academy of Sciences, 1977, P. 10-14.

9. Suzuhi T., Yamahi T. Mass production of poli-beta-hydroxybutyric acid by fully automatic fed-batch culture of methylotrophs // Appl. Microbiol. And Biotechnol. 1986. V. 23. P. 322–329.

10. Andreev L.V. Biosynthesis and lipid metabolism in microorganisms. – Pushchino ONTI NCBI USSR Academy of Sciences, 1979, P. 31.

11. Carver M.A., Jones C.W. The role of C-type cytochromes in the terminal respiratory chain of the methylotrophic bacterium *Methylophilus methylotrophus* // Arch. Microbiol. 1983. V. 47. P. 275–280.

12. Chistoserdov A.Y., Eremashvili M.P. Gene expression of human interferon α_F in obligate methylotroph *Methylobacillus flagellatum KT* // Molecular Genetics, Microbiology and Virology. 1987. V. 8. P. 36–41.

13. Feophilova E.P. Pigments of microorganisms. – Moscow: Nauka, 1974, 218 p.

14. Tzygankov Y.D. Physiological characterization of methylotrophic bacteria / in Biology of thermophilic microorganisms. – Moscow: Nauka, 1986, P. 31–50.

15. Whittenbury R., Phillips K.C., Wilkinson L.F. Enrichment, isolation and some properties of methane-utilizing bacteria // J. Gen Microbiol. 1970. V. 61. P. 205–210.

16. Davies S.L., R. Whittenbury R. Fine structure of metnane and other hydrocarbonutilizing bacteria // J. Gen. Microbiol. 1970. V. 61. P. 227–230.

17. Whittenbury R., Davies S.L., Davey S.I. Exospores and cysts formed by methane utilizing bacteria // J. Gen. Microbiol. 1970. V. 61. P. 219–227.

18. Mosin O.V., Skladnev D.I., Shvets V.I. Biosynthesis of ^{2}H-labeled phenylalanine by a new methylotrophic mutant *Brevibacterium methylicum* // Bioscience, biotechnology, and biochemistry. 1998. V. 62, № 2. P. 225–229.

19. Mosin O.V., Ignatov I., Skladnev D.A., Shvets V.I. Microbial synthesis of deuterium-labeled L-phenylalanine by the facultive methylotrophic bacterium *Brevibacterium meyhylicum* on media with various consentrations of heavy water // Russian Journal of Biopharmaceuticals. 2012. V. 4. № 1. P. 11–22.

20. Mosin O.V., Ignatov I. Biological influence of deuterium on procaryotic and eucaryotic cells // Drugs development and registration. 2014. № 2(7). P. 122–131.

21. Anthony C. Bacterial oxidation of methane and methanol / in: Advances in Microbial Physiology / A. H. Rose, D. W. Tempest (eds.) – New York: Academic Press, 1986, V. 27, p. 114–210.

22. Quayle I.R. The metabolism of one-carbon compounds by microorganisms // Adv. Microb. Physiol. 1972. V. 7. P. 119–124.

23. Mosin O.V., Ignatov I. Preparation of highly deuterated phenylalanine, alanine, valine and leucine/isoleucine using facultative methylotrophic bacterium *Brevibacterium methylicum* // Journal of Medicine, Physiology and Biophysics. 2014. № 1. P. 34-51.

24. Trotsenko Y.A., Murrel J.C. Pathways of primary C_1 assimilation and intermediary metabolism. Metabolosm of aerobic methanotrophs / in: Advances in Applied Microbiology. – London: Academic Press, Elsevier. 2008, V. 63. p. 205–206.

25. Trotsenko Y.A., Khmelenina V.N., Beschastny A.P. The ribulose monophosphate (Quayle) cycle: news and views. Microbial Growth on C1 Compounds / in: Proceedings of the 8th International Symposium on Microbial Growth on C1 Compounds (Lindstrom M.E., Tabita F.R., eds.). 27 August – 1 September, San Diego, Kluwer Academic Publishers, Boston. 1995, p. 86.

26. Anthony C. The biochemistry of methylotrophs. Methylotrophic microorganisms / C. Anthony. – London, New York: Academic Press. 1982, p. 351–378.

27. Ignatov I., Mosin O.V. Possible processes for origin of life and living matter with modeling of physiological processes of bacterium *Bacillus subtilis* in heavy water as model system // Journal of Natural Sciences Research. 2013. V. 83, № 8. P. 132–139.

28. Ignatov I., Mosin O.V. Modeling of possible processes for origin of life and living matter in hot mineral and seawater with deuterium // Journal of Environment and Earth Science. 2013. V. 3, № 14. P. 103–118.

29. Kelly D.P. Autotrophy: concepts of lithotrophic bacteria and their organic metabolism // Ann. Rev. Microbiol. 1970. V. 25. P. 177–185.

30. Koch A. How did bacteria come to be? / A. Koch // *Adv. Microb. Physiol.* 1998. V.40. P. 353–399.

31. Ignatov I. Modeling of possible processes for origin of life and living matter in hot mineral and seawater with deuterium / I. Ignatov, O.V. Mosin // Journal of Environment and Earth Science. 2013. V. 3. № 14. P. 103–118.

32. Romano A. Evolution of carbohydrate metabolic pathways / A. Romano, T. Conway // *Res. Microbiol.* 1996. V. 147(6–7). P. 448–455.

33. Keller M.A. Non-enzymatic glycolysis and pentose phosphate pathway-like reactions in a plausible Archean ocean / M.A. Keller, M. Ralser, A.V. Turchyn // *Mol. Syst. Biol.* 2014. V. 10. P. 725.

34. Smith E. Universality in intermeditary metabolism / E. Smith, H. Morowitz // *Proc. Natl. Acad. Sci. USA.* 2004. V. 101(36). P. 13168–13173.

35. Ebenhöh O. Evolutionary optimization of metabolic pathways. Theoretical reconstruction of the stoichiometry of ATP and NADH producing systems / O. Ebenhöh, R. Heinrich // *Bull. Math. Biol.* 2001. V. 63(1). P. 21–55.

36. Meléndez-Hevia E. The puzzle of the Krebs citric acid cycle: assembling the pieces of chemically feasible reactions, and opportunism in the design of metabolic pathways during evolution / E. Meléndez-Hevia, T. Waddell, M. Cascante // *J. Mol. Evol.* 1996. V. 43(3). P. 293–303.

37. Ouzounis C. The emergence of major cellular processes in evolution / C. Ouzounis, N. Kyrpides // *FEBS Lett.* 1996. V. 390(2). P. 119–123.

38. Schmidt S. Metabolites: a helping hand for pathway evolution? / S. Schmidt, S. Sunyaev, P. Bork, T. Dandekar // *Trends Biochem. Sci.* 2003. V. 28(6). P. 336–341.

39. Mosin O. Evolution, metabolism and biotechnological usage of methylotrophic microorganisms / O. Mosin, I. Ignatov // *European Journal of Molecular Biotechnology.* 2014. V. 5(3). P. 104–119.

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Метаболизм и физиология метилотрофов

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Аннотация. Метилотрофы – аэробные хемогетеротрофные микроорганизмы, представленные кокковидными и палочковидными подвижными формами, являются обитателями водоемов и почв различного типа, протекают процессы разложения органических веществ с образованием одноуглеродных C_1-соединений и некоторых C_2- и C_3-соединений, способных ассимилироваться метилотрофами. Эти микроорганизмы, ассимилирующие углерод по рибулозомонофосфатному и сериновому циклам, выделяют из почвы, сточных вод, содержащих гниющие растительные остатки, из рубца жвачных животных и других источников. Метилотрофные бактерии в последнее время привлекают все большее внимание биотехнологии как удобные источники многочисленных природных биологически активных соединений – кормового белка и незаменимых аминокислот, каротиноидных пигментов, липидов и полисахаридов. Для получения этих соединений используют генетически модифицированные метилотрофные штаммы продуценты, полученные в результате генно-инженерных подходов и селекции. Разработанные за последнее время генно-инженерные методы манипулирования геномом метилотрофов позволяют создавать векторы экспрессии эукариотических белков медицинского и ветеринарного назначения, прежде всего человеческих инсулинов на основе микробной ДНК метилотрофных бактерий. В данной обзорной статье приводятся данные, включая результаты собственных исследований авторов, по метаболизму и физиологии метилотрофов.

Ключевые слова: метилотрофы, таксономия, физиология, метаболизм, эволюция