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The influence of artificial and biogenic magnetic nanoparticles on the metabolism of fungi

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The aim of this work is to show the effect of artificial magnetic nanoparticles of different concentrations in the soil on the metabolism of fungi and their interaction with BMNs during cultivation. It is established by methods of comparative genomics, experimental methods, methods of high-gradient magnetic separation, taking into account the unified mechanism of biomineralization of BMNs in all organisms that a number of unicellular fungi and all higher fungi are producers of BMNs. BMNs in fungi, as in animals, plants, humans, and a number of microorganisms, form chains and are part of the transport system. BMNs in fungi are located on the walls of the conducting tissue — on the walls of vascular hyphae. When artificial magnetite nanoparticles are added to the soil during mushroom growth, nanoparticle conglomerates are formed on the walls of the conducting tissue, which include both BMNs and artificial magnetite nanoparticles. At the same time, the number and size of formed magnetite conglomerates significantly affects the morphology and maturation time of fungi.

Keywords: biogenic magnetic nanoparticles, biomineralization, Magnetospirillum gryphiswaldense MSR-1, atomic force microscopy, magnetic force microscopy, methods of comparative genomics, Agaricus bisporus, Lentinula edodes.

Вплив штучних та біогенних магнітних наночастинок на метаболізм грибів.
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Показано вплив штучних магнітних наночастинок різної концентрації у грунті на метаболізм грибів та їх взаємодію з БМН під час вирощування. Методами порівняльної геноміки, методами магнітного розділення з високим градієнтом з урахуванням уніфікованого механізму біомінералізації БМН у всіх організмах встановлено, що ряд одноклітинних грибів та всіх вищих грибів є продуцентами БМН. БМН у грибів, як і у тварин, рослин, людини та ряду мікроорганізмів, утворюють ланцюги та є частиною транспортної системи. БМН у грибів розташовані на стінках провідної тканини — на стінках судинних гіф. При додаванні штучних наночастинок магнетиту у грунт під час росту грибів на стінках провідної тканини утворюються конгломерати наночастинок, до складу яких входять як БМН, так і наночастинки штучного магнетиту. Водночас, кількість і розмір утворених конгломератів магнетиту суттєво впливає на морфологію та час дозрівання грибів.

Показано влияние искусственных магнитных наночастиц различной концентрации в почве на метаболизм грибов и их взаимодействие с БМН во время выращивания. Методами сравнительной геномики, методами магнитного разделения с высоким градиентом с учетом унифицированного механизма биоминерализации БМН во всех организмах установлено, что ряд одноклеточных грибов и всех высших грибов являются продуцентами БМН. БМН у грибов, как и у животных, растений, человека и ряда микроорганизмов, образуют цепи и является частью транспортной системы. БМН у грибов расположены на

стенках проводящей ткани — на сосудистых стенках гиф. При добавлении искусственных наночастиц магнетита в почву во время роста грибов на стенках проводящей ткани образуются конгломераты наночастиц, в состав которых входят как БМН, так и наночастицы искусственного магнетита. В то же время, количество и размер образуемых конгломератов магнетита существенно влияет на морфологию и время созревания грибов.

1. Introduction

It is known that the magnetic fields accompanying electrical processes in organisms are extremely small, and therefore the biomagnetic phenomena associated with the influence of intrinsic magnetic fields in living organisms on their metabolism, in contrast to bioelectric phenomena, have not been investigated [1–3]. This point of view was maintained for decades even after Blackmore discovered in 1975 in magnetotactic bacteria (MTB) strong natural nanoscale magnets (biogenic magnetic nanoparticles) [4], the synthesis of which is genetically programmed and carried out by the microorganisms themselves.

Biogenic magnetic nanoparticles (BMNs) are currently experimentally found in algae and protozoa [5], worms [6], shellfish [7], insects [8–12], crustaceans [13], migratory and non-quick-moving fishes [14–19], birds [20], bats [21], sea animals [22, 23], pigs [24] and in humans [25–30].

Methods of comparative genomics have shown that the genetic apparatus for the biosynthesis of BMNs is the only one in representatives of all the kingdoms of living organisms and is based on genes that originate from a common ancestor even before the appearance of multicellular organisms [31–33]. Many years BMNs was experimentally studied mainly in connection with ideas about magnetotaxis and magnetoreception, but BMNs, which are the source of their own gradient magnetic fields, non-migrating animals, plants and fungi, have not been studied much. The gradients of the intrinsic magnetostatic scattering fields of the BMNs have a sufficient value to affect the transport systems of cells components — vesicles, granules et al. [32]. Under the influence of an external gradient magnetic field a shift of intracellular amyloplasts in plants was experimentally observed, and as a result, the seedlings of barley *Hordeum vulgare* bent in the direction of the magnetic field gradient [34].

For the first time, BMNs were experimentally detected in unicellular fungi of the generas *Fusarium oxysporum* and *Verticillium dahlia*, which form BMNs of irregular quasi-spherical shape, particle size varies in the range of 20–50 nm, nanoparticles are well separated from each other and embedded in a matrix-like structure containing proteins [35]. The phenotype of these fungi (externally cellular biomineralization of BMNs, the absence of proteins that regulate the shape and size of BMNs) is confirmed by bioinformatic analysis [36, 37]. In [36, 37], using comparative genomics methods, it was predicted that among the studied representatives of the most common fungi of the *Ascomycota* and *Basidiomycota* divisions, the genomes of which are decoded by more than 50% in the GenBank NCBI database [https://www.ncbi.nlm.nih.gov], all species are producers extracellular crystalline BMNs and it has been experimentally shown that BMNs in higher fungi form chains [38, 39] that are localized on the walls of hyphae [40].

The study of fungi, which are representatives of various departments of the kingdom of Fungi, is of both fundamental and practical interest. From a fundamental point of view, the identification of potential producers of BMNs among fungi will help to find an answer to the open question about the functional purpose of BMNs in both fungi and other organisms. From a practical point of view, the identification of potential producers of BMNs among fungi is promising for the use of magnetic technologies for the neutralization of unicellular fungi and microorganisms pathogenic for humans and plants [41–43], for the manufacture of gold, silver, magnetic nanoparticles for biomedical and technical [44, 45], for the manufacture of magnetically controlled biosorbents based on mushroom biomass [46, 47].

The aim of this work is to show the effect of artificial magnetic nanoparticles of various concentrations in the soil on the grows and morphology of fungi and interaction of artificial magnetic nanoparticles with BMNs during mushroom cultivation.

2. Experimental

The study of BMNs in fungi was carried out on the example of higher shiitake, champignon and oyster mushrooms, which are the most common edible mushrooms. Shiitake, mushrooms and oyster mushrooms were grown according to standard methods [48, 49] in triplicate on blocks with a substrate without the addition of magnetite (control) and blocks with a substrate with

the addition of magnetite with a concentration of 0.1 mg/ml and 1 mg/ml. The addition of magnetite nanoparticles of various concentrations to the substrate, namely 1 mg/ml and 0.1 mg/ml (the concentration is close to the magnetite content in the soil [50–53], was carried out by injecting into the substrate for each fungal fruit body. The introduction of magnetite was carried out to a depth of 1 cm, and this is the optimum depth to ensure the absorption of the magnetite solution by the mycelium of the fungi that have sprouted. The addition of magnetite to the substrate was carried out for three days.

3. Results and discussion

The use of artificial magnetic nanoparticles for growing fungi is due to the fact that mushrooms, like other living organisms, have a high-gradient magnetic separator (HGMS) in the form of BMNs chains [37, 46, 47]. Changes in the characteristics of such a separator (the magnitude of the external magnetic field, the size and number of particles or clusters of particles in the BMNs chain, the increase in the number of BMNs chains themselves and their localization) will undoubtedly affect metabolic processes, since biologically active substances (proteins, including hormones, lipids, carbohydrates, metal ions) will accumulate in the vicinity of the BMNs chains [1, 31, 36, 53], accelerating biochemical reactions and, accordingly, the growth of fungi. From this point of view, it is important to investigate how the morphology of fungi and their maturation will change when they are grown on

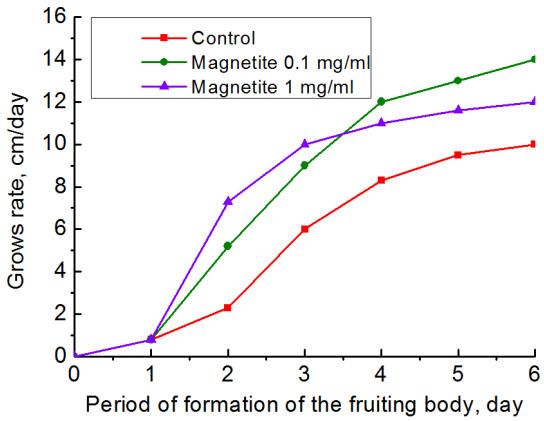

Fig. 1. Shiitake growth dynamics on substrates without addition (control) and with the addition of magnetite of various concentrations (I — lag phase; II — acceleration phase; III — exponential phase; IV — growth retardation phase; V — stationary phase).

soils with different concentrations of artificial magnetic nanoparticles, the sizes of which are also characteristic of BMNs.

Certain differences between the fruiting bodies of fungi from different blocks. Table 1 compares the morphological changes of *Lentinula edodes*, *Agaricus bisporus* and *Pleurotusostreatus* grown on substrates with different concentrations of magnetite.

From the results obtained (Table 1), it can be concluded that at high concentrations of magnetite (1 mg/ml) in the substrate, which is an order of magnitude higher than the characteristic concentration of soils (0.1 mg/ml), growth is accelerated at the beginning of the maturation of the

Table 1. Comparison of the morphological characteristics of shiitake mushrooms, champignons and oyster mushrooms grown on substrates with different concentrations of magnetite (* means the increase in mass, length of the fungus and diameter of the cap of the fungus relative to the control)

	Magnetite concentration	*A. bisporus*	*P. ostreatus*	*L. edodes*
Average weight, g	Control	40±4	15±1	14,4±0,8
	0.1 mg/ml	56±4 (*40 %)	24±1 (*60 %)	24,2±1,1 (*68 %)
	1 mg/ml	44±4 (*10 %)	19±1 (*27 %)	18±1 (*25 %)
The average length of the mushroom, cm	control	6.1±0,2	10±0.1	5.1±0.1
	0,1 mg/ml	7.2±0.2 (*18 %)	14±0.1 (*40 %)	7,9±0,1 (*54 %)
	1 mg/ml	7,0±0,2 (*15 %)	12±0,1 (*20 %)	7,9±0,1 (*54 %)
The average diameter of the cap, cm	Control	7.0±0.3	7±0.1	5,7±0.1
	0,1 mg/ml	8.2±0.3 (*17 %)	9±0.1 (*29 %)	6.9±0,1 (*21 %)
	1 mg/ml	8,2±0.3 (*17 %)	9±0.1 (*29 %)	6.2±0.1 (*9 %)

Fig. 2. Comparison of the morphology of *Lentinula edodes* grown on a substrate without the addition of magnetite (a) with the addition of magnetite at a concentration of 0.1 mg/ml, (b) and 1 mg/ml, (c) on the 6th day of cultivation.

fungus (in comparison with a concentration of 0.1 mg/ml and control), and deceleration starting from the exponential growth phase (Fig. 1). At a magnetite concentration of 0.1 mg/ml, a significant acceleration of growth and faster maturation of fungi are observed in comparison with the control. Figure 1 shows the growth graph of the *Lentinula edodes* grown on substrates without addition (control) and with the addition of magnetite of various concentrations.

Differences in the morphology of fungi are also noticeable (Fig. 2). The shape of the cap: it is convex in the control sample, when magnetite is added at a concentration of 0.1 mg/ml, it is noticeably smoothed, and at a concentration of 1 mg/ml it becomes flat, as can be seen in Fig. 2. The flat shape of the cap in fungi is formed during their aging.

By the methods of atomic force microscopy (AFM) and magnetic force microscopy (MSM), the presence of BMNs and artificial magnetic nanoparticles in the fruit body of *Lentinula edodes* after growing on a substrate with the addition of magnetite of various concentrations (0.1 and 1 mg/ml) in comparison with the control was confirmed. AFM and MSM images of tissue samples of shiitake *Lentinula edodesare* shown in Fig. 3–5, in which the BMNs are reflected in black and white dots.

The AFM image shows the topography (relief) of the surface section of the fruit body *Lentinula edodes*, shows the interweaving of hyphae, the spaces between the hyphae are highlighted in dark color (Fig. 3–5). As can be seen from Fig. 3–5, BMNs in the shiitake are located on the walls of vascular hyphae. The vascular hyphae of *Lentinula edode* are presented in Fig. 3–5 have typical morphology and sizes presented in [54, 55].

In the MSM image, the presence of BMNs (black and white dots) on the walls hyphae *Lentinula edodes*, as well as the formation of chains of BMNs (Fig. 3–5) are clearly visible. Black and white dots that reflect magnetic nanoparticles can represent either a single particle or cluster of magnetic nanoparticles of artificial or biogenic origin.

As can be seen from Fig. 3–5, with an increase in the concentration of artificial magnetite in the substrate, the length of the chain formed by BMN and artificial magnetite particles increases. So, on samples without the addition of magnetite we observe chains length of 3–7 BMNs, with the addition of magnetite in the substrate with a concentration of 0.1 mg/ml — 4–8, and with a concentration of 1 mg/ml — 6–18 magnetic nanoparticles/clusters of magnetic nanoparticles of artificial and biogenic origin (Table 2). Based on the results of AFM and MSM images samples shiitake, the maximum size of BMNs (as the average distance between adjacent black or white points) and the number of BMNs in the chain of studied fungi were also estimated (Table 2).

Thus, the particle sizes and / or clusters of artificial magnetite and BMN in the *Lentinula edodes* grown on a substrate with the addition of magnetite at a concentration of 0.1 mg/ml and 1 mg/ml increase by 22 % and 35 %, respectively, compared with the control. In *Agaricus bisporus* grown on a substrate with the addition of magnetite at a concentration of 0.1 mg/ml and 1 mg/ml, it increases by 25 % and 37 %, respectively, compared with the control. This proves that artificial magnetite is embedded in the chains of BMN. Moreover, it is known that the pore sizes of hyphae in fungi are in the range of 200–400 nm [de Souza Pereira 1999] and the sizes of indi-

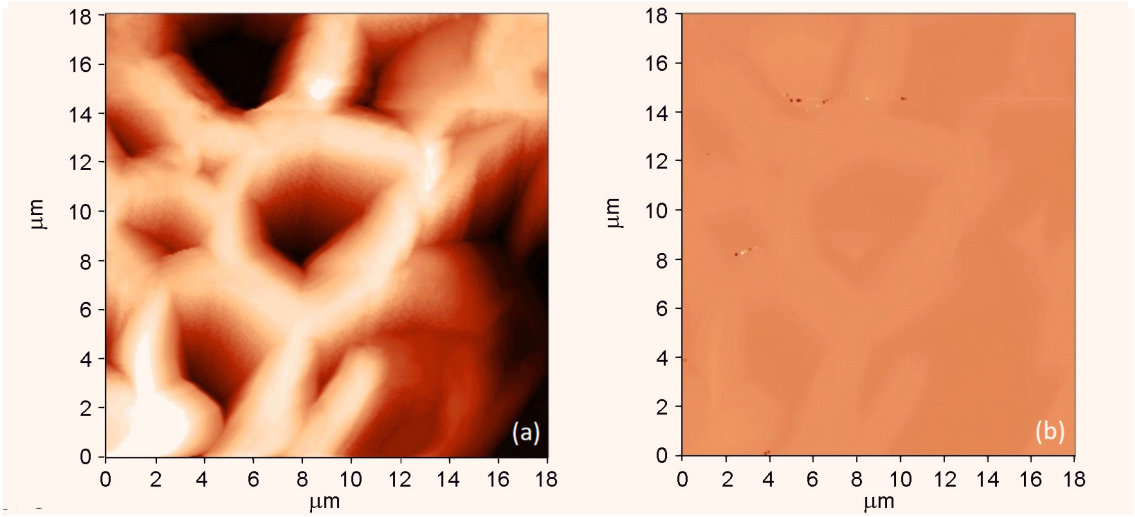

Fig. 3. AFM (a) and MSM (b) of a fruiting body *Lentinula edodes* grown on a substrate without the addition of magnetite (control).

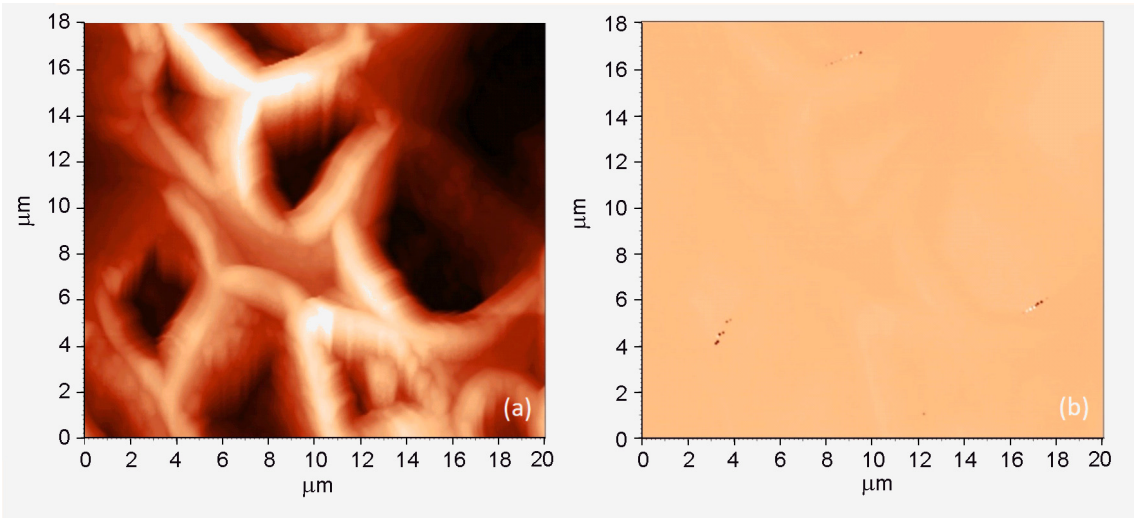

Fig. 4. AFM (a) and MSM (b) a sample of the fruiting body *Lentinula edodes* grown on a substrate with the addition of magnetite at a concentration of 0.1 mg/ml.

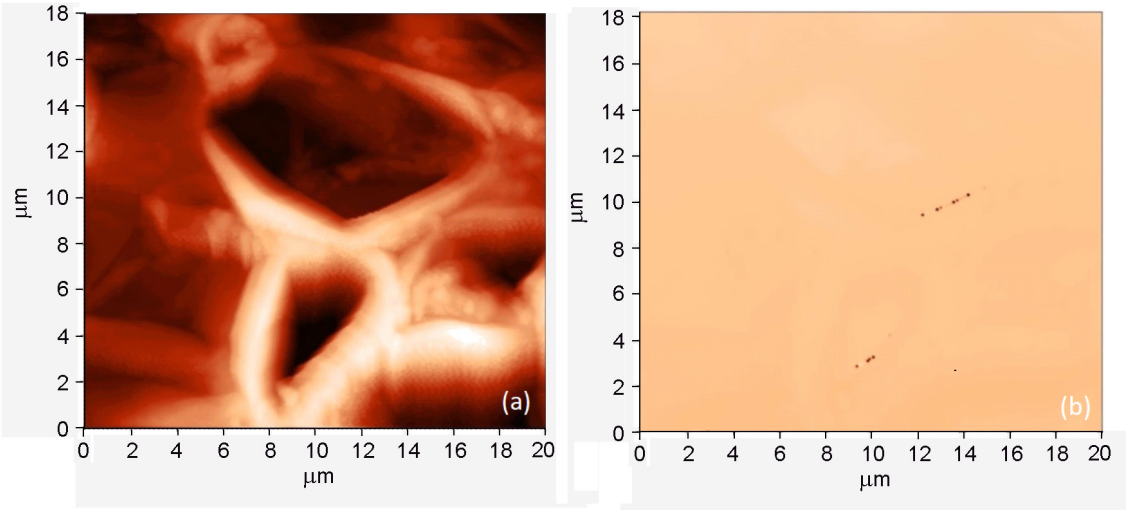

Fig. 5. AFM (a) and MSM (b) of a sample of the fruiting body *Lentinula edodes* grown on a substrate with the addition of magnetite at a concentration of 1 mg/ml.

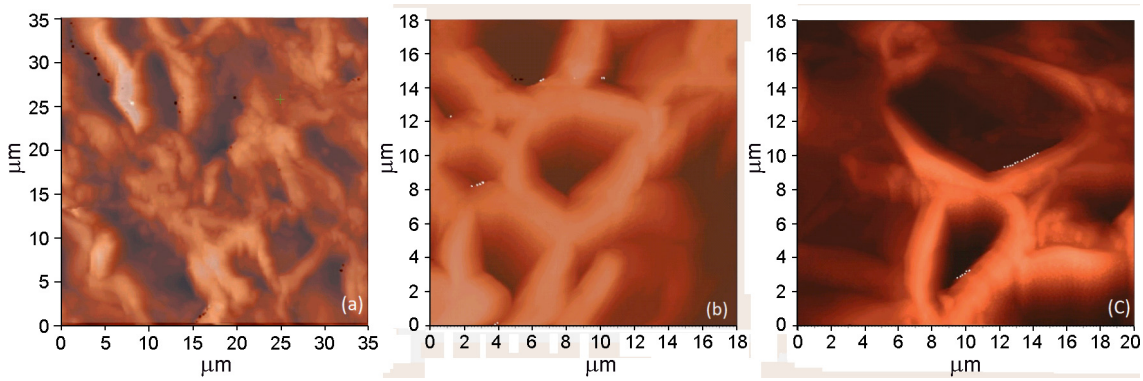

Fig. 6. Samples of the fruiting body of the *Lentinula edodes* obtained by the MSM method, superimposed on AFM images: a) a shiitake grown on a substrate without magnetite, b) with magnetite at a concentration of 0.1 mg/ml, and c) with magnetite at a concentration of 1 mg/ml

vidual particles and / or clusters of BMN and artificial magnetic nanoparticles in samples of fungi grown with the addition of magnetite with a concentration of 1 mg/ml are 170–220 nm for the *Lentinula edodes* and *Agaricus bisporus* (Table 2), it can be assumed that blocking of the small pores of hyphae is possible at a magnetite concentration that exceeds the characteristic maximum concentration in soils.

For greater clarity, the AFM and MFM image of the shiitake (Fig. 3–5) was superimposed one on one (Fig. 6). From Fig. 3–5 and Fig. 6 shows that the BMN in Fig. 6a (control), BMN and artificial magnetic nanoparticles in Fig. 6b (the concentration of magnetite in the substrate is 0.1 mg/ml), BMN and artificial magnetic nanoparticles in Fig. 6c (the concentration of magnetite in the substrate is 1 mg/ml) in the fungi form chains that are localized on the walls of vessel-like hyphae most likely extracellularly, since for mushrooms this localization is fully confirmed by the methods of comparative genomics [56, 57, 58], as well as experimental data [35].

Based on the experimental data (Table 2) and theoretical data [31, 36, 53], it can be argued that gradient magnetic forces in the vicinity of the BMNs are sufficient for the accumulation of vesicles, granules and other cluster components. With BMNs sizes from 20 nm to 150 nm and a vesicle size of the order of 100 nm, the energy of the magnetic dipole interaction of the BMNs with the vesicles is sufficient to hold the vesicles in a fragment of the BMNs chain. Since the sizes of the vesicles (60–300 nm) [59] and the sizes of BMNs in *Lentinula edodes* (132 nm 156 nm) and *Agaricus bisporus* (135–nm — 152 nm) grown on a substrate without the addition of magnetite (control) are in this range (Table 2), it can be argued that natural BMN in fungi perform the same function as in humans, animals and plants, namely the function of concentrators of vesicles and granules in vesicular transport, as well as concentrators of other components with sizes from 100 nm and more [31, 36, 53].

Table 3 shows the size and amount of BMN in the chain in a number of organisms and in fungi.

Table 2. The number and size of BMNs in *Lentinula edodes* and *Agaricus bisporus*, grown on a substrate with the addition of magnetite of various concentrations

Organism	Maximum size estimateBMNs, nm	The number of BMN in chains
L. edodes (control)	132–156	3–7
L. edodes (magnetite concentration in the substrate 0.1 mg/ml)	158–192 (22 %)*	4–8
L. edodes (magnetite concentration in the substrate 1 mg/ml)	170–220 (35 %)*	6–18
A. bisporus (control)	135–152	1–5
A. bisporus (magnetite concentration in the substrate 0.1 mg/ml)	160–200 (25 %)	3–8
A. bisporus (magnetite concentration in the substrate 1 mg/ml)	172–220 (37 %)	5–19

* increase in the size of BMN in relation to control (%).

Table 3. The number and size of BMN in the tissues of a number of organisms

Organisms	Estimation of the maximum size of BMNs, nm	Number of particles in chains
Magnetotaxis bacteria *Magnetospirillum gryphiswaldense MSR-1*	10–40, 35–120 [63]	4–200 [63]
Mushroom *Agaricus bisporus*	55–85 [38], 135–152	1–7, [38] 1–5
Mushroom *Lentinula edodes*	132–156	3–7
Leaf *Nicotianatabacum*	110–210 [62]	4–10 [62]
Root *Nicotianatabacum*	80–190 [62]	6–10 [62]
Potato stem *Solanumtuberosum*	60–120 [62]	4–8 [62]
Potato tuber *Solanumtuberosum*	35–60 [62]	2–8 [62]
Stem *Pisumsativum*	102–104 [62]	3–7 [62]
Termite	≈10 [66]	
Beak of *Gallusgallusdomesticus Columbalivia Erithacusrubecula*	≈1000 [67]	10-15 [67]
The brain of carp *Cyprinus carpio*	350–400 [38]	12 [38]
Human cerebral cortex	90–200 [68]	≈ 80 [68]
Lungs of pigs	≈ 30 nm [24]	

4. Conclusion

Given the studies of fungi carried out by methods of comparative genomics [31, 36, 57], experimental methods [35, 38], methods of high-magnetic magnetic separation [46, 47], taking into account the unified mechanism of biomineralization of BMNs in all organisms, it can be suggested that a number of unicellular fungi and higher mushrooms are producers of BMN.

At the same time, experimental studies of BMN in mushroom samples carried out in this work and others methods, showed that:

— BMNs in fungi, as in animals [24, 60], including humans [61], plants [62]; microorganisms [63], form chains;

— BMN in fungi, as in animals and plants, are part of the transport system. So, BMN in fungi is located on the walls of the conducting tissue — on the walls of vascular hyphae.

BMNs chains are components of cells that form on the walls of a conducting tissue — on the walls of vascular hyphae. At the same time, the conducting tissue of fungi serves to transfer organic and inorganic substances, hormones and the like throughout the body [64]. The identical localization of the chains of BMNs (namely, on the walls of the conducting tissue in the Fungi cannot be accidental, taking on account that the genetically programmed mechanism of biosynthesis of BMN appeared at the beginning of evolution [65]. Such localization supports the idea that the BMN chains in fungi have the same metabolic functions as in animals (including humans) of plants and microorganisms. As it has already been noted that BMNs chains create magnetic scattering fields of the order of several thousand Oe and magnetic field gradients that can significantly accelerate mass transfer processes near the membrane of vesicle cells and granules, structural elements and other membranes.

In addition, when artificial magnetite nanoparticles are added to the soil during mushroom cultivation, conglomerates of nanoparticles, which include both BMNs and artificial magnetite nanoparticles, are formed on the walls of the conductive tissue (vessel-like hyphae). Moreover, the number and size of the formed magnetite conglomerates and their number of chains differs from the control and significantly affects the morphology and maturation of mushrooms.

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